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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Potential of leek (*Allium ampeloprasum*) waste for microalgae *Chlorella vulgaris* cultivation: A preliminary evaluation

Pırasa (*Allium ampeloprasum*) atığının mikroalg *Chlorella vulgaris* kültürü için potansiyeli: Ön değerlendirme

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Abstract: Leek is an economical and healthy plant species. It contains rich dietary fibers, amino acids, bioactive compounds that increase its antioxidant capacity and more than 20 different fatty acids. It is rich in potassium, iron and selenium and can be used as a valuable source for microalgae cultivation. For impotance of leek, this study investigated the biomass production of *Chlorella vulgaris* microalgae species with leek leaf waste. To obtain the leek extract to be used for the experiment, leek leaves were dried in an oven at 40 °C and crushed in a mortar and pestle and filtered. Leek leaves were first dissolved with 10 ml DMSO (Dimethylsulfoxide) to 0.1 g/L and diluted with distilled water to a final volume of 100 ml. *Chlorella vulgaris* was exposed to leek extract concentrations of 0.01, 0.025, 0.05, 0.1 and 1.0 g/L for 72 hours and BG-11 enrichment medium was used in the control group. According to the data obtained, when leek leaves were used in the cultivation of *C. vulgaris* microalgae, a very high increase of 160% was observed at a concentration of 0.05 g/L compared to BG-11 enrichment medium. However, in the group where leek leaves were used completely, 64% increase was observed compared to the control group. This study proved that *C. vulgaris* have significant potential for food industries and the biocompost of vegetables is a suitable medium for microalgae cultivation. This study has proven that the use of vegetable wastes is suitable for obtaining a culture with high biomass of C. vulgaris microalgae, which has been used intensively in different areas of the food industry, and that leek wastes in particular provide high biomass growth. Therefore, the lower concentration of leek served as the best medium to increase the growth and biomass of C. vulgaris. This study proves that costs can be reduced and sustainable effective culture techniques can be used in microalgae culture by using vegetable wastes such as leek waste, which provides high biomass growth even at low concentrations.

Keywords: *Allium ampeloprasum*, phytoplankton, biomass, aquaculture, recycling, agricultural waste

Öz: Pırasa ekonomik ve sağlıklı bir bitki türüdür. Zengin diyet lifleri, amino asitler, antioksidan kapasitesini artıran biyoaktif bileşikler ve 20'den fazla farklı yağ asidi içerir. Potasyum, demir ve selenyum açısından zengindir ve mikroalg yetiştiriciliğinde değerli bir kaynak olarak kullanılabilir. Bu çalışmada, pırasa yaprak atıkları ile *Chlorella vulgaris* mikroalg türlerinin biyokütle üretiminin araştırılması amaçlanmıştır. Deneyde kullanılacak pırasa ekstraktını elde etmek için pırasa yaprakları 40 °C'de bir fırında kurutuldu ve bir havan ve tokmakla ezilerek süzüldü. Pırasa yaprakları önce 10 ml Dimetilsülfoksit ile 0,1 g/L'ye kadar çözüldü ve damıtılmış su ile son hacim 100 ml'ye kadar seyreltildi. *C. vulgaris* 0.01, 0.025, 0.05, 0.1 ve 1.0 g/L pırasa ekstraktı konsantrasyonlarına 72 saat süreyle maruz bırakılmış ve kontrol grubunda BG-11 zenginleştirme ortamı kullanılmıştır. Elde edilen verilere göre, pırasa yaprakları *C. vulgaris* yetiştirilmesinde kullanıldığında, 0.05 g/L konsantrasyonda BG-11 zenginleştirme ortamına göre %160 gibi çok yüksek bir artış gözlenmiştir. Ancak pırasa yapraklarının tamamen kullanıldığı grupta kontrol grubuna göre %64 oranında artış gözlenmiştir. Bu çalışma, gıda endüstrisinde farklı alanlarda yoğun olarak kullanılmaya başlanan *C. vulgaris* türünün yoğun biyokütleye sahip kültür elde edilmesinde sebze atıklarının kullanılmasının uygun olduğunu ve özellikle pırasa atıklarının yüksek biyokütle artışını imkan sağladığını kanıtlamıştır. Bu çalışma düşük konsantarasyon da dahi yüksek biyokütle artışını sağlayan pırasa atığı gibi bitkisel atıkların mikro alg kültüründe kullanılarak maliyetlerin düşürülebildiğini ve sürdürülebilir etkili kültür tekniklerinin kullanılabilirliğini ispatlamaktadır.

Anahtar kelimeler: *Allium ampeloprasum*, fitoplankton, biyokütle, su ürünleri yetiştiriciliği, geri dönüşüm, tarımsal atık

INTRODUCTION

Global energy consumption is largely powered by 87% of energy from fossil fuels, which are major contributors to greenhouse gas emissions and climate change. Therefore, energy production from renewable biological sources is important for energy security and environmental sustainability. Reliance on alternative energy sources can increase energy security by stabilizing economic fluctuations. Biomass-based renewable biofuels can be produced from sources such as energy crops, forestry residues and agricultural wastes derived from traditional biomass (Singh et al., 2014). Global challenges such as food security, population growth and environmental pressures require the development of new processes for the utilization of industrial food waste to provide social and economic benefits for the development of global sustainable agriculture (Goula and Lazarides 2015; Wang et al., 2017).

Today, the consumption of vegetables and agricultural products is increasing due to the increasing importance of vegetarian diets. The FAO (Food and Agriculture Organization of the United Nations) estimates the amount of agricultural waste at over 60%. The utilization of powders obtained from fruit waste as a new method has recently attracted the attention of many researchers (Bhandari et al., 2013; Karam et al., 2016; Neacsu et al., 2015). Similarly, Bas-Bellver et al. (2020) produced powders from vegetable waste (carrot, leek, celery and cabbage) and used them as functional food additives.

Leek (*Allium ampeloprasum*) is an economically important plant species of the Amaryllidaceae family. It is widely distributed throughout the Middle East, Russia and the Mediterranean Basin. World leek production has increased steadily since 2001. Turkey, the leading country in Europe in leek production, has an annual production of over 200,000 mmt tons (Celebi-Toprak and Alan, 2021).

Leek is a good source of carbohydrates, protein, fat, dietary fiber and a high source of the minerals. The carbohydrate and protein composition available ranges from 0.5-16.60 and 1.5-2.1 g/100 g. The amino acids (Najda et al., 2016), organic acids and phytochemicals are present in higher amount and increase the positive health effects (Shelke et al., 2020). The edible parts of leeks contain more than 20 different fatty acids such as linoleic, palmitic, oleic and α-linolenic acids. Leek plant, which is a source of Ca (30.24-81.7 mg/100 g) and Fe (0.20-2.1 mg/100 g), has also been reported to be rich in Mg (10-28 mg/100 g), Na (5-54.6 mg/100 g), P (35 mg/100 g) and Cu (0.06-0.30 mg/100 g) elements. (García-Herrera et al., 2014). In addition, leek showed that antibacterial properties against many bacteria, viruses and fungi. (Shelke et al., 2020). Due to the widespread production of leek worldwide and its rich nutritional content, it attracts attention in terms of utilization of agricultural industry wastes. However, there are limited studies on the recycling of leek wastes and the effects of nutrient content.

Microalgae are living organisms with significant potential in areas such as biotechnology and sustainable energy production. Researchers have recognized the importance of algae for the green bioeconomy, considering their functions in the environment and their potential for commercial use that reduces dependence on land-based fossil fuel products (Ahmad et al., 2020; Peter et al., 2022).

In a study, it was found that the use of compost mixture provided higher biomass concentration compared to *Chlorella vulgaris* cultivation in culture media (Chew et al., 2018). Tekin et al. (2021) used carrot pulp as agricultural waste and investigated the effects of carrot pulp on biodiesel production of *Chlorella vulgaris* microalgae. According to the data the biodiesel produced with carrot pulp complies with international standards used for biodiesel production. In addition, the performance of biodiesel production with microalgae cultivation by utilizing the wastes of the palm oil producing plant was also examined and it was reported that these wastes can also be used (Cheah et al., 2018).

The aim of this study was to investigate the potential of using the unused leaf parts of the leek plant, both during and after harvest, as enrichment in the culture medium of the microalgae species *Chlorella vulgaris*. Microalgae cultivation is an important field in terms of biomass production, biofuel production and environmental sustainability, and the use of waste products has great potential in this process. The results will help us to better understand the impression of leek green waste on microalgae cultivation. It will also contribute to waste management by promoting a sustainable approach.

MATERIALS AND METHODS

Preparation of leek extract

The leek leaves to be used for the experiment were first dried in an oven at 40 °C, then crushed in a porcelain mortar and filtered. Leek leaves were first dissolved with 10 ml DMSO (Dimethylsulfoxide) to a value of 0.1 g/L and diluted with pure water to a final volume of 100 ml. During the hot-air drying, a laboratory oven (Nuve FN 400P/500P) was used at 40°C for 12 h. Leek wastes were sliced and dried at 40°C on a basket in a laboratory oven with an air speed of 2.5 m/s (±0.03 m/s) and an adjustable temperature (±1°C) (Doymaz, 2008). Thus, the risk of DMSO-induced inhibition was minimized. The dilutions in which the experiment was performed and the amounts added are shown in Table 1.

Tablo 1. Dilutions made in experiments and amounts added

Dilution Groups (g/L)	Phytoplankton Culture Media (BG11) (ml)	Leek Extract (ml)	Chlorella Culture (ml)
Control Group	1,8	0	0,2
0,01	1,782	0,018	0,2
0,025	1,755	0,045	0,2
0,05	1,71	0,09	0,2
0,1	1,62	0,18	0,2
1	0	1,8	0,2

Cell culture and microalgae media

In the Ecotoxicology laboratory at the Faculty of Fisheries, Ege University, the test organism was cultured *Chlorella vulgaris* using BG-11 medium (OECD, 2011) (Turan and Çakal Arslan, 2023). Prior to the experiment, a pre-culture was established and incubated at a temperature of 25±1°C. The initial cell concentration in the test cultures was approximately 4-5x10^{-4} cells/mL for *Chlorella vulgaris.* The experiments were carried out in 24-well plate containers with a final volume of 2 ml and 3 repetitions. Samples were exposed to a photoperiod of 14:10 (Light: Dark) hours under constant illumination at around 2000 lux, while being agitated at 100 rpm. Cell density was measured at 24, 48 and 72 hour time points on a Biontech plate reader at 660 wavelength (Turan and Çakal Arslan, 2023).

Determining the growth rate

Microalgae growth rate was performed in accordance with the OECD 201 standard (OECD, 2011). At 0 and 72 hours, cell count data were evaluated based on growth rate as described in standard protocols.

The mean specific growth rate (μ), the logarithmic increase in biomass for each control and experimental groups, was calculated as follows,

$\mu i\text{-}j = \ln x_j - \ln x_i / t_j - t_i$ (day^{-1})

$\mu_{i\text{-}j}$: the average specific growth rate from i to j;

X_i: biomass at time i,

X_j: biomass at time j,

The SPSS software was used for probit analysis and the statistical significance of the data on growth rates was compared with controls using ANOVA with the assistance of the SPSS program (Ozdamar, 1999). The cell number averages among the different culture media tested were compared using one-way ANOVA testing The level of significance considered for all the analyses was P<0.05. t-test.

RESULTS

Nutrients, carbon dioxide and light are important contributors to sustain microalgae growth. Among nutrients, especially nitrogen is an important source to increase microalgae biomass (Hsieh and Wu, 2009). In order to examine the effect of leek extract on the growth rate of *C. vulgaris*, different concentrations (0.01, 0.025, 0.05, 0.1 g/L) were diluted with nutrient media. According to the data obtained in the study, when leek extract was used in the cultivation of *C. vulgaris* microalgae, a very high increase of 160% was observed at a concentration of 0.05 g/L compared to the conventional enrichment medium used in the control group. In the group using purely leek extract, an increase of 64% was observed compared to the control group, but not as large as in the groups containing 0.05 g/L and lower leek extract (Figure 1). It is thought that the reason why *C. vulgaris* increases its growth rate and cell density at a concentration of 0.05 g/L (50 mg/L) is due to the amount of nitrogen and ammonium. At low concentrations, it is not sufficient for the increase of algal biomass, and above 0.005, it has a restrictive effect. This depends on the tolerance of the species. It turns out that this is the ideal amount of leek waste for the growth and biomass increase of this species.

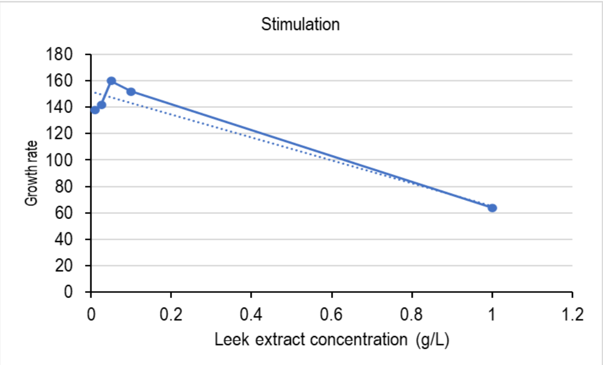

Figure 1. The effect of leek extract on the growth rate of *C. vulgaris*

DISCUSSION

Microalgae need to be produced in a high quality and sustainable manner, both for food supplements and biofuels. For this purpose, the use of agricultural wastes should be investigated and suitable ones should be combined with enrichment media to reduce costs and increase environmentally friendly production processes.

Chew et al. (2018) examined the specific growth rate of *C. vulgaris* by mixing BG-11 and compost media in different ratios. According to the results, the highest specific growth rate was measured in the combination of 75% organic compost + 25% culture medium. *C. vulgaris* grown in 75% compost content was found to increase by 19% compared to the control group. In our study, the highest growth rate was observed at 0.05 g/L, which corresponds to 4.5% of the proportional content of leek extract. Although the percentage of leek extract in the culture medium is low, the 160% increase compared to the control group shows that the nutrient content of leek extract is richer. In terms of nitrogen compounds, algae tend to prefer ammonium over nitrate, and wastewater with high ammonium concentrations promotes rapid algal growth. However, ammonium requirements vary depending on the algae. *Chlorella* and *Scenedesmus* sensivity range reported as 30 to 300 mg/L NH4+–N (Kligerman and Bouwer, 2015; Cai et al., 2013).

Tekin et al. (2021) used carrot pulp hydrolysate to increase the biomass production of *C. vulgaris* to produce biodiesel. For this reason, *C. vulgaris* were grown in BG-11 medium containing carrot pulp at concentrations of 0.25, 0.5, 1 and 2 g/L. It was determined that the addition of 0.5 g/L carrot pulp caused a 1.38-fold higher increase in growth compared to photoautotrophic conditions. When we compared the growth of *C. vulgaris* with carrot pulp, which is 10 times more concentrated than our study with leek extract, the increase rate values were close. These results revealed that leek extract, which is present at lower concentrations in the medium than carrot pulp, is more effective in the growth rate of *C. vulgaris*.

For sustainable environment, Microalgae feedstock is suitable for biodiesel production. However, microalgae biomass has higher biomass productivity compared to terrestrial feedstock production in bioenergy production. However, microalgae production does not have a sufficient market share due to its high-cost production technology (Chung et al., 2017). In order to reduce the high costs of microalgae cultivation, studies on cost-effective and efficient biomass production have increased in recent years (Ak et al., 2013; Benas and Ak, 2022; Cheirsilp et al., 2023; Zhu et al., 2022). According to our study that leek, an agricultural waste, increased the growth rate of microalgae at certain values. The findings suggest that leek waste, traditionally viewed as a byproduct with limited utility, can serve as a valuable resource in the cultivation of microalgae. By converting agricultural waste into a nutrient source, not only is the environmental burden of waste disposal mitigated, but we also pave the way for enhanced production of microalgae. In the light of the results, we think that the use of leek waste in microalgae cultivation will contribute to sustainable microalgae production, reducing resource waste and contributing to green technology for environmental protection.

Leek, which we used in the study, is an important and economic crop widely cultivated in our country and especially in our region. Leek leaves remain as waste in large quantities during and after harvesting. Therefore, utilization of waste leek leaves will contribute to sustainable agriculture. In addition, it is thought to be economically beneficial by reducing the use of chemicals used in microalgae cultivation.

CONCLUSION

In our study, *Chlorella vulgaris* was exposed to leek extract concentrations of 0.01, 0.025, 0.05, 0.1 and 1.0 g/L for 72 hours. According to the results obtained, a very high increase of 160% was observed at a concentration of 0.05 g/L compared to the enrichment medium. However, the growth rate of *C. vulgaris* was 64% in the group with only leek extract in the medium.

This study showed that leek has the potential to be used in microalgae cultivation. With further analysis, the usability of agricultural wastes in the cultivation of *Chlorella vulgaris* species to be used in different fields of use can be revealed more clearly. However, if it is to be used for this purpose, some further research (nutrient analysis, heavy metal and pesticide residue analysis in leaves, etc.) must be carried out.

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AUTHORSHIP CONTRIBUTIONS

Koray Benas: Conceptualization, resources, investigation, methodology, writing-reviewing and editing. Muhammet Ali Karaaslan: Conceptualization, resources, investigation, methodology, writing-reviewing, formal analysis. Özlem Çakal Arslan: Conceptualization, resources, methodology, investigation.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

ETHICS APPROVAL

No specific ethical approval was necessary for the study.

DATA AVAILABILITY

All relevant data is inside the article.

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