GEÇİCİ KAPAK

Kapak tasarımı devam ediyor.

BİDGE Yayınları

Selected New Interdisciplinary Scientific Studies in Biology

Editör: ALİ BİLGİLİ

ISBN: 978-625-8989-87-8

1. Baskı
Sayfa Düzeni: Gözde YÜCEL
Yayınlama Tarihi: -
BİDGE Yayınları

Sertifika No: 71374

www.bidgeyayinlari.com.tr - bidgeyayinlari@gmail.com
Krc Bilişim Ticaret ve Organizasyon Ltd. Şti.
Güzeltepe Mahallesi Abidin Daver Sokak Sefer Apartmanı No: 7/9 Çankaya / Ankara

İÇİNDEKİLER

BÖLÜM 0

Scenedesmus spp.: ISOLATION FROM DIFFERENT FRESHWATER ENVIRONMENTS AND LABORATORY CULTIVATION

RIDVAN ÖZTÜRK[1]
ELİF TEZEL ERSANLI[2]

Introduction

Presently, with the increasing population of the world, which has not been matched by commensurate food production,

---

[1] Bakırköy Çatı Private Social Activities and Development Center, Istanbul/Türkiye, ORCID: 0000-0001-8362-8511, ozturkridvan.1990@gmail.com

[2] Prof. Dr., Sinop University, Faculty of Arts and Science, Department of Biology, Sinop/Türkiye, ORCID: 0000-0003-0608-9344, eersanli@sinop.edu.tr

"*This study is based on the master's thesis prepared by the first author under the supervision of the second author and was supported by the Scientific Research Projects Coordination Unit at Sinop University (Project No: FEF-1901.13-08).*"

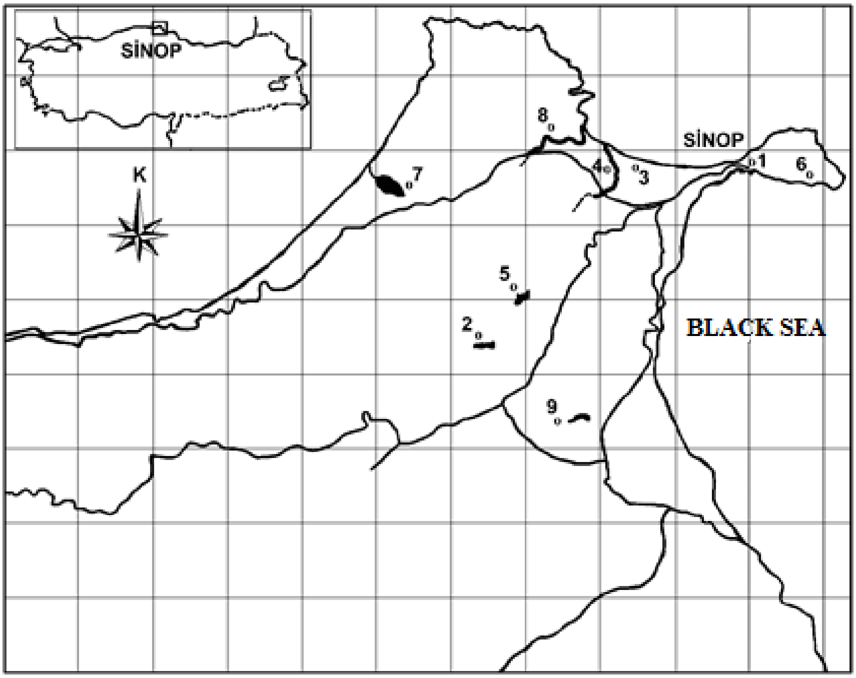
there exist serious problems in the world today like imbalance in nutrition and undernourishment. In response to the increasing population, the existing means of production in agriculture have proved insufficient; thus, intensive efforts are being made to discover alternative efficient methods of production. Algae are the primary producers of organic matter synthesis in aquatic environments. Algae are the bioreactors of aquatic systems. They represent an important source of many biochemical compounds, including proteins, carbohydrates, lipids, vitamins, pigments and mineral salts. Microalgae can be referred to as the most important biological raw material of the future in biotechnology. Microalgae and macroalgae are economically important because they have made millions of dollars worth of contributions to the world economy annually (Borowitzka, 1992: 268). Microalgae are economically important because they produce huge amounts of biomass. Moreover, due to their rapid growth rate, adaptation to different conditions, and broad tolerance ranges, microalgae are of particular interest for biotechnology. Currently, algae are among the most widely researched microorganisms for producing total cellular protein (TCP). At the same time, they are potentially applicable in animal and human nutrition. The main feature of algae in producing TCP is that they are photosynthetic organisms and therefore do not need organic carbon as a source of energy (Beyatlı, 1996: 54). The development of cultures by means of production systems and techniques may be conducted through several ways (Stein, 1973: 8; Borowitzka, 1992: 267; Sukatar, 2002: 101; Andersen, 2005: 83). In the case of algal cultivation, the most important purpose is to obtain the highest possible biomass per unit time. The choice of an appropriate culture technique will not be possible without considering production costs and economic aspects as well. The last few decades have been devoted to researching the *Scenedesmus* genus, especially because of their biofuel properties. This way, different approaches to obtaining maximum biomass in a short period of time and at a minimum cost while utilizing certain culture media, nutrient salts, pH levels, and other environmental factors are being designed. Moreover, experiments focusing on the efficient extraction of products from algae are gaining popularity. The aim of this work is the isolation of *Scenedesmus* spp. from the freshwater environments of Sinop province and conducting laboratory culture. With a focus on growth rates achieved in varying culture media, the study seeks to establish culture conditions that will achieve maximum cell concentration per unit time, thus showing growth optimization. The expectation is that the results will be useful in formulating production procedures with improved yield and lower cost for future *Scenedesmus* spp. experiments.

Scenedesmus spp. used for the study were isolated from freshwater environments and cultured in laboratory conditions. *Scenedesmus* species belong to the genus *Scenedesmus* as defined by Meyen (1829) and are placed in the class Chlorophyceae of the division Chlorophyta. *Scenedesmus* species are cosmopolitan algae usually inhabiting stagnant or slowly flowing water. Morphologically, the species are usually represented by single, double, or sometimes triple rows of colonies; colonies of these species contain either two, four, or eight cells, whereas colonies containing sixteen or thirty-two cells are occasionally observed. Cell morphology of *Scenedesmus* varies and is represented by cylindrical, ellipsoidal, oval, or ovoid-shaped cells; cell walls can be smooth or granulated, while in some cases long spines can be found. Every cell contains a single chloroplast with a pyrenoid and asexual reproduction occurs by means of autospore formation. Algae of the genus *Scenedesmus* exhibit planktonic behavior but can also be sedentary and associated with macrophytes; the alga is widespread in stagnant and slow flowing environments (John, Whitton & Brook, 2002: 384).

Materials and Methods

Sinop province is located at 35°09′ east longitude and 42°01′ north latitude, and it constitutes the most northern province in Turkey. The classification of species into the *Scenedesmus* genus has been made according to the basic taxonomic references of Prescott (1973: 273) and John, Whitton & Brook (2002: 384). The scientific name of species and their taxonomy has been checked via the AlgaeBase website database (Guiry & Guiry, 2013). The sampling sites in Sinop province are shown in Figure 1 below.

Figure 1 Sampling stations in Sinop province (1: Pond in Museum of Archaeology, 2: Bektaşağa Pond, 3: Airport Zone, 4: Karasu Stream, 5: Muhsinli Pond, 6: Nisi Pond, 7: Sarıkum Lake, 8: Sırakaraağaçlar Creek, 9: Taşmanlı Pond



The abbreviations and alternative names for the sampling stations in the Sinop are shown in Table 1.

Table 1 Species found in the sampling stations in Sinop

Serial Number	**Location**	**Coordinates**	**Code**	**Species**
1	Pond in Museum of Archaeology	N 42°0.1'42.6" E 035°09'05.9"	ASA	*Tetradesmus obliquus* (Turpin) M.J. Wynne, 2016 Syn: *S. acutus* Meyen, 1829
2			ASE	*Scenedesmus ecornis* (Ehrenberg) Chodat, 1926 Syn: *S. ecornis* (Ehrenberg) Chodat, 1926
3			ASO	*Scenedesmus obtusus* Meyen, 1829 Syn: *S. ovalternus* Chodat, 1926
4			ASQ	*Scenedesmus communis* E. Hegewald, 1977 Syn: *S. quadricauda* Chodat, 1926
5	Bektaşağa Pond	N 41°56'25.5" E 034°59'11.8"	BSA	*Tetradesmus obliquus*
6			BSE	*Scenedesmus ecornis*

7	Airport Area	N 42°01'10.2" E 035°05'18.8"	HSA	*Tetradesmus obliquus*
8			HSE	*Scenedesmus ecornis*
9			HSO	*Scenedesmus obtusus*
10	Karasu Stream	N 42°01'57.1" E 035°03'36.0"	KSA	*Tetradesmus obliquus*
11			KSE	*Scenedesmus ecornis*
12	Muhsinli Pond	N 41°57'52.1" E 035°00'32.7"	MSA	*Tetradesmus obliquus*
13			MSE	*Scenedesmus ecornis*
14			MSO	*Tetradesmus obliquus*
15	Nisi Pond	N 42°01'15.07" E 35°11'10.90"	NSA	*Tetradesmus obliquus*
16	Sarıkum Lake	N 42º00'43.76" E 34º55'45.20"	SGSA	*Tetradesmus obliquus*
17			SGSO	*Scenedesmus obtusus*
18	Sırakaraağaçlar Creek	N 42°02'31.4" E 035°02'41.3"	SSA	*Tetradesmus obliquus*
19			SSE	*Scenedesmus ecornis*
20			SSQ	*Scenedesmus communis*

21	Taşmanlı Pond	N 41°54'21.3" E 035°02'39.3"	TSA	*Tetradesmus obliquus*
22			TSE	*Scenedesmus ecornis*
23			TSO	*Scenedesmus obtusus*

Sampling was conducted in lake, stream, creek and ponds of Sinop province where the *Scenedesmus* species abounded especially during spring and summer season by bringing back samples from these locations in laboratory. pH levels were measured in these collected samples, which were made to have their pH decreased to 4 to eliminated zooplankton that were present and after 24 hours, adjusted back to the same level (Brett, 1989: 409; Andersen, 2005: 215). These samples were allowed to enrich for a week. Ten milliliters of these enriched samples were then inoculated into the BG-11 to get a mixed culture.

Culture Medium

BG-11 (Blue-Green Media) (UTEX, 2014) was used as the culture medium to isolate species from the genus *Scenedesmus* in fresh water in the Sinop Province. To examine the cultivation of isolated species under laboratory conditions, urea containing *Scenedesmus* medium (U-S) (İnaç, 1994: 17), Bristol-Proteose medium (B-P) (UTEX, 2014) and MWC (Modified Woods Hole Medium) (Anonymous, 2014) were examined in relation to the cultivation of isolated species under laboratory conditions. The amount of culture medium, considering the surface-to-volume ratio of the growing medium and CO_2 limitation, was set to be 1/3 of the volume of erlenmeyer flasks (Apha-Awwa-Wef, 2005: 52).

Sterilization was done through an autoclave Nüve OT 032. Sodium penicillin ($C_{16}H_{17}N_2NaO_4S$) was added to the culture medium to prevent contamination by prokaryotes (Andersen, 2005:

123). A single-cell isolation method using a micropipette was applied to purify and isolate the algal cells (Pringsheim, 1946: 19; Andersen, 2005: 122). Identification of species from the genus *Scenedesmus* was done with a microscope Leica DM500.

The isolated cultures were then introduced to 500 mL flasks that contained 150 mL volume each. A total of three inoculations was carried out. Moving on to the experimentation stage, 15 mL volume was extracted from the inoculum cultures, and then mixed with 135 mL volume of culture medium (Apha-Awwa-Wef, 2005: 54). This liquid culture medium was kept at 24 ± 2 °C (Stein, 1973: 7), in 65% humidity, under Philips light bulb having 220-240 V, 30 W and 1800 lumen for 7 days in a Nüve cooled ES110 incubator. To ensure equal lighting exposure and to avoid sedimentation at the bottom of the cultures, agitation was done twice daily.

Both direct and indirect techniques were employed for the measurement of biomass. Direct methods include the estimation of algal biomass through the counting of cell numbers using a microscope. Some examples of indirect methods are measurements of dry weight, wet weight and spectrophotometry (Rodrigues et al., 2011: 225; Sivakumar & Rajendran, 2013: 53; Santos-Ballardo et al., 2015: 88).

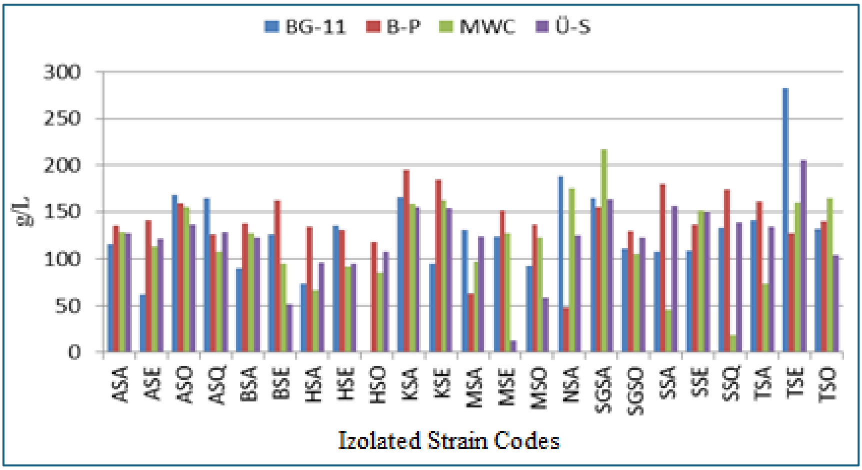
Results

To find out the biomass of the *Scenedesmus* strains isolated from 9 distinct freshwater environments in the province of Sinop and to know which media is suitable for maximum growth, experimental work was carried out using 23 pure strains of algae grown in 500 mL erlenmeyer flasks with 150 mL of media, as per standards.

Wet Weight Determination

After observing the average values of wet weight obtained from the strains grown in the media, it was observed that they are 126.8 g/L for the BG-11 media, 140.5 g/L for Bristol-Proteose media, 119.6 g/L for the MWC media and 121.3 g/L for urea-containing *Scenedesmus* media (U-S) (Figure 2).

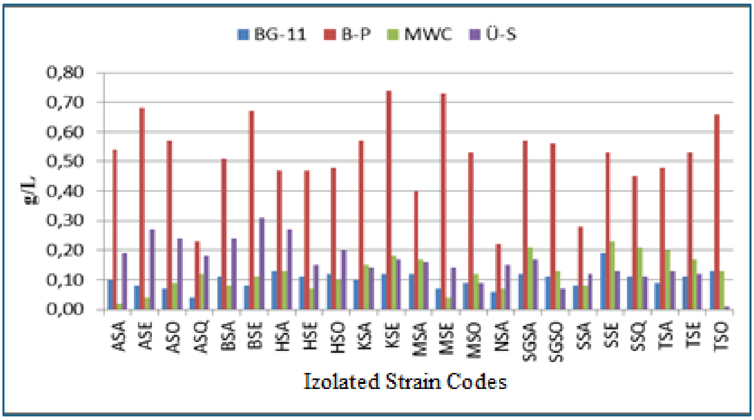
Figure 2 Comparison of the wet weight values of 23 strains of the isolated media in BG-11, Bristol-Proteose (B-P), MWC and U-S medium



Dry Weight Determination

Based on average values, dry weights were determined to be 0.10 g/L in BG-11 medium, 0.52 g/L in Bristol-Proteose medium, 0.12 g/L in MWC medium and 0.16 g/L in urea-containing *Scenedesmus* medium (Figure 3).

Figure 3 Comparative dry weight analysis of 23 isolated strains in BG-11, Bristol-Proteose (B-P), MWC and U-S media



Count of Cells

When the average numbers of strains developed in various media are considered, it can be observed that the cell counts varied from 222283 to 1126087 c/mL. Among the highest counts of cells in all media were 9750000 c/mL obtained on the twelfth day in the Bristol-Proteose medium, then again 9750000 c/mL on the tenth day, 14 days, seventh day, fifth day and fourth day in the same medium. Afterwards, the number of cells was 3141667 c/mL in the urea-containing *Scenedesmus* on the fourteenth day, then 2966667 c/mL on the twelfth day, 2941667 c/mL on the tenth day, and 2608333 c/mL on the fourth day, 2500000 c/mL on the fifth day, 2166667 c/mL on the seventh day, and 2166667 c/mL on the third day. Next comes the count of 1150000 c/mL on the fourteenth day in the MWC medium, 1100000 c/mL on the tenth day, and 1083333 c/mL in the BG-11 on the first day.

Spectrophotometric Growth of Cultures

Based on calculations for the average of all readings taken per day, it is observed that spectrophotometric growth at 500 nm wavelength was the least at 4.99 mg/L in the MWC medium and maximum at 6.50 mg/L in the Bristol-Proteose (B-P) medium. Spectrophotometric growth at 680 nm wavelength was the least at 5.03 mg/L in the MWC medium and maximum at 6.56 mg/L in the Bristol-Proteose (B-P) medium. Based on calculations, spectrophotometric growth at 750 nm wavelength was the least at 5.02 mg/L in the MWC medium and maximum at 6.51 mg/L in the Bristol-Proteose (B-P) medium.

Whereas in terms of spectrophotometric growth at a wavelength of 500 nm for the isolated 23 strains on BG-11 medium, the most favorable growth was achieved by MSE strain at an average value of 5.69 mg/L, the ASE strain at 5.65 mg/L and MSO strain at 5.63 mg/L. On the other hand, concerning spectrophotometric growth at a wavelength of 680 nm on the average basis, the best growth was achieved by ASA strain at 5.71 mg/L, the MSE strain at 5.66 mg/L and the ASE strain at 5.64 mg/L. On the other hand, when spectrophotometric growth was analyzed at a wavelength of 750 nm on the average basis, the best growth was obtained by HSE strain at 5.74 mg/L, the ASO strain at 5.73 mg/L and MSE strain at 5.70 mg/L.

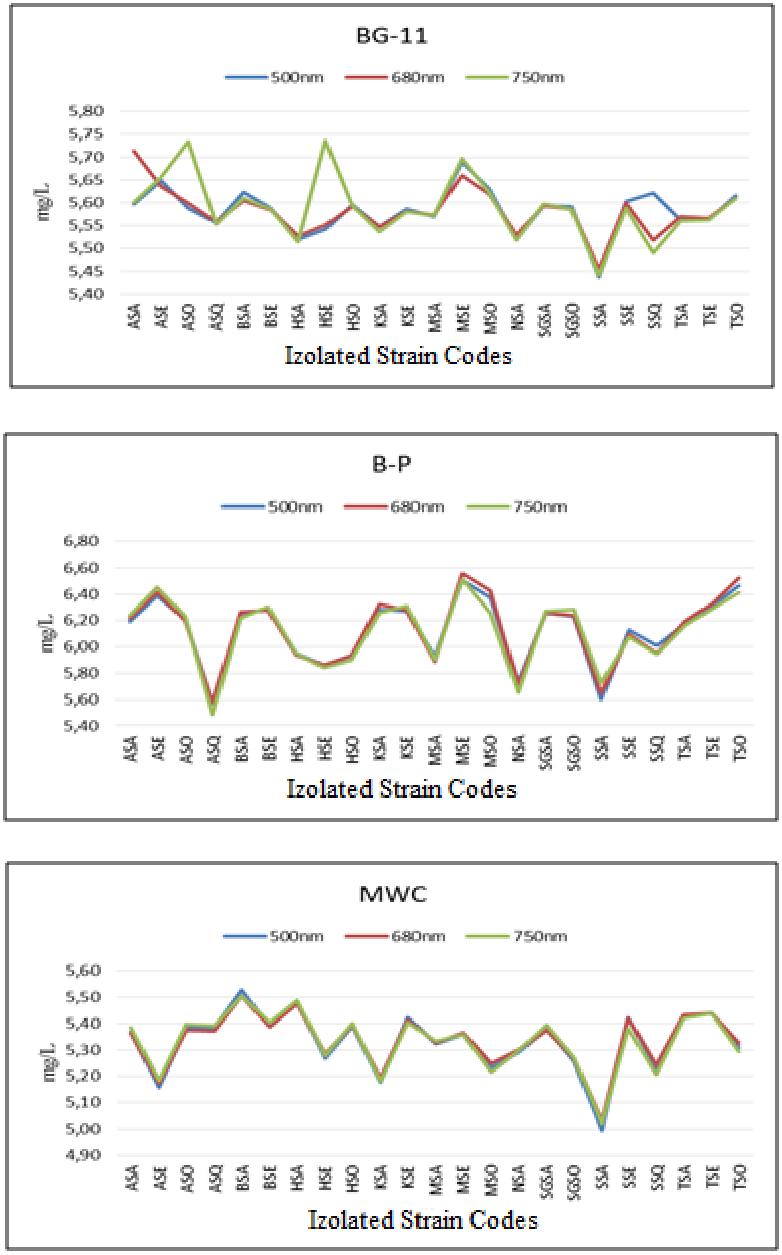
When the spectrophotometric growth of the 23 isolated strains was examined at 500 nm, based on their mean values on B-P medium, it was noted that the highest growth was observed in the TSO strain with 6.47 mg/L, ASE strain with 6.39 mg/L and MSO strain with 6.38 mg/L. When the spectrophotometric growth of the strains was examined at 680 nm, it was found that the highest growth was observed in MSE strain with 6.56 mg/L, TSO strain with 6.52 mg/L and MSO strain with 6.43 mg/L. When the spectrophotometric growth of the strains was examined at 750 nm, it was noted that the highest growth was observed in MSE strain with 6.51 mg/L, ASE strain with 6.45 mg/L and TSO strain with 6.41 mg/L.

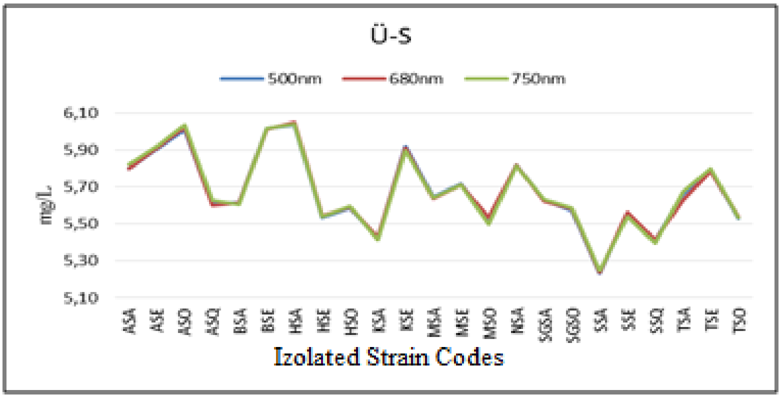
The spectrophotometric growth of the 23 isolated strains at a wavelength of 500 nm, when considering their average values at the MWC medium, revealed that the highest growth rate was for the BSA strain (5.53 mg/L), the HSA strain (5.48 mg/L) and the TSE strain (5.44 mg/L). The spectrophotometric growth at a wavelength of 680 nm showed that the highest growth rate was for the BSA strain (5.51 mg/L), the HSA strain (5.48 mg/L) and the TSE strain (5.44 mg/L). The spectrophotometric growth at a wavelength of 750 nm showed that the highest growth rate was for the BSA strain (5.51 mg/L), the HSA strain (5.49 mg/L) and the TSE strain (5.44 mg/L).

In relation to the spectrophotometric growth of the 23 isolated strains at a wavelength of 500 nm using U-S medium, according to the average values obtained, the most favorable growth was obtained for the HSA strain (6.04 mg/L), the BSE strain (6.02 mg/L) and the ASO strain (6.01 mg/L). For spectrophotometric growth at a wavelength of 680 nm, the most favorable growth was observed for the HSA strain (6.05 mg/L), the ASO strain (6.02 mg/L) and the BSE strain (6.01 mg/L). As for spectrophotometric growth at a wavelength of 750 nm, the most favorable growth was observed for the HSA and ASO strains (6.04 mg/L), the BSE strain (6.02 mg/L) and the ASE strain (5.92 mg/L).

Spectrophotometric growth of the 23 isolated strains at a wavelength of 500, 680 and 750 nm in four different media is illustrated through a graph (Figure 4).

Figure 4 Spectrophotometric growth of the 23 isolated strains at a wavelength of 500, 680 and 750 nm in four different media





Discussion and Conclusion

Present day researches into microalgal culture have become very important as algae are widely used in the production of food, industry and fuels on the globe. The methods and media used in the cultivation of algae can be improved in such a way to attain maximum growth and to produce maximum yield of desired metabolites. There are several microalgal culture media that have been reported for freshwater algal cultures. But, the productivity level depends on the strain. The production of natural metabolites of the same strain can be improved through re-designing to obtain maximum biomass in different media, with different nutrient compositions, at different nutrient levels and pH levels (Al-Shatri et al., 2014: 1719).

Microalgae do not require equal amounts of all nutrients; therefore, they are added to culture media in varying quantities. Many culture media contain higher concentrations of supporting nutrients than those found in natural habitats to achieve high biomass yields. Different species require different amounts of certain nutrients (Anonymous, 2015).

The study conducted with *Scenedesmus abundans* was carried out using three different mediums, BBM, modified CHU-13 and BG-11, and growth rates for the cultures were evaluated at a wavelength of 680 nm. The most effective medium for algal growth was found to be modified CHU-13. The reason for the increase in algal biomass and lipid was due to the nitrogen level of 0.32 g/L (Mandotra et al., 2014: 47). However, in the case of our research, on the basis of cellular analysis, it was noticed that the best growth was seen in the Bristol-Proteose medium, followed by urea Scenedesmus, BG-11 and MWC media, respectively.

A review of other studies in the literature reveals that in a study by Crofcheck et al. (2013: 590), *Scenedesmus acutus* was cultured under low CO_2 conditions in KNO_3 and urea-containing media, and similar results were observed. They found that a decrease in urea levels did not have a growth-limiting effect, whereas the pH of the medium significantly affected growth. They reported that urea could be preferred in the medium due to its lower cost. In another study investigating optimal conditions for obtaining the best biomass of *Scenedesmus sp.* used in biofuel production, different nitrogen sources, different concentrations, various CO_2 levels and salts were used (Makarevičienė et al., 2011: 21). It was determined that the best biomass was obtained when urea was used as the nitrogen source or in a modified BG-11 medium containing reduced $NaNO_3$. In this study as well, it was found that the urea-containing Scenedesmus medium supported the growth of the species more effectively than BG-11 and MWC media. The fertilizing effect of the urea used in this medium was observed.

In a study by Chaichalerm et al. (2012: 296) on the cultivation of certain isolated microalgal strains in various media, the species *Scenedesmus acutus* was cultured in 3NBBM medium, N-8 medium, Kuhl medium and BG-11 medium; the best growth was observed in the BG-11 medium due to its high nitrogen concentration. In a study investigating the effect of different nitrogen sources on the biomass productivity of the *Scenedesmus bijugatus* species, it was determined that a medium with a nitrate concentration of 5-10 mM was suitable for Scenedesmus growth. Similar results to those in the nitrate medium were obtained in media using low urea concentrations (5 and 10 mM). This study emphasized the universal availability and affordable cost of urea, highlighting its potential for use in large-scale algal cultures (Arumugam et al., 2013: 249). In another study examining the effects of different nitrogen and phosphorus limitations on the growth and lipid content of *Scenedesmus sp.* was found to be high under limiting conditions. However, due to lipid productivity and accumulation rates, the algal biomass was found to be lower (Xin et al., 2010: 5497).

Different types of algal cultures have different nutrient and light requirements. Generally, microalgae use natural light in the wavelength range of 400 to 700 nm for photosynthesis (Andersen, 2005: 307). In our study, culture growth was monitored spectrophotometrically at wavelengths of 500, 680 and 750 nm (Ma et al., 2002: 63; Demirel, 2006: 75). It was found that the growth of the 23 isolated strains showed similar changes across different wavelengths. The spectrophotometric growth measured at 500, 680 and 750 nm was highest in the Bristol-Proteose (B-P) medium, followed by the urea-containing *Scenedesmus* and BG-11 media, while the lowest growth was observed in the MWC medium.

Based on the wet weight values of the strains produced in the media, the best growth was observed in the Bristol-Proteose medium, followed by the BG-11, U-S and MWC media, in that order. When considering the dry weight of the produced strains, the highest yield was obtained in the Bristol-Proteose medium, followed by the U-S medium, the MWC medium and the BG-11 medium. The literature indicates that dry weight data provide more accurate results in biomass monitoring (Anonymous, 2016).

Strains of the genus *Scenedesmus* collected from different sources of fresh water have been cultured under four different media: BG-11, Bristol-Proteose, MWC and U-S Medium. The growth rate of the strains of *Scenedesmus* in these media has been observed through direct and indirect methods. It has been found that growth rates in strains of *Scenedesmus* are optimum when grown in Bristol-Proteose medium, followed by urea containing Scenedesmus medium. Use of Bristol-Proteose medium which gives the optimum yield, growth and cell concentration per unit time is of particular importance while conducting research on *Scenedesmus* spp.

References

Al-Shatri, A.H., Ali, E., Al-Shorgani, N., & Sahaid, M. (2014). Growth of *Scenedesmus dimorphus* in different algal media and pH profile due to secreted metabolites. *African Journal of Biotechnology, 13*(16), 1714-1720. Doi: 10.5897/AJB2013.13455

Andersen, R.A. (2005). *Algal culturing techniques*. Elsevier Academic Press.

Anonymous. 2014. http://www.ccap.ac.uk/media/recipes.htm# (Accessed August 1, 2014).

Anonymous. 2015. http://www.marine.csiro.au/microalgae/methods/Making %20 Media.htm) (Australian National Algae Culture Collection-Methods) (Accessed: April 1, 2015).

Anonymous. 2016. https://www.noisebridge.net/images/7/70/TruCell.TN.AUvsOD.pdf, Technical Note, Finesse, LLC 3350 Scott Boulevard #1 Santa Clara, CA 95054 9351 Irvine Boulevard Irvine, CA 92618 800-598-9515 www.finesse-inc.com.

Apha-Awwa-Wef (2005). Standard methods for the examination of water and wastewater. 21th Edition. New York, Total Solids Suspended, Method 2540 D, 2-55 a 2-59.

Arumugam, M., Agarwal, A., Arya, M.C., & Ahmed, Z. (2013). Influence of nitrogen sources on biomass productivity of microalgae *Scenedesmus bijugatus*. *Bioresource technology*, *131*, 246-249. Doi: 10.1016/j.biortech.2012.12.159

Beyatlı, Y. (1996). *Biotechnology and Bioprotein Production*. KÜKEM Association Scientific Publications.

Borowitzka, M.A. (1992). Algal biotechnology products and processes: Matching science and economics. *Journal of Applied Phycology*, *4*, 267-279.

Brett M.T., (1989). Zooplankton Communities and Acidification Processes. *Water, Air, & Soil Pollution*, *44*, 387-414.

Chaichalerm, S., Pokethitiyook, P., Yuan, W., Meetam, M., Sritong, K., Pugkaew, W., Kungvansaichol, K., Kruatrachue, M. & Damrongphol, P. (2012). Culture of microalgal strains isolated from natural habitats in Thailand in various enriched media. *Applied Energy*, *89*, 296-302.

Crofcheck, C.E.X.Y., Shea, A., Montross, M., Crocker, M. & Andrews, R. (2013). Influence of media composition on the growth rate of *Chlorella vulgaris* and *Scenedesmus acutus* utilized for CO_2 mitigation. *Journal of Biochemical Technology*, *4*(2), 589-594.

Demirel, Z. (2006). *Investigation of the antimicrobial and antioxidant properties of the green microalga (Chlorophyta) Scenedesmus protuberans Fris. isolated from Lake Eğirdir* [Master's Thesis, Ege University].

Guiry, M.D., & Guiry, G.M. (2013). *AlgaeBase: World-wide electronic publication*. National University of Ireland, Galway. https://www.algaebase.org.

İnaç, A. (1994). *Isolation of Scenedesmus* spp. *from freshwater and investigation of its growth under laboratory conditions* [Master's Thesis, Gazi University].

John, D.M., Whitton, B.A. & Brook, A.J. (2002). *The freshwater algal flora of the British Isles: An identification guide to freshwater and terrestrial algae*. Cambridge University Press.

Ma, J., Zheng, R., Xu, L. & Wang, S. (2002). Differential sensitivity of two green algae, *Scenedesmus obliquus* and *Chlorella pyrenoidosa*, to 12 pesticides. *Ecotoxicology and Environmental Safety*, *52*(1), 57-61. Doi: 10.1006/eesa.2002.2146

Makarevičienė, V., Andrulevičiūtė, V., Skorupskaitė, V. & Kasperovičienė, J. (2011). Cultivation of microalgae *Chlorella sp.* and *Scenedesmus sp.* as a potential biofuel feedstock. *Environmental Research, Engineering and Management, 57*(3), 21-27. Doi: 10.5755/j01.erem.57.3.476

Mandotra, S.K., Kumar, P., Suseela, M.R. & Ramteke, P.W. (2014). Fresh water green microalga Scenedesmus abundans: A potential feedstock for high quality biodiesel production. *Bioresource technology*, *156*, 42-47. Doi: 10.1016/j.biortech.2013.12.127

Prescott, G.W. (1973). *Algae of Western Great Lake Area*. Fifth Printing, William C. Brown Publishers, Dubuque. 1977.

Pringsheim, E. (1946). *Pure Culture of Algae*, Cambridge University Press.

Rodrigues, L.H.R., Arenzon, A., Raya-Rodriguez, M.T. & Fontoura, N.F. (2011). Algal density assessed by spectrophotometry: A calibration curve for the unicellular algae *Pseudokirchneriella subcapitata*. *Journal of Environmental Chemistry and Ecotoxicology*, *3*(8), 225-228.

Santos-Ballardo, D.U., Rossi, S., Hernández, V., Vázquez Gómez, R., Rendón-Unceta, M.C., Caro-Corrales, J. & Valdez-Ortiz, A. (2015). A simple spectrophotometric method for biomass measurement of important microalgae species in aquaculture. *Aquaculture*, *448*, 87-92.

Sivakumar, R. & Rajendran, S. (2013). Growth measurement technique of microalgae. *International Journal of Current Science*, *7*, 52-54.

Stein, J.R. (1973). *Handbook of phycological methods: Culture methods and growth measurements*. Cambridge University Press.

Sukatar, A. (2002). *Algae Culture Methods*. Ege University Press.

UTEX Culture Collection of Algae. (2014). *The UTEX Culture Collection of Algae at the University of Texas at Austin*. Retrieved August 1, 2014, from https://utex.org/.

Xin, L., Hu, H.Y., Ke, G. & Sun, Y.X. (2010). Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake and lipid accumulation of a freshwater microalga *Scenedesmus sp. Bioresource Technology*, *101*, 5494-5500.

BÖLÜM 0

PHYTOPLANKTON DYNAMICS AND SEASONAL CHANGES IN A STREAM (SİNOP, TÜRKİYE)

ELİF TEZEL ERSANLI[1]
RIDVAN ÖZTÜRK[2]

Introduction

Water provides a vital habitat for numerous organisms and is essential for cellular functions (Akın & Akın, 2007: 107). In recent times, while it is difficult to find suitable water sources, existing water resources are continually polluted. For this reason, the physical, chemical and biological conditions of the water resources should be revealed and an effective planning to be made as a result and the development of currently applied techniques should be the first step to increase production (Kelly & Whitton, 1998: 55). Water quality affects species composition, productivity, abundance and physiological status of aquatic species. Algae are ecologically used to detect changes related to ecosystem changes

---

[1] Prof. Dr., Sinop University, Faculty of Arts and Science, Department of Biology, Sinop/Türkiye, ORCID: 0000-0003-0608-9344, eersanli@sinop.edu.tr

[2] Bakırköy Çatı Private Social Activities and Development Center, Istanbul/Türkiye, ORCID: 0000-0001-8362-8511, ozturkridvan.1990@gmail.com

"*This research has been supported by Sinop University Scientific Research Projects Coordination Unit. Project Number: FEF-1901.14-05, 2016*"

and to distinguish favorable from unfavorable environmental conditions. Effective ecological management and remediation, before any adverse impacts occur in aquatic environments, by identifying changes in nutrient status, can be ensured by monitoring or using the right sources.

In rivers, the physical and chemical properties of water shift because of displacement, climate changes, and more human interference. This also messes with the biological makeup of rivers. The main reasons for the degradation of river ecosystems are industrial wastes, irregular agricultural practices, domestic wastes and damage to river basins (Yıldırım, 2011: 54). Damage to river basins and the mixing of foreign substances into the water, which will disrupt the bottom and coastal structure of the river, damage the habitat of aquatic organisms. The first organisms affected by this situation are algae, which are primary producers (Kalyoncu, Barlas & Yorulmaz, 2008: 15). The effect of water movements in rivers also affects the organisms in the environment.

The most dominant communities in rivers are benthic. Phytoplankton is observed in the upper tributaries of rivers, but it is either consumed by filter-feeding organisms or lost with the velocity of the water flow. In places where the water stagnation time is longer than the generation time of phytoplankton, phytoplankton can be transported in the direction of the water flow (Lampert & Sommer, 2007: 225). In the researches conducted in our inland waters in our country, the studies on rivers are fewer than on lakes. The reason for the limited number of these studies is that the irregular water flow volumes of the rivers and especially the weak tributaries are not suitable for periodic studies.

The aim of this study was to determine the phytoplankton flora and dynamics of Çakıroğlu Stream in Sinop, which has not been investigated ecologically until now, to determine the physical and chemical factors affecting the changes in phytoplankton, to calculate the phytoplankton biomass and to describe its current ecological status.

Materials and Methods

Sinop province is located on the Boztepe Peninsula in the Central Black Sea Region, the northernmost region of Turkiye, between parallels 41.2-43.5 and meridians 34.5-35.5 (ÇOB, 2006: 3). The total river surface in Sinop province is 1220 ha. Some of these rivers flow into the Black Sea and some into the Kızılırmak, which passes through the southern border of the province (ÇOB, 2004: 23). The Çakıroğlu Stream originates from Dranas Mountain, flows in a wide alluvial valley and flows into the sea between Sinop and Gerze. It also forms a small delta where it flows into the Black Sea.

In order to investigate the physical and chemical properties of the phytoplankton and water of Çakıroğlu Stream, 3 stations were determined between July 2015 and June 2016, starting from the location where the stream flows into the sea to the upper basin region. Depending on the season, significant decreases in the water level were observed and it was observed that the river bed narrowed from time to time.

We looked at the physical and chemical properties of Çakıroğlu Stream water at all stations. During sampling, we used portable digital Hach Lange brand HQ40D equipment to measure water temperature (T), dissolved oxygen (DO), pH, conductivity (C), and oxidation reduction potential (ORP). For ammonia, nitrite, nitrate, phosphorus, and total hardness (TH), we followed standard methods from Apha-Awwa-Wef (2005: 37, 114, 118, 126, 153). Total organic matter was determined using Şengül & Türkman's (1991: 91) method.

To study the phytoplankton in Çakıroğlu Stream, we took water samples from set stations. Then, we processed the samples and counted plankton using the Utermohl method from Sournia (1978: 99), with Micros Austria MCXI600 and Leica DM500 microscopes. We evaluated our results by standard methods from Apha-Awwa-Wef (2005: 14).

Organic matter in the diatom samples was removed by oxidation using a 1:1 mixture of concentrated nitric acid (HNO_3) and potassium dichromate ($K_2Cr_2O_7$). Potassium dichromate was added until the solution color changed from green to yellow. The samples were then boiled until their volume decreased to approximately one-third of the original volume (Apha-Awwa-Wef, 2005: 34), thereby improving the visibility of diatom frustule structures.

Algal species were identified through detailed microscopic examination using established taxonomic references and identification manuals (Anagnostidis & Komárek, 1988: 472; Komárek & Anagnostidis, 1986: 226, 1989: 345, 1999: 548; Hartley, 1996: 601; Krammer & Lange-Bertalot, 1991a: 576, 1991b: 437, 1999a: 876, 1999b: 596; John, Whitton & Brook, 2002: 702; Krammer, 2003: 530; Wehr & Sheath, 2003: 918; Prescott, 1973: 977). The nomenclature and systematic classification of all taxa were standardized according to the AlgaeBase database (Guiry & Guiry, 2013).

Results

Seasonal variation of phytoplankton, physical and chemical properties of Çakıroğlu Stream were investigated, and in addition, the pollution status of the water was determined and its current ecological status was revealed.

The physicochemical characteristics of the water were determined at all sampling stations along Çakıroğlu Stream.

Descriptive statistics of the measured physicochemical parameters are presented in Table 1.

Table 1 Summary statistics of physicochemical parameters of Çakıroğlu Stream

	St.1			**St.2**			**St.3**		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
T (°C)	8.50	28.60	18.90	8.10	27.60	18.56	8.20	26.90	18.36
DO (mg/L)	8.67	12.89	10.20	8.29	11.79	9.85	7.93	11.57	9.79
Conductivity (µS/cm)	252.00	433.00	370.33	355.00	457.00	394.08	382.00	467.00	411.42
ORP (mV)	86.20	228.40	171.44	87.40	224.60	170.76	87.80	220.70	171.17
pH	7.45	8.65	8.18	7.39	8.40	8.17	7.26	8.39	8.16
Nitrite (mg/L)	0.00	0.07	0.03	0.00	0.10	0.03	0.00	0.06	0.03
Nitrate (mg/L)	0.00	0.56	0.21	0.00	1.50	0.28	0.00	0.50	0.16
Ammonia (mg/L)	0.00	9.59	1.96	0.00	9.94	2.34	0.00	8.09	1.47
Si (mg/L)	2.99	5.48	4.40	2.48	5.67	4.61	3.73	5.24	4.55
Phosphate (mg/L)	0.16	51.95	7.58	0.09	63.47	15.95	0.09	39.06	6.64
Organic Matter (mg/L)	1.80	12.20	4.78	2.20	7.00	4.32	2.00	17.00	5.02
Total hardness (FS°)	11.20	26.00	20.90	18.80	24.80	21.80	17.60	31.60	22.07

During the study period, the average values of water temperature, conductivity, pH, and oxidation reduction potential (ORP) were 18.61 °C, 391.94 µS/cm, 8.17, and 171.12 mV, respectively. Furthermore, the average concentrations of dissolved oxygen, organic carbon, and phosphorus were 9.95 mg/L, 4.71 mg/L and 10.06 mg/L, respectively, while the average water hardness was 21.59 FS^{o}. The nitrite values of water were variable between 0.00-0.56 mg/L, the nitrate values were between 0.00-1.50 mg/L, the ammonia values were between 0.00-9.94 mg/L, the silica values were between 2.48 to 5.67 mg/L throughout the year.

In the Çakıroğlu Stream phytoplankton, 30 taxa belonging to Cyanobacteria, Charophyta, Chlorophyta, Bacillariophyta and Ocrophyta divisions were identified. Of the 30 taxa, 13% belonged to Cyanobacteria, 20% to Charophyta, 10% to Chlorophyta, 54% to Bacillariophyta and 3% to Ocrophyta. Frequency values of algae taxa detected in Çakıroğlu Stream are given in Table 2.

Table 2 Checklist of algal taxa identified in Çakıroğlu Stream and their relative frequencies of occurrence. Frequency classes were categorized as continuous (81-100%), common (61-80%), frequent (41-60%), occasional (21-40%), and rare (1-20%)

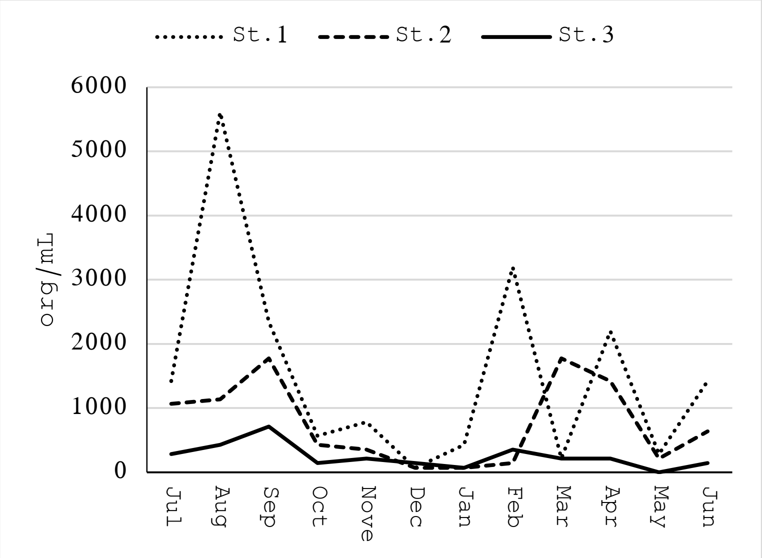
ORGANISM	**St.1**	**St.2**	**St.3**
Phylum Cyanobacteria			
Chroococcus dispersus (Keissler) Lemmermann		8	17
Merismopedia glauca (Ehrenberg) Kützing	8		
Oscillatoria princeps Vaucher ex Gomont	8		
Pseudanabaena limnetica (Lemmermann) Komárek			17
Phylum Bacillariophyta			

Brachysira brebissonii R.Ross	8		
Cymatopleura solea (Brébisson) W.Smith	17		
Cymbella affinis Kützing	42	33	58
Cymbella gracilis (Ehrenberg) Kützing	8	33	8
Diatoma vulgaris Bory	8	17	17
Encyonema elginense (Krammer) D.G.Mann	17	25	8
Fragilariforma constricta (Ehrenberg) D.M.Williams & Round		8	
Gyrosigma acuminatum (Kützing) Rabenhorst	25	8	8
Hantzschia amphioxys (Ehrenberg) Grunow	8		
Meridion circulare (Greville) C.Agardh	42	50	58
Navicula capitatoradiata H.Germain	42	25	25
Pantocsekiella ocellata (Pantocsek) K.T.Kiss & E.Ács	33		
Peridinium cinctum (O.F.Müller) Ehrenberg		8	
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	8		
Tabellaria fenestrata (Lyngbye) Kützing	8		
Ulnaria ulna (Nitzsch) Compère	83	67	50
Phylum Charophyta			
Closterium archerianum Cleve ex P.Lundell	8	17	
Closterium dianae Ehrenberg ex Ralfs	8	8	
Cosmarium laeve var. *octangulare* (Wille) West & G.S.West	8	17	8

Cosmarium trilobulatum Reinsch	8		
Spirogyra majuscula Kützing	17	8	8
Zygnema cruciatum (Vaucher) C.Agardh		8	8
Phylum Chlorophyta			
Coelastrum microporum Nägeli	8	8	8
Monoraphidium mirabile (West & G.S.West) Pankow	8	17	17
Scenedesmus communis E.Hegewald	8		
Phylum Ochrophyta			
Dinobryon sociale (Ehrenberg) Ehrenberg	8	8	

The seasonal variation of total number of organisms in Çakıroğlu Stream phytoplankton are given in Figure 1.

Figure 1 Seasonal variation of total number of organisms in Çakıroğlu Stream



In the Çakıroğlu Stream, in July, the lowest number of organisms was detected at St.3 with 284 org/mL, and the highest number of organisms was detected at St.1 with 1420 org/mL. While *Meridion circulare* was the dominant species at all stations in the range of 40-75% this month, *Ulnaria ulna* became the subdominant species in the range of 20-25% at all stations. In August, the total number of organisms varied between 426-5609 org/mL. *Navicula capitoradita* was dominant at St.1 with 47%, *Scenedesmus communis* was subdominant with 25%, and *Ulnaria ulna* was dominant at St.2 with 25%.

In September, the lowest number of organisms was detected at St.3 with 710 org/mL, and the highest number of organisms was detected at St.1 with 2343 org/mL. In this month, *Ulnaria ulna* became the dominant species at St.2 and *Navicula capitoradita* at St.1. In October, the total number of organisms ranged from 142-568 org/mL. *Ulnaria ulna* became dominant and *Meridion circulare* subdominant at all stations. The total number of organisms was recorded between 213 org/mL and 781 org/mL in November. *Cymbella affinis*, *Encyonema elginense* and *Meridion circulare* became dominant at St.3, and *Ulnaria ulna* at St.1 and St.2 this month.

A decrease was observed in the total number of organisms in December and the highest number of organisms was recorded as 142 org/mL. The total number of organisms also varied between 71-426 org/mL in January. It was recorded that the number of organisms varied between the lowest 142 org/mL and 3195 org/mL in February. In this month, *Cymbella affinis* became dominant organisms at St.2 and *Coelastrum microporum* at St.1.

In March, the total number of organisms varied between 213-1775 org/mL, with *Cymbella affinis* at the 2nd station and *Pantocsekiella ocellata* at the 1st station. While the total number of organisms was recorded between 213-2201 org/mL in April, the total number of organisms decreased in May and no species were found at the 3rd station. In June, the total number of organisms was determined between 710-5609 org/mL, and *Closterium archerianum* was dominant at the 1st station, *Cymbella affinis* at the 2nd station, *Cymbella affinis* and *Meridion circulare* at the 3rd station.

Discussion

Çakıroğlu Stream is located within the borders of Sinop province, was examined and the pollution status of the water was determined and its current ecological status was revealed. The 30 taxa identified in the study area belong to the divisions Cyanobacteria (4), Charophyta (6), Chlorophyta (3), Bacillariophyta (16) and Ocrophyta (1).

Temperature is an important factor in controlling the rate of photosynthesis (Davison, 1991: 2) and is also effective in the use of nitrogen and phosphorus in algae (Mallin et al., 1999: 825). Change of temperature in streams can be caused by many reasons such as air temperature, water flow rate, water volume, depth and changes in stream structure. During the research period, the highest temperature was measured in August with 28.60 ^{o}C, while the lowest temperature was measured in February with 8.10 ^{o}C.

Dissolved oxygen levels are governed by the equilibrium between surface aeration and oxygen consumption via chemical and microbial processes (Williams & Boorman, 2012: 104). In this study, values ranged from 7.93 to 12.89 mg/L. The increase in the flow rate and surface area of water in streams will also increase the contact surface with air, allowing the amount of oxygen to increase (Çiçek et al., 2010: 87).

Electrical conductivity, which is proportional to total dissolved solids in water (Hayashi et al., 2012: 102), showed a maximum value in November (467.00 μS/cm) and a minimum value in August (252.00 μS/cm).

The amount of organic matter is correlated with organic carbon. The decomposition of particulate organic carbon plays an important role in the quality of water by reducing the dissolved oxygen in the water and increasing the biological oxygen demand. With the increase in precipitation, the amount of organic carbon entering the rivers increases (Ouyang, 2003: 823). Organic matter consists of living things living in the environment and organic wastes coming from outside. During the research, the average amount of organic matter was determined as 4.71 mg/L. The fact that the amount of organic matter is not too high may be related to the fact that the stream is away from the forest effect.

During the research period, the highest total hardness value was 31.60 FS^o, and the lowest total hardness value was 11.20 FS^o. The average hardness value was determined as 21.59 FS^o. According to this, the water of the creek is in the group of slightly hard and medium hard waters (Egemen & Sunlu, 1999: 43).

The pH in water changes with oxygen production and carbon dioxide consumption, so pH is affected by photosynthetic activity (Odum, 1956: 103). The mean pH value was determined as 8.17 during the sampling period.

Oxidation reduction potential (ORP) refers to the self-renewal and cleaning potential of the aquatic ecosystem under study. In other words, it refers to the rate at which organic parts of contaminated materials and dying organisms are converted into inorganic parts. Generally, a high ORP value means that the environment is healthy and ORP in clean waters is between 300-500 mV (Wetzel, 1983: 291). During the research, the highest ORP value was measured as 228.40 mV in November and the lowest as 86.20 mV in June. According to the measured values, the stream water appears to have a low potential for self-renewal and self-cleaning.

Phosphorus, one of the important nutritional minerals for phytoplankton, is very important for algae and is the limiting factor for phytoplankton. Phosphorus value was measured between 0.09-63.47 mg/L in the research. The different phosphorus values observed in the study area suggest that some parts of the stream may be under human influence.

Nitrogen, the element required for amino acid and protein synthesis, is the primary need of algae (Reynolds, 1984: 164). In the study conducted on Çakıroğlu Stream, the average ammonia value was 1.92 mg/L, the average nitrite value was 0.03 mg/L, and the average nitrate value was 0.22 mg/L. The measurement of the highest nitrite, nitrate and ammonia values at the 2nd station may be due to the fact that the environment of the station is under the influence of the settlements and fields around it.

The phytoplankton distribution in Çakıroğlu Stream was examined, and it was observed that the lowest total organism count was recorded in December and January, and the highest value was determined in August and then in March. In the Çakıroğlu Stream phytoplankton, the Bacillariophya division was present throughout the research period, while *Cymbella* spp., *Ulnaria sp.* and *Meridion sp.* became the dominant organisms.

In July, the *Meridion circulare* became the dominant species at all stations, while *Ulnaria ulna* became the subdominant species at all stations. *Navicula capitoradita* became dominant, *Scenedesmus communis* subdominant in August, *U. ulna*, *N. capitoradita* dominant species in September, *U. ulna* dominant and *M. circulare* subdominant species in October. In November, *Cymbella affinis*, *Encyonema elginense* and *M. circulare* became *U. ulna* dominant species.

In December, *C. affinis* and *Coelastrum microporum* became dominant organisms. In March, *C. affinis* and *Pantocsekiella ocellata* became dominant organisms. While the total number of organisms was recorded between 213-2201 org/mL in April, the total number of organisms decreased in May. In June, *Closterium archerianum*, *C. affinis* and *M. circulare* became dominant species.

In river studies conducted in our country, it is seen that the division with the highest value in terms of number of organisms and species diversity is Bacillariophyta (Ertan & Morkoyunlu, 1998: 254; Kara & Şahin, 2001: 185; Şahin, 2003: 114; Çiçek et al., 2010: 87; Baykal et al., 2011: 499).

Four species were identified from the class Cyanophyceae belonging to the Cyanobacteria division. *Chroococcus dispersus*, *Merismopedia glauca*, *Oscillatoria princeps* and *Pseudanabaena limnetica* were detected from this division. Members of this division increased especially in June and then in August.

Closterium archerianum and *C. dianae*, *Cosmarium trilobulatum, C. laeve* var. *Octangulare*, *Spirogyra majuscula* and *Zygnema cruciatum* were recorded from Charophyta. An increase was recorded in the species belonging to the Charophyta division in June and August.

Coelastrum microporum, *Monoraphidium mirabile* and *Scenedesmus communis* from Chlorophyta division, *Dinobryon sociale* from Ochrophyta division were recorded. Chlorophyta members increased in number in June, February and August, while Ochrophyta was only represented in June and September.

The European Union Water Framework Directive (WFD) program aims to protect water resources, aquatic ecosystems and improve the aquatic environment. These targets are set for all surface waters, groundwaters and protected areas. For surface waters, the aim is to achieve good ecological and good chemical conditions. River basin management, which is considered to be implemented in rivers, is extremely important in terms of maintaining the quality of river ecosystems (Grontmij, 2004: 25). Although there is no leakage to the stream due to industrial wastes around Çakıroğlu Stream, human influence can create a stress effect on the natural life of the region. Of the stream water it is necessary to take measures for the sustainable usage. These measures may be to reduce the uncontrolled use of stream water, to organicize agricultural fertilization and to prevent domestic waste from mixing with water. Bacillariophyta division was dominant in the stream water. According to the quality criteria of inland water resources, Çakıroğlu Stream water is class I in terms of average temperature and dissolved oxygen values, and class II in terms of average pH and total phosphorus values. In terms of water requirement, Çakıroğlu Stream is an important water source for the aquatic, semi-aquatic and terrestrial organisms it feeds around.

References

Akın, M. & Akın, G. (2007). Suyun önemi, Türkiye'de su potansiyeli, su havzaları ve su kirliliği. *Ankara Üniversitesi Dil ve Tarih-Coğrafya Fakültesi Dergisi*, *47*(2), 105-118.

Anagnostidis, K. & Komárek, J. (1998). Modern approach to the classification system of Cyanophytes. *Algological Studies/Archiv für Hydrobiologie*, 80 (1-4), 327-472.

Apha-Awwa-Wef (2005). *Standard methods for the examination of water and wastewater*. 21th Edition. New York.

Baykal, T., Açıkgöz, İ., Udoh, A.U. & Yıldız, K. (2011). Seasonal variations in phytoplankton composition and biomass in a small lowland river-lake system (Melen River, Turkey). *Turkish Journal of Biology*, *35*(4): 485-501. Doi: 10.3906/biy-0904-5

Çevre ve Orman Bakanlığı (ÇOB). (2004). *Sinop Province environmental status report for 2004*. Ministry of Environment and Forestry.

Çevre ve Orman Bakanlığı (ÇOB). (2006). *Sinop Province environmental status report for 2006*. Ministry of Environment and Forestry.

Çiçek, N.L., Kalyoncu, H., Akköz, C. & Ertan, Ö.O. (2010). Epilithic algae and their seasonal distributions in Darıören Stream and Isparta Creek (Isparta, Turkey). *Journal of FisheriesSciences.com*, *4*(1), 78-90.

Davison, I.R. (1991). Environmental effects on algal photosynthesis: Temperature. *Journal of Phycology*, *27*(1), 2-8. Doi: 10.1111/j.0022-3646.1991.00002.x

Egemen, Ö. & Sunlu, U. (1999). *Water quality* (3rd ed.). Faculty of Fisheries Publications.

Ertan, Ö. O. & Morkoyunlu, A. (1998). The algae flora of Aksu Stream (Isparta-Turkey). *Turkish Journal of Botany, 22*(2), 239-255.

Grontmij (2004). *Implementation of the Water Framework Directive in Turkey (WFD), Implementation Manual (Handbook)*. Project MAT01/TR/9/3, Document No: 13/99044324/MJH, Grontmij Consulting Engineers, Houten.

Guiry, M.D. & Guiry, G.M. (2013). *AlgaeBase: World-wide electronic publication*. National University of Ireland, Galway. (Accessed on 01/08/2016 from https://www.algaebase.org).

Hartley, B., Barber, H.G., Carter, J.R. & Sims, P.A. (1996). *An atlas of British diatoms*. Biopress Ltd., Bristol, UK.

Hayashi, M., Vogt, T., Mächler, L. & Schirmer, M. (2012). Diurnal fluctuations of electrical conductivity in a pre-alpine river: Effects of photosynthesis and groundwater exchange. *Journal of Hydrology*, *450-451*, 93-104. Doi: 10.1016/j.jhydrol.2012.05.020

John, D. M., Whitton, B. A. & Brook, A. J. (2002). *The freshwater algal flora of the British Isles: An identification guide to freshwater and terrestrial algae*. Cambridge University Press.

Kalyoncu, H., Barlas, M. & Yorulmaz, B. (2008). Aksu Çayı'nda (Isparta-Antalya) epilitik alg çeşitliliği ve akarsuyun fizikokimyasal yapısı arasındaki ilişki. *Ekoloji*, *17*(66), 15-22.

Kara, H. & Şahin, B. (2001). Epipelic and epilithic algae of Değirmendere River (Trabzon-Turkey). *Turkish Journal of Botany*, *25*, 177-186.

Kelly, M.G. & Whitton, B.A. (1998). Biological monitoring of eutrophication in rivers. *Hydrobiologia*, *384*, 55-67.

Komárek, J. & Anagnostidis, K. (1986). Modern approach to the classification system of Cyanophytes. 2-Chroococcales. *Algological Studies*, *434*, 157-226.

Komárek, J. & Anagnostic, K. (1989). Modern approach to the classification system of Cyanophites 4-Nostocales. *Algological Studies/Archiv für Hydrobiologie*, *56*, 247-345.

Komárek, J. & Anagnostidis, K. (1999). Cyanoprokaryota. 1. Teil: Chroococcales. In H. Ettl, G. Gärtner, G. Heynig, & D. Mollenhauer (Eds.), *Süßwasserflora von Mitteleuropa* (Vol. 19/1). Gustav Fischer.

Krammer, K. (2003). *Diatoms of Europe* (Vol. 4). R.G. Gantner Verlag K.G.

Krammer, K. & Lange-Bertalot, H. (1991a). Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. In H. Ettl, J. Gerloff, H. Heynig, & D. Mollenhauer (Eds.), *Süßwasserflora von Mitteleuropa*. Gustav Fischer Verlag.

Krammer, K. & Lange-Bertalot, H. (1991b). Bacillariophyceae. 4. Teil: Achnanthaceae. Kritische Ergänzungen zu *Navicula* (Lineolatae) und *Gomphonema*. In H. Ettl, G. Gärtner, J. Gerloff, H. Heynig, & D. Mollenhauer (Eds.), *Süßwasserflora von Mitteleuropa*. Gustav Fischer Verlag.

Krammer, K. & Lange-Bertalot, H. (1999a). Bacillariophyceae. 1. Naviculaceae, *Süßwasserflora Von Mitteleuropa*. Stuttgart, New York, Gustav Fischer Verlag, 2/1.

Krammer, K. & Lange-Bertalot, H. (1999b). Bacillariophyceae. 2. Bacillariaceae, Epithemiaceae, Surirellaceae, *Süßwasserflora Von Mitteleuropa*. Stuttgart, New York, Gustav Fischer Verlag, 2/2.

Lampert, W., & Sommer, U. (2007). *Limnoecology: The ecology of lakes and streams* (2nd ed.). Oxford University Press.

Mallin, M.A., Cahoon, L.B., McIver, M.R., Parsons, D.C. & Shank, G.C. (1999). Alternation of factors limiting phytoplankton production in the Cape Fear River estuary. *Estuaries*, *22*(4), 825-836.

Odum, H.T. (1956). Primary production in flowing waters. *Limnology and Oceanography*, *1*(2), 102-117. Doi: 10.4319/lo.1956.1.2.0102

Ouyang, Y. (2003). Simulating dynamic load of naturally occurring TOC from watershed into a river. *Water Research*, *37*, 823-832.

Prescott, G.W. (1973). *Algae of Western Great Lake Area*. Fifth Printing, William C. Brown Publishers, Dubuque.

Reynolds, C.S. (1984). *The ecology of freshwater phytoplankton*. Cambridge University Press.

Sournia, A. (1978). *Phytoplankton manual*. UNESCO.

Şahin, B. (2003). Epipelic and epilithic algae of lower parts of Yanbolu River (Trabzon, Turkey). *Turkish Journal of Biology*, *27*, 107-115.

Şengül, F. & Türkman, A. (1991). *Water and wastewater analyses*. Dokuz Eylül University Publication.

Wehr, J.D. & Sheath, R.G. (2003). *Freshwater algae of North America: Ecology and classification*. Academic Press.

Wetzel, R.G. (1983). *Limnology* (2nd ed.). Saunders College Publishing.

Williams, R.J. & Boorman, D.B. (2012). Modelling in-stream temperature and dissolved oxygen at sub-daily time steps: an application to the River Kennet, UK. *The Science of the total environment*, *423*, 104-110. Doi: 10.1016/j.scitotenv.2012.01.054

Yıldırım, V. (2011). Physicochemical characteristics and epipelic algae of the Dicle River (Diyarbakır). *Ecological Life Sciences*, *6*(2), 53-92. Doi: 10.12739/10.12739

BÖLÜM 0

MACHINE LEARNING AND DEEP LEARNING METHODOLOGIES FOR PROFILING FATTY ACID DYNAMICS IN STRESSED INDUSTRIAL CROPS

NARİN ASLAN[1]
FADİME KARABULUT[2]

Introduction

High yields in stressed and non-stressed environments can be achieved through abiotic stress adaptation, which can be based on the early warning system of plant stress, which occurs before symptoms in the plant manifest. In order to quantify physiological reactions to stress, several detection techniques have been developed. Large-scale surveys prefer the remote sensing method because it records, measures, and interprets digital representations of energy patterns and images to detect the chemical or physical characteristics of crops from any distance. Thanks to incredibly creative and advanced data analysis techniques, these methods enable the surveying of entire fields during all cropping seasons and enable the accurate, timely, and reliable detection of crop stress.

---

[1] Assist. Prof. Dr., Department of Mechatronics Engineering, Faculty of Technology, Firat University, Orcid: 0000-0002-7609-1557

[2] Assist. Prof. Dr., Department of Plant and Animal Production, Hizan Vocational School, Bitlis Eren University, Orcid: 0000-0001-5186-2303

However, since remote sensing techniques are qualitative, accurate measurements cannot be made. Compared to VOC-based methods, the two main acquisition strategies supporting quantitative methods at the cellular level are nuclear magnetic resonance (NMR) and mass spectrometry (MS). Even though these lab techniques frequently yield incredibly accurate results, they harm samples and are only suitable for monitoring a very small number of crops or plant component parts. As a result, this method is not appropriate for instantly identifying shifts in the physiological responses of crops over time, nor for regulating, forecasting, and managing spatial variability within fields. Therefore, the current frontier of stress assessment in plant science is the development of novel quantitative and continuous imaging methods for both dynamic functional imaging (positron emission tomography) and precise morphological studies (computed tomography, magnetic resonance) (Galieni et al., 2021: 1).

Variations in genotype result in variations in the biochemical structure of cells. These variations, when combined with environmental factors, impact the formation of organs, the growth of crops, and ultimately, crucial traits for agriculture like pest and stress resistance and yield. Comprehending the effects of environment on phenotypes helps to predict quality and yield traits from genotypes in specific environments and provides fundamental insights into the regulation of important processes in plant development and physiology. Processing and integrating huge, noisy, and heterogeneous datasets becomes more and more necessary when analysing phenotypes measured at these various levels or relating these phenotypes to genotypes. Machine learning (ML), a group of computational methods for finding predictive patterns in data, is playing an increasingly important role in these endeavours (Crossa et al., 2017: 961).

In light of its applications in fatty acid analysis, this review examines how machine learning is advancing the field of plant science. The goal is to demonstrate to non-experts that machine learning (ML) provides a variety of techniques that enable us to identify significant patterns in pertinent plant data. Along with encouraging advancements and potential future paths, we go over recently created prediction strategies and techniques.

Metabolites of plants in stressful environments

The metabolome is very dynamic, and metabolomics is helpful in identifying metabolic and cellular signalling pathways as well as the ongoing metabolite changes that represent the phenotypes and traits of organisms. For instance, a number of metabolites vary with sampling time according to the bioactivity profiles of Fucus vesiculosus, which were sampled every month for a year. Accordingly, fluorotannin total ion number (TIC) is the highest in summer, whereas lipids, carotenoids, and chlorophylls reach their highest levels in winter and spring (Heavisides et al., 2018: 1). GC-MS metabolomics reveals that different tissues and types of common wheat have different compositions of cuticular wax and different crystal microstructures (Lavergne et al., 2018: 1). Crop production is always at risk from abiotic stresses, which have an impact on photosynthesis, yield, quality, germination, nutrient uptake, and primary and specialised metabolites and other aspects of plant growth and development. Relative to animals or microbes, crops generate a greater amount of metabolites (Carrington et al., 2018: 823). In response to shifting environmental conditions, crops, being inert organisms, have evolved mechanisms for adaptation. One mechanism involves the synthesis of different metabolites related to defence, which include glucosinolates, alkaloids, and flavonoids, which are specialised metabolites. Additionally, crops responses modify lipids, protein kinases, reactive oxygen species (ROS), phytohormones signalling pathways, and redox homeostasis (Viant and Sommer, 2013: 144; Faizan et al., 2024a:1,b:3191). To comprehend how crops react to particular stresses, it is therefore essential to comprehend the molecular and chemical mechanisms underlying signalling and metabolic processes (such as the synthesis of defence metabolites, osmoprotectants, and components that strengthen cell walls). Changes in the concentration of different metabolites within biochemical networks serve as mechanistic indicators for defining the phenotypic and physiological responses of crops to environmental fluctuations (Littlejohn et al., 2021: 3088).

Despite over a million metabolites, plants still have a wide diversity, many of which remain unknown (Fang et al., 2019: 83). Through intricate biochemical processes, primary (central) and specialised metabolites are constantly produced. The biosynthesis of carbohydrates, lipids, and amino acids depends on primary metabolites. Glycolysis, tricarboxylic acid cycle, and photosynthesis are mediated by them and have an impact on the growth and development of crops. Crops that experience changes in the synthesis of primary metabolites may experience compromised photosynthesis, unstable osmotic adjustment, and reduced respiration. Even though the reactions of primary metabolism are conserved, these pathways function as precursors to produce specialised metabolites in different crop lineages, such as alkaloids, carotenoids, flavonoids, glucosinolates, phenolics, and phytic acids. These metabolites were not previously thought to be essential for plant growth and development. They were originally referred to as natural products or secondary metabolites because of this. It's common knowledge that crops respond to various abiotic stress conditions, like high temperatures, cold temperatures, droughts, and salinity, by producing specialised metabolites (Razzaq et al., 2019: 1; Faizan et al., 2024a: 1, b: 3191). These specialised metabolites, which are linked to specialised metabolite pathways, are able to adapt over time to various environmental challenges. Alongside the genetic diversity in the biosynthesis of these specialised metabolites, their products seem to have a highly diverse chemical structure (Razzaq et al., 2019: 1).

Heat stress causes cytotoxic carbonyl groups to be produced, membrane fragmentation, separation of lipids forming bilayers, protein denaturation, organelle swelling, and membrane hyperfluidity (Higashi and Saito, 2019: 1). Crops increase the total amount of phosphatidylglycerol, phosphatidylinositol, saturated phosphatidylcholine, and phosphatidylethanolamine in response to these detrimental effects on the composition of their membrane lipids (Bian et al., 2023:1). Under stress conditions, further research has shown that the endoplasmic reticulum, plasma membrane, and chloroplasts have higher levels of phospholipids (stearate, palmitate, and oleate), triacylglycerols (hexadecatrienoic acid and α-linolenate), and galactolipids (linoleate) (Higashi and Saito, 2019: 1). While polyunsaturated fatty acids are usually associated with reduced heat tolerance, higher membrane stiffness resulting from an increase in saturated and monounsaturated fatty acids (16:0, 18:0, and 18:1) counteracts the effects of heat stress (Bian et al., 2023: 1). Lipids are necessary for membrane remodelling as well as for signalling pathway activation and energy provision during stress reactions. The effective opening of stomata in Arabidopsis under heat stress is dependent on the breakdown of fats (Korte et al., 2023: 81). Cold stress decreases membrane fluidity, in contrast to heat stress. Tolerance responses result in an increase in the ratio of unsaturated to saturated lipids, which in turn causes the membranes of cells and plastids to become more fluid (Dhaliwal et al., 2024: 1). For example, lipid remodelling in maize mediates cold stress tolerance by regulating the amounts of various lipids, including polyunsaturated phosphatidic acid, TAG, DAG, and MGDG, as well as mono- and di-unsaturated lysophosphatidic acid, phosphatidylinositol, PC, and lysophosphatidylcholine, (Gao et al., 2021: 326). The primary lipid classes found in maize roots under cold stress include PC, phosphatidylethanolamine, phosphatidic acid, and phosphatidylinositol (Zhao et al., 2024: 369). It has also been noted that high concentrations of MGDG, phosphatidic acid, and lysophosphatidic acid in barley can promote cold tolerance (Zhao et al., 2024: 369).

Mild drought might cause some of the mentioned effects, but the plant can recover. A severe or prolonged drought is what leads to irreversible damage and death (Sato et al., 2024:1873; Kim et al., 2024:170). According to Liang et al. (2023: 361), phospholipids like phosphatidylcholine, phosphatidylethanolamine, and phosphatidylglycerol degrade first as a result. According to Sharma et al. (2023: 1), during a drought, the synthesis of lipid byproducts and signalling lipids rises, but the production of essential oils varies based on the species. Increased levels of malondialdehyde, a crucial stress marker, result from membrane peroxidation (Hassan et al., 2022: 1). Thus crops are able to control the synthesis and composition of different classes of lipids to better withstand drought. As per Zhang et al. (2021: 285), the cuticle's wax accumulation and increase in cutin can decrease transpiration by closing the leaf surface. This could be a significant tolerance response to drought stress. Furthermore, transpiration may benefit from changes in the composition of very long-chain lipids associated with cuticles. By linolenic acid synthesis, MGDG, and reduced fatty acid saturation, membrane stability is enhanced during drought (Rabeh et al., 2023: 1781). Because they are fundamental elements of photosynthetic membranes, greater total DGDG and MGDG content as well as DGDG/MGDG ratios are associated with photosynthetic capacity and membrane fluidity during droughts (Hassan et al., 2022: 1). The functions of phosphatidylcholine, phosphatidic acid, sulfoquinovosyldiacylglycerol, phosphatidylglycerol, phosphatidylinositol, and lyso-phosphatidylcholine, among other lipids, in membrane fluidity and lipid signalling are primarily indicated by their modulation under drought stress (Shen et al., 2022: 1). Combining with dehydrins, phosphatidyl serine, phosphatidyl glycerol, phosphatidyl choline, and phosphatidic acid can also increase water-holding capacity, chlorophyll content, antioxidant defence, and osmoregulation, all of which can help promote drought tolerance (Riyazuddin et al., 2022: 519). Furthermore, these lipids interact with nitric oxide and abscisic acid to control stomatal closure, as do sphingolipids and oxylipins (Liang et al., 2023: 361). On the other hand, abscisic acid also triggers the biosynthesis of fatty acids (Kulak, 2020: 1). Thyme plants that can withstand drought have higher levels of signalling lipids and much lower levels of oxylipins brought on by ROS (Moradi et al., 2017: 1). Waterlogging, also known as inundation stress, is the result of too much water in the root zone of land crops. This stressor reduces aerobic respiration and energy production, initiates anaerobic fermentation, results in poor soil aeration, and depletes the roots of oxygen (hypoxia or anoxia) (Xie et al., 2021: 1). Stress damages chloroplast membranes, which lowers photosynthetic capacity and results in ROS accumulation that causes membrane peroxidation and electrolyte leakage (Basu et al., 2021: 1764). According to Xu et al. (2020: 1), hypoxia has been shown to lower the total amount of lipids, inhibit their biosynthesis, and cause degradation that leads to the accumulation of free fatty acids. Lipid remodelling, which varies depending on the species and length of stress, has been linked to tolerance to waterlogging stress. For instance, lipid droplet formation increases in the inter-chloroplast cytoplasm of ramie (Boehmeria nivea), while the contents of fatty acids and 18:3 (n3) Coenzyme A (C18:3-CoA) gradually increase (Shao et al., 2024: 1). Waterlogging adaptation is also linked to the modulation of wax accumulation. It has been observed that unsaturation of acyl-CoAs and very long-chain ceramides mitigates hypoxia-induced damage by means of their interactions with ethylene pathways. It has been observed that under hypoxia, signalling lipids like phosphatidic acid rise (Xu et al., 2020: 1). On the other hand, by enhancing antioxidant capacity, lipids like oxylipin jasmonate play a significant role in regulating how plants react to reoxygenation (Xie et al., 2021:1).

According to Henschel et al. (2024: 1), salt stress is the result of an accumulation of ions, primarily sodium and chloride, in the root zone that lowers the osmotic potential and hinders the uptake of water and nutrients. Furthermore, because of the excessive buildup of sodium in cells, salt stress damages electrochemical gradients and results in protein denaturation (Zhao et al., 2021: 1). Alkaline stress, also known as saline-alkali stress, is the result of neutral or alkaline salts building up in soils. This raises pH and can lead to ion toxicity, osmotic stress, chemical degradation, and nutrient and oxygen shortage (Rao et al., 2023: 1). For the permeability and integrity of the membrane, it is crucial. In species that are sensitive to salt, salt stress causes lipid peroxidation and lipolysis, which lowers the overall amount of lipid in membranes by inhibiting lipid biosynthesis. As a result of increased lipid homeostasis, species that are tolerant to salt have higher total lipid contents (Rawat et al., 2021: 653). Additionally, salt stress causes sensitive species' fatty acid saturation to rise, stiffening their membranes. But halophytes retain the fluidity of their membranes and exhibit high concentrations of unsaturated fatty acids (Rawat et al., 2021: 653). Phosphatidylserine and phosphatidylinositol have also been found to increase in response to salt stress, and they are involved in signalling responses through Ca^{2+} causing Na^{+} efflux (Helm et al., 2020: 1). In salt-tolerant species, there is typically an increase in the ratio of sterols to phospholipids, which lowers the permeability of the membrane to Na^{+} and Cl^{-} (Salama et al., 2007: 351). It has also been observed that LD accumulation enhances recovery from salt stress, possibly by supplying fatty acids necessary for membrane remodelling (You et al., 2019: 510). Alkaline stress is also known to cause significant lipid remodelling in addition to salinity (Lu et al., 2021: 66). Significant lipid remodelling occurs in maize roots when exposed to alkaline stress; phosphatidylcholine content is reduced, phospholipid and galactolipid synthesis in plastids is increased, and C34:6 galactolipids are produced, all of which point to the induction of the prokaryotic metabolic pathway (Xu et al., 2021: 1). Alterations in root exudates are a crucial defensive reaction to alkaline stress. Alkaline stress in wheat roots has been shown to increase the synthesis of fatty acids, phenolic acids, and glycolysis (Wang et al., 2024: 1).

Lipid peroxidation is one of the primary changes associated with heavy metal stress that can compromise the integrity of the plasma membrane (Zhang et al., 2007:44). Furthermore, modifications in membrane lipids, both in terms of quality and quantity, can result from heavy metal stress, modifying the permeability and functionality of the membrane (Devi and Prasad, 1999: 99). For instance, halophyte plant leaves' lipid pattern is altered when mercury (Hg) is present in the surrounding air. Crop tolerance to mercury is impacted by this shift in the membrane's degree of unsaturation, fluidity, and stability (Figueira et al., 2023: 1). The content of lipophilic compounds in roots and leaves is also greatly impacted by the heavy metal cadmium (Cd). A mechanism for maintaining membrane fluidity under sublethal Cd toxicity is likely to be the lipid composition changes (Andresen et al., 2020: 1628).

Nutrient deficiencies occur when crop requirements are not met for any of the 14 essential mineral nutrients. Growth, productivity, and even a plant survival are impacted by this mineral shortage. The effects of a nutrient deficiency vary depending on the nutrient, its levels, source, and plant species because these nutrients have distinct roles in plants (de Bang et al., 2021: 2446). A deficiency in nitrogen, for instance, can impact the overall metabolism of crops since it is a crucial component of proteins and macromolecules like RuBisCo and chlorophylls (Terrer et al., 2019: 684). Increased fatty acid unsaturation and chloroplast membrane stability are associated with increased tolerance to stress, whereas decreases in total lipid content are associated with nitrogen depletion (Li et al., 2024:1). Reduced phosphatidylglycerol, glucuronosyl diacylglycerol, and MGDG/DGDG ratios are further indicators of nitrogen deficiency (Peng et al., 2023: 904). The capacity to preserve the amount of phospholipids is a crucial tolerance response because phosphorus deficiency leads to significant membrane lipid remodelling, which includes the conversion of phospholipids to non-phospholipids (Honda et al., 2023:1). Reduced phospholipid concentrations cause crops to produce more membrane galactolipids, which raise the amount of triacylglycerol in the leaves and roots (Pfaff et al., 2020: 1).

According to Kargiotidou et al. (2008: 2043), increased solar radiation can cause a change in the composition of seeds, increasing the proportion of oleic acid (C18:1) fatty acids and decreasing the amount of linoleic acid (C18:2) fatty acids, as well as higher levels of fatty acids stored in seeds. Light has a major impact on lipid levels, as demonstrated by an analysis of how crops' membrane lipid composition responds to varying light intensities. Glycerolipids are reduced in the dark and increased in the presence of strong light (Burgos et al., 2011: 656).

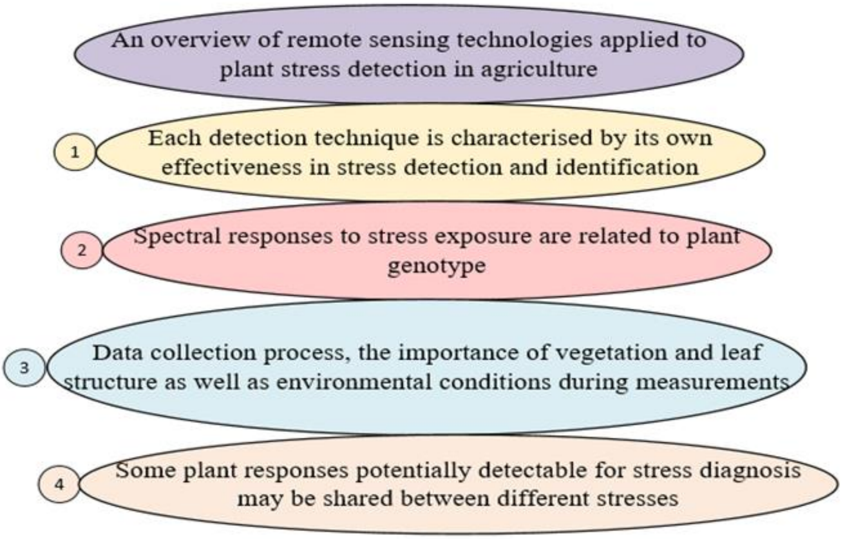
An outline of recent studies on crop lipidomics

Lipidomics is a new field of study that focusses on the diverse group of biomolecules known as lipids and their roles in different biological processes (Han and Gross, 2022: 1). Lipidomics is a result of recent developments in analytical techniques. According to Kehelpannala et al. (2021: 894) these analytical techniques sheds light on the intricate biological mechanisms underpinning plant growth, development, and stress adaptation. Since abiotic stresses pose major constraints to agricultural productivity, lipidomics is an essential tool for comprehending the function of lipids in plant stress responses. The majority of publications on lipidomics and plant stresses currently focus on abiotic stresses. Most of these studies report overall lipid profile alterations and associate them with responses related to either signalling or membranes. The mechanism underlying stress responses is often determined by analyzing lipid profiles of genotypes with altered stress tolerance (Zhang et al., 2021:285). Additionally, lipidomics of plants treated with stress reducers is used to better investigate these responses. The methods most frequently employed in crop lipidomics are known to be mass spectrometry (MS), gas chromatography (GC), and liquid chromatography (LC). Furthermore, researchers can make more thorough inferences about the observed responses thanks to multiomics and integrated approaches, such as the combination of transcriptomics, metabolomics, and lipidomics (Li et al., 2022: 1). Multiomic analyses can highlight the ways in which stress conditions impact protein abundance and activity (proteomics), metabolic profiles (metabolomics and lipidomics), and gene expression (genomics and transcriptomics) concurrently by merging these varied datasets. By applying an integrative approach, important regulatory networks, innovative biomarkers, and stress-responsive pathways can be identified that may be missed when examining each omic layer independently.

Qualitative remote sensing techniques for stress evaluation

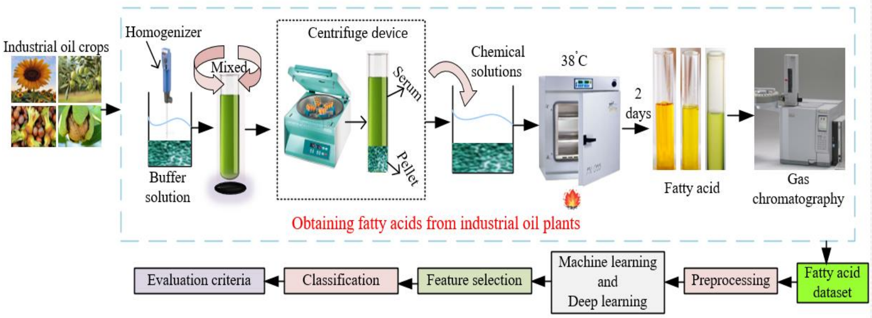
A collection of methods that record, measure, and interpret energy patterns and digital representations obtained from non-contact sensor systems to determine the chemical or physical characteristics of physical objects at any distance is known as remote sensing. It is employed in precision agriculture and resistance breeding as a quick and non-destructive way to identify abiotic stressful situations (Omia et al., 2023: 1). The mechanisms at play are mostly based on how electromagnetic radiation interacts with crops. Given particular circumstances, changes in the crop-electromagnetic radiation relationship can be used to identify healthy crop conditions. This is because stressful conditions can induce a wide range of intricate physiological and biochemical reactions in crops, such as altered stomatal conductance, pigment concentration, and biochemistry. Agricultural science in the past has relied heavily on reflectance (visible, VIS, in the 0.4-0.7 μm region; near infrared, NIR, 0.7-1.3 μm; and shortwave infrared, SWIR, in the 1.3-2.5 μm region); thermal (thermal infrared, TIR, in the 7.0-20.0 μm region); and fluorescence (at wavelengths of 0.68 with 0.74 μm). These sensors are all well-suited for a variety of applications, including airborne and satellite remote sensing, microscopic observation (e.g., laboratory spectroscopy or hyperspectral microscopy), and ground-based close sensing (i.e., detector within 2 m from the observed object). As such, the spatial resolution of each sensor is characterised not only by its resolution in differentiating signal variations but also by the distance between the sensor and the object of analysis. Furthermore, sensors can be classified according to their application in (i) non-imaging techniques (such as VIS, IR, and fluorescence spectroscopy) and (ii) imaging techniques (such as VIS, multispectral and hyperspectral imaging, thermal imaging, fluorescence imaging, and x-ray imaging). Since they don't provide spatial information, non-imaging sensors are generally more useful for measurements made at the laboratory or leaf scales. Crop electromagnetic patterns after stress exposure and subtle tissue processes may be correlated, thanks to the high resolution of current sensors (Thomas et al., 2018:1). When collecting data on crop responses to stress, spatial resolution at the canopy or field levels is essential (Kuska and Mahlein, 2018: 987). While remote sensing offers promise for stress detection, there are certain general issues and drawbacks to consider. Initially, every sensing technique has a unique effectiveness in identifying and detecting stress, which is determined by the inherent features of the applied technology as well as the technological advancements made. The kind and intensity of stressful situations determine these. Because of this, it is not always clear how to diagnose stressful conditions early—that is, before symptoms manifest. For instance, temperature-based indices can accurately predict crop responses prior to visual inspection in the event of water stress, while certain crop indices based on reflectance in the VIS/NIR range are only useful for responses observed later in the growth cycle (Gerhards et al., 2019: 1). Second, the genotype of the crop influences the spectral responses to stress exposure within the same sensing crop technique and stressed condition. Deriving indicators or parameters for particular demands may necessitate a thorough investigation of particular stress-genotype combinations, along with a comprehension of the physiological and biochemical mechanisms resulting in alterations in spectral characteristics. Third, during measurements, data collection procedures should consider the state of the environment in addition to the vegetation and leaf structural architecture. Improvements are required for (i) pre-processing data, (ii) using multi-sensor platforms with 3D shape sensors, and (iii) integrating integrated calibration systems into automated systems (Mishra et al., 2020: 43). Fourth, it may be challenging to pinpoint a single stressor, particularly in open-air environments where several stress scenarios could arise, because certain crop responses that could be detected for stress diagnosis may be similar to plant reaction to various abiotic stresses by various stresses (such as salinity, temperatures, drought, and mineral toxicity) (Figure 1). It is determined that while a single sensing technique can exhibit high specificity in identifying individual stress signals under controlled experimental conditions, a comprehensive and integrated approach is necessary to identify potential multiple causes in agricultural practices (Jones and Schofield, 2008: 19). The aforementioned factors offer a fresh perspective on how to advance vegetation sensing for stress detection by utilising both established methods and introducing and improving cutting-edge imaging approaches that are useful for the agriculture industry (Figure 1). Figure 1 provides a more comprehensive summary of remote sensing technologies used for plant stress detection in agriculture (Gerhards et al., 2019: 1).

Figure 1 It displays advances in vegetation sensing for stress detection by utilising both established methods and introducing and enhancing new imaging techniques appropriate for the agricultural sector



Before classifying the changes in fatty acid metabolism of industrial crops exposed to abiotic stress, the treatments applied to the crops are presented in Figure 2.

Figure 2 The flow chart for examining the changes in fatty acid metabolism of industrial crops exposed to abiotic stress using machine learning and deep learning methods



Methanol extracts are prepared in a 50/250 ratio for the samples extracted from the seeds of corn, sunflower, sesame, olive, safflower, hazelnut, and walnut, among other agricultural crops. In the homogeniser, the sample crop group is broken down in solvents to produce the extracts. Following their dissolution in the homogeniser, each group is centrifuged at 5000 rpm and 4 °C. After centrifugation, 2% methanolic H2SO4 (v/v) is added to the supernatant, which is then vortexed and incubated for 16 hours at 38 oC. 5 ml of 5% NaCI is added after cooling at the conclusion of the incubation period. 5 ml of n-hexane is added to the fatty acid methyl esters that have formed in the reaction medium. After treating the fatty acid methyl ester mixture with 2% KHCO3, the solvent is removed by evaporation. N-hexane is used to dissolve fatty acid methyl ester mixtures, which are then subjected to gas chromatography analysis (Christie 1990). The fatty acid dataset created is preprocessed before being analyzed in machine learning and deep learning methods. The dataset, which undergoes pre-processing such as normalization and data augmentation, is trained on machine or deep learning networks. Feature extraction is performed on the trained data. These extracted features are classified and performance measurements are calculated.

These machine learning and deep learning stages and stress-induced fatty acid analyses in industrial crops are described in detail below.

Data preprocessing

Novel approaches to data analysis in numerous domains can be facilitated by machine learning. At its core, machine learning employs methods for learning from data without the need for explicit programming instructions (Singh et al., 2016: 110). It can therefore lead to the discovery of novel patterns that conventional analytical techniques might overlook. Data collection and storage, preprocessing, classification, and feature extraction are the primary steps in a machine learning process (Ramos-Giraldo et al., 2020: 27). A condensed route map for techniques related to machine learning data analysis is presented. Rather than analysing features one at a time, machine learning can simultaneously combine multiple factors to detect patterns, which is advantageous in fields of agriculture (Singh et al., 2016: 110; Elahi et al., 2023:1). Because the environment around crops is frequently very complex, using a variety of factors is important. Thus, factors like direction, light intensity, and leaf angle can alter the outcome. Machine learning can be applied to pre-processing tasks like feature extraction and dimensionality reduction in addition to classification. Stress identification, discrimination, and quantification are all part of the crop health assessment process. Identification searches for signs of a specific stress in a time-dependent fashion. Discrimination includes identifying a particular stress and distinguishing its symptoms from those of other stresses; quantification is the measurement of the stress's intensity. There is currently no universal approach that can be recommended for all applications, as the best ML way or method depends on the specific problem being addressed. The following sections provide a summary of data processing techniques in machine learning applied to different agricultural applications.

To guarantee the precision and repeatability of classification results, data preprocessing is crucial (Tsaftaris et al., 2016: 989).

Preprocessing is the process of transforming data into a more standardised and easily accessible format in order to enhance the performance of classification algorithms. Principal component analysis (PCA), image thresholding, colour space conversion, dimensionality reduction, contrast enhancement, normalisation, and noise removal with filters are a few examples of image preprocessing techniques (Singh et al., 2016: 110). While imaging techniques are the main focus of this section, some of the listed methods, like PCA, can also be used to process spectral data.

Normalization

Data preparation for machine learning usually involves the normalisation process. Normalisation aims to preserve information and avoid distorting the value range differences by applying a common scale to the values of the dataset's numerical columns. Typically, the following Equation is used to perform normalisation.

$$X(:,i)^N = \frac{X(:,i) - \min(X(:,i))}{\max(X(:,i)) - \min(X(:,i))}$$

where X is the feature vector of dimension N, $max\ (X(:,i))$ is the maximum value of the feature vector, $min\ (X(:,i))$is the minimum value of the feature vector. Thus, in order to achieve the best outcomes, the normalisation process should be used (Aslan et al. 2022:1).

Color space conversion

Because different colour spaces present colour data in ways that facilitate specific computations or offer a more naturalistic means of describing colours, they arise. The combined percentage of red, green, and blue hues, for instance, is how the RGB colour space defines a colour. Hue, saturation, and brightness are three other colour models that describe colour. Hue is the degree of grey or pure colour, while brightness is the intensity or total brightness of a colour. Feature extraction and image classification can benefit from the extraction of extra colour features from images using colour spaces. Numerous research have used features from colour space transformation to process RGB data for crop stress detection. Moreover, leaf senescence brought on by environmental stressors can be seen with RGB imaging. Maize leaf redness is known to be induced by a variety of abiotic stressors (Ahmad and Reid, 1996: 185). Many techniques are available for analysing the findings of both imaging types (Kior et al., 2021: 1).

Dimensionality reduction

A more condensed representation of the data with maximum information preservation is the goal of the dimensionality reduction process. Feature summaries are produced by principal component analysis (PCA), a popular method for reducing dimensionality that geometrically projects the data into lower dimensions (principal components) (Lever et al., 2017: 641). PCA can reduce the dimensionality of the data by combining dependent (or highly correlated) variables into a common variable with the least amount of information loss. The initial principal component (PC1) is assessed using the dataset. After that, PC2 is assessed in comparison to the others, and the procedure is repeated. The variances in the data are represented by principal components, or PCs. Therefore, they can be plotted as PCA score plots, which are 2D or 3D plots (for two or three PCs) depending on the situation. Every PC can also be fed into various machine learning models as a preprocessing step for dimensionality reduction. According to Lu et al. (2017:369), PCA has been a crucial preprocessing step in numerous studies for managing spectral and imaging data. PCA is typically chosen over more recent techniques as an unbiased dimensionality reduction method, even though more effective methods have surfaced, such as linear discriminant analysis (LDA), which can maximise class separation. A useful tool to help with data interpretation is PCA. But one drawback of this approach is that data outliers may have an impact on it (Wold et al., 1987: 37; Saha and Manickavasagan, 2021: 28).

Segmentation

The technique of "image segmentation" can help divide an image into key areas, like the object and its background. Because it can minimise mistakes or misclassifications brought on by background noise, this method is helpful in agricultural applications (Lei et al., 2024: 1). Crucial techniques include clustering-based strategies like K-means, which are helpful for locating stressed crop parts in an image (Al Bashish et al., 2010: 113).

Analysing crop stress images requires segmentation. Using patches taken from nearby pixels, a CNN can classify a pixel in an image and represent the classified pixel (Li et al., 2014:1). Overlapping input patch boundaries and repeatedly computing the same convolution are known drawbacks of this approach. Thankfully, convolutions can be written as linear operators, such as the dot product and convolution (Mallat, 2016:1). In lieu of producing a pixel-by-pixel output, a CNN with a fully connected layer can process a larger input image than the trained image. The entire input image can therefore be effectively processed using a fully convolutional network (Taye, 2023:1). Particularly in industrial oil crops like sunflower, hazelnut, and olive, research on deep learning and machine learning techniques has gathered steam.

Deep Learning Architecture

Deep learning is a representation learning algorithm that can automatically perform high-level abstraction on data, contains multiple layers of intermediate processing, and consists of multiple complex nonlinear transformation structures. Recent years have seen a notable acceleration of deep learning work due to the success of the deep convolutional architecture known as AlexNet (Au 2012:1), which served as the foundation for the ImageNet Large-Scale Visual Recognition Challenge (ILSVRC) held in 2012. (Da'u and Salim, 2020:2709). The deep learning architecture's layer composition is described below.

Convolutional neural networks (CNNs) are a popular model in agriculture that convolutionally processes data for image classification (Jin et al., 2017: 4509). Additionally, pre-trained CNN models like VGG (Ferentinos, 2018:311), AlexNet (Ma et al., 2018:18), ResNet (Zhang et al., 2018:1), and GoogleLeNet (Mohanty et al., 2016:1) have been utilised. Several studies have used databases like Crop Village (Saleem et al., 2019:1) and Wheat Disease Database (Lu et al., 2017:378) in conjunction with deep learning models when a large set of training data is needed. Working well with unprocessed data is one benefit of deep learning techniques (Brahimi et al., 2017:299). As a result, it shortens the time needed for segmentation, feature extraction, dimension reduction, and colour space conversion during data preprocessing. LeCun et al. (2015:436) also mention that feature extraction can occasionally be carried out by the deep learning model without the requirement for an external processing step. The requirement for sizable datasets to yield precise results is, nevertheless, a major disadvantage (Lu et al., 2019:369).

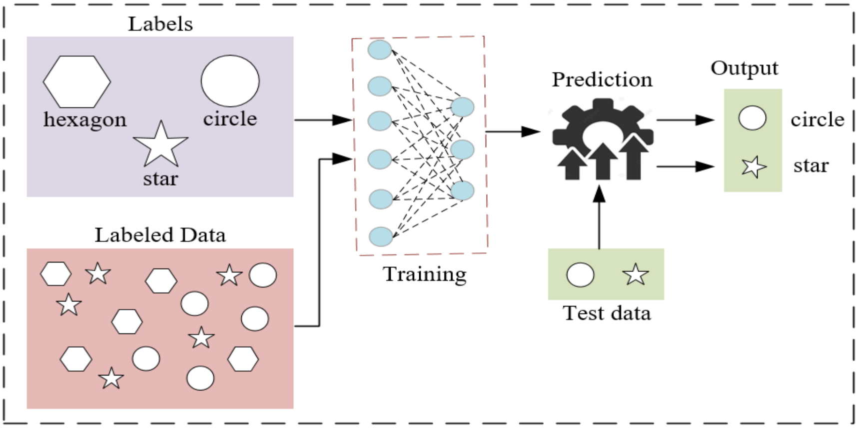
Machine learning

Computer systems use machine learning, a subset of artificial intelligence, to carry out particular tasks (Koza et al., 1996:151). It is not possible to process and analyse very large data with human power. In order to solve and analyse these data, machine learning has been introduced. Numerous machine learning techniques exist. The common goal of these methods is to create the most appropriate models for new data by using past data. Reinforcement learning, semi-supervised learning, unsupervised learning, and supervised learning are the four components of machine learning methods.

Supervised learning

Using labelled data sets to train a model is known as supervised learning. Labelled data are data sets where the correct output of each input is known. In this type of learning, the model learns the relationship between inputs and their corresponding correct outputs (Harrington, 2012: 1). In order to do this, the learning algorithm must "reasonably" extrapolate from the training set to untested scenarios. The term "generalisation error" refers to the statistical quality of an algorithm. Test data are used to calculate the generalisation error on datasets that are not labelled. Among the algorithms used in supervised learning are K-nearest neighbour methods, logistic regression, decision trees, neural networks, support vector machines, and neural networks (Couprie et al., 2013:1). The supervised learning model's structure is depicted in Figure 3.

Figure 3. Supervised learning model

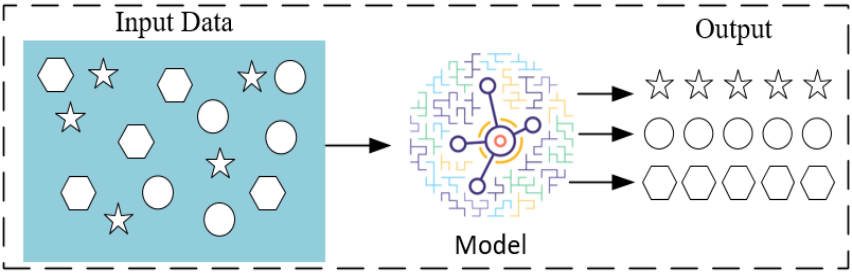


As seen in Figure 3, the most important point in supervised learning is the labelled data set. It is known which data corresponds to which information. It takes a known input set and the corresponding outputs and produces the most appropriate output for new data that the algorithm has never seen before.

Unsupervised learning

A training data set is not required for this type of learning. The computer is not initially given a training set. The computer creates a prediction by self-learning the relationships and structures between the data. The data scientist who creates the algorithm creates the data and enables the algorithm to learn on its own by analysing it on its own. An artificial intelligence created with unsupervised machine learning can categorise data into groups according to their similarity even if no specific category is given for grouping. It is analysed in two main sections: clustering and association techniques. In order to cluster data, a set of uncategorised data must be examined for patterns. Algorithms for clustering analyse the data and identify any naturally occurring clusters. These clusters' specifics can all be changed appropriately. The association technique is used to identify patterns in large databases between variables. Relationships between data objects are simple for experts to establish. The model of unsupervised learning is shown in Figure 4.

Figure 4. Unsupervised learning model



As seen in Figure 4, there is unlabelled data in unsupervised learning. It learns by itself by trying to find the hidden structure between these unlabelled data.

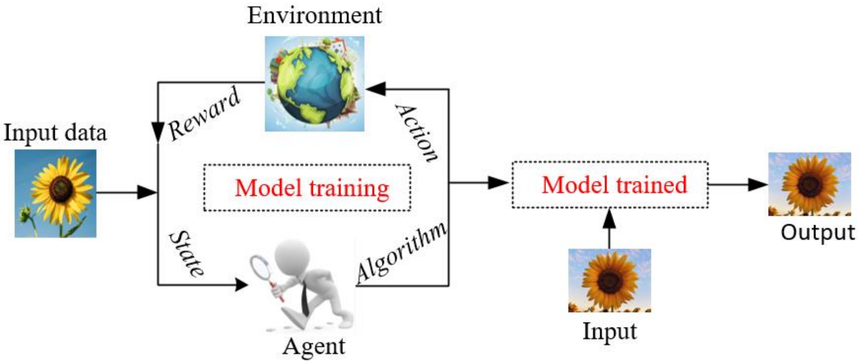
Semi-supervised learning

To facilitate training, machine learning uses either labelled or unlabelled data. In between these two approaches is semi-supervised learning. Data labelling is a costly and time-consuming procedure that needs personnel with extensive training. Here, there are situations where only a small percentage of observations have labels; in these situations, semi-supervised machine learning is useful.

Reinforcement learning

In order to act in a way that maximises reward and minimises risk, reinforcement learning makes use of observations from interactions with the environment. As an algorithm, it looks around it all the time until it finds every possibility. No other machine learning algorithm can match reinforcement learning's capacity to tackle a wide range of challenging issues. In order to achieve maximum efficiency, it allows machines to independently determine the best course of action in a given situation. Q-learning, temporal difference, and deep adversarial networks are examples of common algorithms in this category.

Figure 5 Structure of the Reinforcement Learning Method



Reinforcement learning is a machine learning approach that deals with what actions subjects should take in order to achieve the highest amount of reward in an environment. This learning mimics the way humans learn. Reinforcement learning, unlike other machine and deep learning frameworks, consists of unlabelled data. There is an agent and an environment. The agent basically makes observations about this agent and the action takes place. The environment gives a reward to this agent as feedback. In this process, the main goal for the agent is to maximise the reward.

Feature selection

An attribute is each column/column in a data set that will allow us to create the targeted model output. Attribute selection is the process of deciding which attributes within the data set are most useful. The machine learning model's performance is significantly impacted by this process. Superfluous characteristics may lengthen the training period of the model. The model ought to be clear-cut and understandable. Too many attributes can reduce the interpretability of the model. Because of overfitting, the model's performance may be good in the training dataset but poor in the test dataset. The model's error rate will be high if the records in the test data set and the training data set are not comparable. Some important feature selection methods used in machine learning and deep learning methods are mentioned below.

Relief method

The weights of the estimator when its output depends on a multiclass variable are determined by a well-known feature selection algorithm called Relief. It reacts differently to features. After applying the Relief algorithm to select a sample from the data set, an attribute ranking is generated for the sample that takes into consideration the sample's proximity and distance from other classes in its class. The feature selection process is finished by running this feature ranking model with both positive and negative weights. To improve the classification capacity, apply the feature reduction method (Karabulut and Ozmen Koca, 2020: 2972).

It is taken from a dataset that contains n instances of p features that belong to two designated classes. All the features in the dataset are scaled to fall between 0 and 1 (binary data should never be greater than 0). M times are done with the algorithm. The weight vector (W), which is p-long and made up of zeros, is the initial value.

Every time we perform an iteration, we take the feature vector (X) of a random sample and the feature vectors of the samples belonging to each class that are closest to X (based on the Euclidean distance). A sample is considered to be "near miss" if it deviates significantly from the closest same class sample, and it is considered a "near hit" if it is the closest. This update for the weight vector is given in the following Equation :

$$W_i = W_i - (x_i - nearHit_i)^2 + (x_i - nearMiss_i)^2$$

where i, which spans from 1 to p, is the components index.

Fisher Score

The Fisher Score method calculates a relationship score by taking the mean and standard deviation of the feature values for each class. This Equation is used to calculate the Fisher Score (Yıldız et al., 2012:1; Bolon-Canedo et al., 2014:111).

$$F(x_i) = \frac{|\mu_i^+ - \mu_i^-|}{\sigma_i^+ - \sigma_i^-}$$

In the formula; + and - signs indicate different classes for a two-class problem, $\mu_i^+$and μ_i^-values indicate the arithmetic means of the classes, σ_i^+ and σ_i^-values indicate the standard deviation values of the classes. In order to select the desired number of features, the features are ranked from large to small based on the calculated scores. This method looks for features that will help distinguish two classes from one another. The process begins with the top row of features. A high Fisher score means that there are small deviations in the relevant feature's value within the relevant classes and that the relevant feature's mean difference between the two classes is substantial. Consequently, when choosing the features that will most effectively differentiate the two classes from one another, those with high Fisher scores are favoured (Bolon-Canedo et al., 2014:111).

Chi-Square test method

A popular technique for feature selection that assesses the importance of the discrepancy between observed and expected frequencies is the Chi-Square test. The procedure looks into the likelihood that features X and Y are related. When the test is finished, the features that show no correlation with Y are removed from the dataset. The Chi-Square test employs the following Equation (Inc, 2007: 1; Unver and Gamgam, 2006:1).

$$X^2 = \sum_{i=1}^{I}\sum_{j=1}^{J}\frac{\left(N_{ij} - \widehat{N}_{ij}\right)}{\widehat{N}_{ij}}$$

$$\widehat{N}_{ij} = N_{i}.N_{j} /N$$

$$d = (I - 1)(J - 1)$$

In the formula; N_{ij} is the number of observed units at the i th level of Y and j th level of X, N , ij is the expected number of units at the i th level of Y and j th level of X when the two features are independent, d is the degrees of freedom of the Chi-Square distribution to be used for the test statistic. When it is desired to select a certain number of features, the process is carried out by ranking the features from largest to smallest according to the calculated X^2value and then selecting the desired number of features starting from the top row.

Iterative Neighborhood Component Analysis (INCA)

According to Raghu and Sriraam (2018:18), one of the most popular weight-based feature selectors for maximising classification algorithms' prediction accuracy is neighbour component analysis, or NCA. Initially, each feature column is given a set weight. Typically, a value of one is chosen. Stochastic gradient descent, distance-based fitness functions, and optimisers like Adam are used to update the weights. According to Jin and Deng (2018: 35), informative and redundant weights produce only positive weights and represent larger and smaller weights, respectively. NCA has two key issues. First of all, for a given problem, there is no perfect feature vector or classifier. Secondly, the feature that is redundant is chosen for elimination because it lacks a negatively weighted feature. To get around these issues, the INCA feature selector is an augmented and iterative model of NCA. Finding the ideal number of features is the main goal of the CCA feature selector. As a result, CCA employs an iterative error calculation procedure. The best feature vector is determined by taking the feature vector with the lowest error value, and a classifier is chosen to serve as the error/loss value calculator. CCA chooses a variable quantity of characteristics for variable issues (Aslan et al., 2022:1).

Classification architectures

In the supervised machine learning process of classification, the model makes an effort to predict the right label for a given set of input data. Before the model is applied to new, unseen data for prediction, it is thoroughly trained on training data and assessed on test data. Classification methods commonly used in ML and deep learning methods are mentioned below.

Tree: During the machine learning process, the compilation of computational techniques and mathematical equations are used to help identify, generalise and classify the selected dataset. T-technique, classification methods are performed by creating a tree structure. A large dataset is divided into smaller sections by the tree structure, which uses branches and leaves as nodes. Every non-leaf node in this instance is regarded as an input feature. Each leaf node is a member of the target class value or probability distribution (Nnamoko et al., 2014: 50). Individual nodes make up the final product, which is a tree structure. An input feature is used to represent each non-leaf node, and feature values are arranged along the arcs of this representation. There is a class value or probability distribution shared by all of the leaf nodes. One can obtain a number between 0 and 1 using the entropy method. A data set's coherence is indicated by this number. With a root node at the top, it is constructed top to bottom and comprises subsets whose features share the same target (goal) values. Stated differently, entropy refers to a predefined level of components used in distortion measurements. It is frequently found via probability computations, such as the one in Equation (Tran et al., 2015: 57; Nevlud et al., 2013:468).

$$E(S) = \sum_{i=1}^{c} -p_i log_2 p_i$$

$$E(T,X) = \sum_{c \in X}^{c} p_{(l)} E_{(l)}$$

In this equation *S* represents the current state, *Pi* the probability of an event occurring, *I* the fraction of the state *S* and *I* the node of S'. In this case X is the desired quality of the current state *T*.

Linear discriminant: It is a linear classification algorithm that models the distribution of each class using Gaussian distributions. It finds the decision boundary that maximises the separation between classes. As a widely used supervised dimensionality reduction method, linear discriminant analysis looks for a linear combination of features that simultaneously maximises inter-class scatter and minimises intra-class scatter (Sugiyama,2007:1). Therefore, Class-related instances appear as close together as possible in the projected space, while concurrently class-different instances appear as far apart as possible. The linear discriminant vector is shown in the following Equation.

$$y = Ax$$

where x is an input vector and the matrix A is chosen in such a way that the instances of each class are closer to each other and the classes are better separated from each other.

$$P(A|B) = \frac{P(B|A).P(A)}{P(B)}$$

P(A|B) = The probability of A when B is known to be true

P(B|A) = The probability of B when A is known to be true

P(A) = The probability of A

P(B) = The probability of B

Naive Bayes: When a feature is present in a class, the Naïve Bayes classifier assumes that its presence is uncorrelated with the presence of any other feature. For classification tasks like text classification, a well-liked supervised machine learning algorithm is the Naïve Bayes classifier. Because it belongs to the family of generative learning algorithms, it models the distribution of inputs for a particular class or category. This approach makes the algorithm capable of making accurate and timely predictions by assuming that the characteristics of the input data are conditionally independent of the class (Narayan 2021: 3241; Kang et al., 2021:1).

In real-world scenarios, it is frequently not true that all features in the input data are independent of one another, as assumed by the Naïve Bayes classifier. Notwithstanding this oversimplifying supposition, the Naïve Bayes classifier finds widespread usage in numerous real-world scenarios because of its effectiveness and strong performance. Additionally, despite being among the most basic Bayesian network models, Naïve Bayes classifiers can achieve high accuracy when used in conjunction with kernel density estimation.

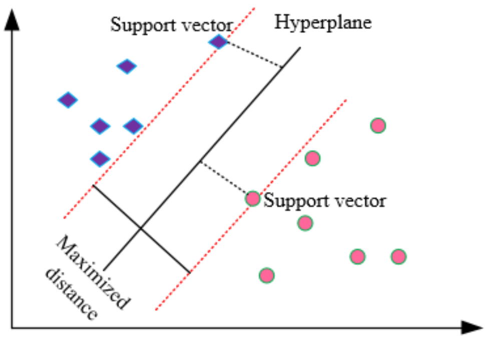
Support vector machine: It is a data analysis algorithm that uses regression and classification processes to analyse data. The working principle of the SVM algorithm is to determine a boundary for a point field of interest (belonging to a particular class). When a boundary is drawn (on the training samples) for any test samples to be categorised, it is necessary to examine whether the test samples fall within the boundary. The training data set (except for data that are very close to or on the boundary) is only considered redundant during boundary construction, so the technique does not depend on them until it has completed its task. It only needs a basic set of points to clearly define and determine the boundaries. These data points are called ‘supports’ because they effectively support the boundary. The name ‘vector’ comes from the fact that each element is a vector: each row of data contains information for a collection of attributes (Suthaharan, 2016: 207). The SVM vector is shown in the following Equation.

$$(x) = w^T x_i + b$$

Where w^T is the vector weight, $f(x)$ is the feature sets for both classes, x is the dataset used in training and b is the bias.

The classifier is displayed as a single direction or a curve in basic scenarios when the data is bidirectional and the data on both sides are in the case of multiple classes, each with its own set of attributes. The basic idea of SVM is the use of hyperplanes to separate different classes. An important component of SVM is the selection of a suitable kernel function and the modification of the kernel parameters. The structure of SVM is shown in Figure 6.

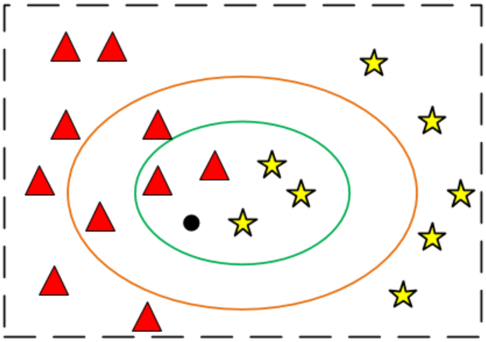
Figure 6 SVM structure



The optimal line to divide the two classes is what SVM looks for. The margin is the area inside this line that is 1. The more space there is between two or more classes, the better the separation. The SVM forecasts the margin's edge on which fresh samples will land (Moraes et al., 2013: 1).

KNN: K-Nearest Neighbour (KNN) is a supervised classification algorithm. This technique basically works by using K neighbouring models from a training set to determine the classification label of a model (Fukunaga and Narendra, 1975:750). It is a technique where the KNN algorithm stores data and categorises new cases based on the similarity score of the data. The value of K in KNN has the most influential and high impact on the performance of the KNN algorithm. Using the training samples as a guide, KNN classifies features based on how close they are to the region of interest (Abrahamsson et al., 2022: 1). The KNN model is shown in Figure 7.

Figure 7 KNN structure



Here the black circle indicates the specimen under test, which must be classified into one of two groups. (yellow star or red triangle). If K=5, the red triangles take it, since three of the five numbers in the green line circle indicate K=5. If K=7, four of the seven numbers in the orange line circles are red and indicate these circles. The parameter K and the total number of nearest neighbours should be ascertained prior to utilising the K-EYK method.

With five convolutional layers, AlexNet is one of the pre-trained networks that is frequently used for image classification because it is a reasonably simple task. AlexNet uses the hyperbolic tangent activation function, which is the most popular option for CNNs (Qayyum et al., 2017: 2662). Deep pre-trained networks later appeared; one example is VGG19, which has 19 deep layers (Simonyan and Zisserman, 2014: 1). Less memory is used during inference in these deeper networks because they employ smaller stacked cores. This enhances the functionality of portable computers, like smartphones (Chen et al., 2016: 130). The GoogLeNet, a 22-layer neural network developed by Szegedy et al. (2016: 2818), makes use of starting blocks. Using starting blocks has the benefit of lowering the number of parameters while improving the efficiency of the training process. After 2014, ImageNet's performance reached saturation, and it is skewed to believe that more intricate architectures result in higher performance. However, even though it's not required, crop stress detection with deeper networks offers a smaller memory footprint. Thus, for crop stress images, other practically used methods like AlexNet or VGG16 are still in use.

The data are fed into a machine learning algorithm for classification after the required pre-processing steps have been finished. In order to classify the unlabelled data into categories (such as stressed or healthy), these algorithms look for patterns in the data (Rumpf et al., 2010: 91). Three types of machine learning algorithms can be distinguished: supervised, weakly supervised, and unsupervised. While unsupervised learning solely uses unlabelled data, weakly supervised learning may use smaller datasets, coarse labels, or incorrectly classified labels for training. Supervised learning uses labelled training data to predict the labels of test data. This is the main distinction between these algorithms (Zhou, 2018: 44). Developing clusters of instances with similar characteristics using clustering algorithms is one of the most well-known applications of unsupervised learning (Rodriguez et al., 2019:1). Data classification in agriculture uses a lot of machine learning algorithms. However, support vector machines (SVM) (Rumpf et al., 2010:91) and artificial neural networks (ANN) (Bindushree and Sivasankari, 2015: 1) are the most widely used techniques. Machine learning-based classifiers have a high degree of reliability. One disadvantage, though, is that they have a propensity to misclassify data due to overfitting, particularly in small datasets. Furthermore, machine learning can be laborious, particularly when dealing with big image files. All of the following procedures, though, can help lessen the severity of both issues. Data augmentation (Sladojevic et al., 2016:1), reflection (Ghosal et al., 2018: 4613), and colour change (Esgario et al., 2020: 1) are some of the techniques used to slightly distort images in order to reduce over-fitting errors in image classification. The data should undergo image processing and data augmentation if the data processing pathway determines that they are required before the data is run through the classification algorithm.

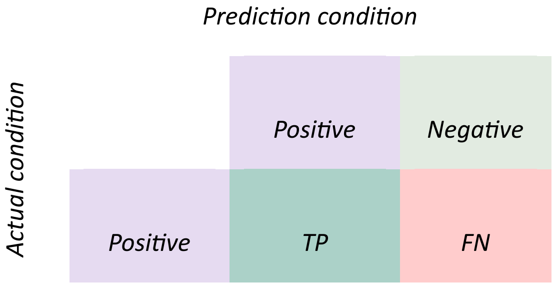
Since SVM uses supervised learning to identify unknown data classes, it needs a training dataset. Assuming dimensionality reduction techniques like PCA can reduce the majority of the training dataset (e.g., >90%) to two dimensions, we can consider a simple scenario. One can plot these data onto a two-dimensional coordinate system. Drawing a line that best divides all data into two classes can be done using known classes of the data (crops under stress or healthy) (Cervantes et al., 2020: 189). When the procedure is applied to three or more data dimensions, the boundary is a plane for three dimensions or a hyperplane for dimensions greater than three. Compared to the raw data's dimensions, which can range widely from hundreds for spectra to millions for images, the number of dimensions needed—roughly ten principal components from PCA—remains significantly smaller. The test data is fed into the same data processing path as the training data and categorised based on the decision boundary that was created during the training process. Non-linear kernels enable non-linear separations even though SVM is a linear algorithm. It is also feasible to classify into multiple classes by employing multiple decision boundaries (Esgario et al., 2020: 1). Data augmentation and image processing should be carried out before the data is sent through the classification algorithm, if they are judged necessary in the data processing path.

Artificial neural networks, or ANNs, are machine learning models that simulate the operation of biological neural networks (Krenker et al., 2011: 1). Artificial neurones that analyse different inputs and weight them based on their significance make up the basic architecture (Sladojevic et al., 2016:1). They then produce comparable outputs. Numerous investigations have effectively employed artificial neural networks (ANNs) to identify and categorise a range of crop stressors. Squash (Pineda et al., 2017: 1), oil palm (Golhani et al., 2019: 639), and wheat (Humpal et al., 2020:1) are a few examples of these, along with the discovery of powdery mildew and soft rot. Utilising ANNs without specialised knowledge of the data and its interpretation is one of their main benefits. Nevertheless, their drawbacks include a propensity for over-fitting and a higher demand on computational power (Tu, 1996:1225). When there is noise in the dataset and it is difficult to identify a clear decision boundary, this becomes especially problematic.

Evaluation criteria

Evaluation metrics are used in machine learning and deep learning techniques to gauge classification performance. A number of evaluation criteria are looked at, including Mean Square Error (MSE), Root Mean Square Error (RMSE), Mean Absolute Error (MAE), Sensitivity, Kappa, and Matthews Correlation Coefficient (MCC). Figure 8 displays the structure of the confusion matrix.

Figure 8 Confusion matrix structure



	Positive	Negative
Positive	TP	FN

Negative	*FP*	*TN*

Here, n number of samples in the dataset, y_i real value (correct label), $\hat{y}_i$ Predicted value (model prediction).

For this special case, assuming that True Negatives (T N), True Positives (T P), False Negatives (F N) and False Positives (F P) values are taken as reference, metrics are calculated with Equation as follows.

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

$$Sensitivity = \frac{TP}{TP + \ FP}$$

$$MCC = \frac{(TP * TN) - (FP * FN)}{\left((TP + FP) * (TP + FN) * (TN + FP) * (TN + FN)\right)^{0.5}}$$

$$po = observed\ fit;$$

$$pe = expected\ fit$$

$$Kappa = \frac{po - pe}{1 - pe}$$

$$Dice = \frac{(2 * \mathrm{TP})}{(\mathrm{TP} + \mathrm{FP} + \mathrm{FN}\ )}$$

$$Jaccard = \frac{dice}{2 - dice}$$

$$MAE\ = \frac{1}{n}\sum_{i=1}^{n}|y_i - \ \hat{y}_i|$$

$$MSE\ = \frac{1}{n}\sum_{i=1}^{n}(y_i - \ \hat{y}_i)^2$$

$$RMSE = \sqrt{MSE}$$

Accuracy is a measure used to evaluate classification models. How well the model can identify positive examples is indicated by precision. The accuracy of a machine learning model's positive predictions is measured by the precision metric, which serves as an indicator of the model's performance. In essence, *Kappa* finds the performance gap between each class and a classifier that estimates frequency at random. An *MCC* score is only high when the prediction performs well across all four categories of the confusion matrix. This statistical ratio is more dependable. *Dice* measures how well the automatic probabilistic segmentation of their images performs and how repeatable the manual segmentations are in terms of spatial overlap. The similarity and diversity of sample sets are measured using the Jaccard algorithm. The *MAE* measures the extent of discrepancy between an observation's true value and its prediction. For a regression model, *RMSE* is one of the two primary performance metrics. It figures out how much a model's predicted values typically differ from the actual values. An estimate of the model's predictive accuracy for the target value is provided. *MSE* is an essential metric for assessing how well predictive models perform. It calculates the average squared difference between a dataset's actual target values and its predicted target values.

Crop ve leaf segmentation from deep learning method

An essential predictor of crop performance in response to salinity and ultimate yield is the accumulation of crop biomass, or fresh weight (FW). In short, by determining the quantitative correlation between FWs and pixel counts, pixels taken from top view images can be transformed into FWs. After the crop segmentation process, the number of pixels from the image area can be used to estimate the actual projected region of okra. The projected area of the crop, which was extracted from the RGB images, and the FW show a strong correlation. The findings indicate that, in cultivation applications, phenotypic measurements of crop biomass production are not the only options available. Since they are dynamic, complex organisms, living crops constantly change during growth in terms of their leaf overlap and leaf angle. The crop segmentation network still faces difficulties with leaf overlap and unequal lighting in digital photos. The significance of sample segmentation in automated crop phenotyping analyses is another reason for the development of the leaf identification technique. A deeper comprehension of the deep learning process is made possible by the feature map's visualisation in intermediate layers. Deep learning is required for the identification of each individual leaf in order to extract the determined leaf spectra from HSI (Feng et al., 2020: 1448).

Conclusions and Future Perspectives

Crop metabolomics has made significant strides in the past 20 years. For the purpose of crop engineering or marker-based breeding to increase yield, quality, and stress resilience, it is assisting in the identification of molecular markers. Lipidomics is one of the omics approaches that are still in their infancy but have a lot of potential to help scientists understand how crops respond to abiotic stresses. There are noticeable variations in lipidomic outcomes between species and abiotic stressors. Lipid signalling and membrane remodelling are suggested by this. The mechanisms underlying lipid catabolism, anabolism, and energy metabolism, however, are the subject of relatively few studies. Moreover, there doesn't appear to be much research on how different abiotic stressors interact with one another or how lipids and crop hormones interact with one another in response to abiotic stressors. Within this framework, the majority of crop lipidomics research has focused on making exploratory modifications to the amounts, ratios, and compositions of lipids. Nevertheless, integrative methods enable a thorough examination of the mechanisms underlying responses in addition to the lipid composition, leading to more profound conclusions. Finding new lipids with abiotic stress-reduction properties is another exciting field.

Machine learning is widely used for data integration in a single study or for the analysis of individual biochemical datasets. It is not, however, currently extensively utilised for extracting and integrating the vast array of scientific data and information that is accessible. Given the rate at which scientific literature and data are being gathered, integrative analyses of this kind will become more and more crucial. By enabling predictive models that are more adaptable to data formats and more adept at capturing non-linear relationships, machine learning techniques hold promise for solving some of these issues. While there are still many obstacles to overcome, deep learning can be successfully used to detect crop stress. Future research should concentrate on deep neural networks that can process 3D images. Many tasks in crop stress detection analysis can be classified, though this approach may not always be the best one because it may necessitate post-processing like segmentation. Furthermore, investigating the use of unsupervised and semi-supervised deep learning in crop stress detection is worthwhile. Still, the majority of research relies on supervised methodologies. One benefit of using unsupervised methods is that they allow network training to proceed without the need for ground truth labels.

References

Abrahamsson D. et al., 2022. “Modeling the transplacental transfer of small molecules using machine learning: a case study on per- and polyfluorinated substances (PFAS)”, *Journal of Exposure Science & Environmental Epidemiology, c. 32, sy 6*, Art. doi: 10.1038/s41370-022-00481-2.

Ahmad, I.S. & Reid, J.F. (1996). Evaluation of colour representations for maize images. *Journal of Agricultural Engineering Research, 63*, 185–196.

Al Bashish, D. Braik, M. & Bani-Ahmad, S. (2010). ;A framework for detection and classification of plant leaf and stem diseases. In Proceedings of the 2010 International Conference on Signal and Image Processing, Chennai, India, 15–17 December pp. 113–118.

Amruthnath, N. & Gupta, T. A research study on unsupervised machine learning algorithms for early fault detection in predictive maintenance. In Proceedings of the 2018 5th International Conference on Industrial Engineering and Applications (ICIEA), Singapore, 26–28 April 2018; pp. 355–361.

Andresen, E. Lyubenova, L. Hubáˇcek, T. Bokhari, S.N.H. Matoušková, Š., Mijovilovich, A., Rohovec, J. & Küpper, H. (2020). Chronic Exposure of Soybean Plants to Nanomolar Cadmium Reveals Specific Additional High-Affinity Targets of Cadmium Toxicity. *Journal of Experimental Botany, 71*, 1628–1644.

Aslan, N., Koca, G. O., Kobat, M. A., & Dogan, S. (2022). Multi-classification deep CNN model for diagnosing COVID-19 using iterative neighborhood component analysis and iterative ReliefF feature selection techniques with X-ray images. *Chemometrics and Intelligent Laboratory Systems, 224,* 104539.

Au, B. 2012. Building on ILSVRC2012-Winning AlexNet for Tiny ImageNet.

Basu, S., Kumari, S., Kumar, P., Kumar, G. & Rajwanshi, R. (2021). Redox Imbalance Impedes Photosynthetic Activity in Rice by Disrupting Cellular Membrane Integrity and Induces Programmed Cell Death under Submergence. *Physiologia Plantarum, 172*, 1764–1778.

Bian, Y., Li, Q., Zhang, X., Hao, T., Liu, N., Yang, Z. & Yu, J. (2023). Lipid Composition Remodeling Plays a Critical Role during the Differential Responses of Leaves and Roots to Heat Stress in Bermudagrass. Environmental and Experimental Botany*, 213*, 105423.

Bindushree, H.B. & Sivasankari, G.G. (2015). Application of Image Processing Techniques for Plant Leaf Disease Detection. International Journal of Engineering Research & Technology *(IJERT)*, 3, 19.

Bolon-Canedo, V., Sanchez-Marono, N., AlonsoBetanzos, A., Benítez, J.M. & Herrera, F. (2014). A review of microarray datasets and applied feature selection methods, *Information Sciences, 282,* 111–135.

Brahimi, M., Boukhalfa, K. & Moussaoui, A. (2017). Deep Learning for Tomato Diseases: Classification and Symptoms Visualization. Applied Artifical Intelligence*, 31, 299–315*.

Burgos, A., Szymanski, J., Seiwert, B., Degenkolbe, T., Hannah, M.A., Giavalisco, P. & Willmitzer, L. (2011). Analysis of Short-Term Changes in the *Arabidopsis Thaliana* Glycerolipidome in Response to Temperature and Light. *The Plant Journal, 66, 656–668.*

Carrington, Y., Guo, J., Le, C.H., Fillo, A., Kwon, J., Tran, L.T. & Ehlting, J. (2018). Evolution of a secondary metabolic pathway from primary metabolism: Shikimate and quinate biosynthesis in plants. *The Plant Journal, 95, 823–833*.

Cervantes, J., Garcia-Lamont, F., Rodríguez-Mazahua, L. & Lopez, A. (2020). A comprehensive survey on support vector machine classification: Applications, challenges and trends. *Neurocomputing, 408, 189–215*.

Chen, C.F., Lee, G.G., Sritapan, V. & Lin, C.Y. Deep convolutional neural network on iOS mobile devices. In Proceedings of the 2016 IEEE InternationalWorkshop on Signal Processing Systems (SiPS), Dallas, TX, USA, 26–28 October 2016; pp. 130–135.

Christie, W.W., 1990. Gas chromatography and lipids. The Oıl Pres, Glaskow.

Couprie, C., Farabet, C., Najman, L. & LeCun, Y. Indoor semantic segmentation using depth information. arXiv 2013, arXiv:1301.3572.

Crossa, J., Pe´ rez-Rodrı´guez, P., Cuevas, J., Montesinos-Lo´ pez, O., Jarquı´n, D., de Los Campos, G., Burguen˜ o, J., Gonza´ lez-Camacho, J.M., Pe´ rez-Elizalde, S., Beyene, Y. & Dreisigacker, S., et al. (2017). Genomic selection in plant breeding: methods, models, and Perspectives. *Trends Plant Science, 22, 961–975.*

Da'u A. & Salim, N. "Recommendation system based on deep learning methods: a systematic review and new directions", *Artifical Intelligence Review, c. 53, sy 4, ss. 2709-2748*, Nis. 2020, doi: 10.1007/s10462-019-09744-1.

de Bang, T.C., Husted, S., Laursen, K.H., Persson, D.P., & Schjoerring, J.K. (2021). The Molecular–Physiological Functions of Mineral Macronutrients and Their Consequences for Deficiency Symptoms in Plants. New Phytologist, 229, 2446–2469.

Devi, S.R. & Prasad, M.N.V. Membrane Lipid Alterations in Heavy Metal Exposed Plants. In Heavy Metal Stress in Plants; Springer: Berlin/Heidelberg, Germany, 1999.

Dhaliwal, L.K., Shim, J., Auld, D., Angeles-Shim, R.B. (2024). Fatty Acid Unsaturation Improves Germination of Upland Cotton (Gossypium Hirsutum) under Cold Stress. *Frontiers in Plant Science, 15, 1286908.*

Elahi, M., Afolaranmi, S.O., Martinez Lastra, J.L. et al. (2023). A comprehensive literature review of the applications of AI techniques through the lifecycle of industrial equipment. *Discover Artifical Intelligence, 3, 43*. https://doi.org/10.1007/s44163-023-00089-x.

Esgario, J.G.M., Krohling, R.A. & Ventura, J.A. (2020). Deep learning for classification and severity estimation of coffee leaf biotic stress. *Computers Electronics Agriculture, 169, 105162.*

Faizan, M., Alam, P., Kumari, A., Suresh, G., Sharma, P., Karabulut, F., ... & Hayat, S. (2024). Unraveling the nano-biochar mediated regulation of heavy metal stress tolerance for sustaining plant health. Plant Stress, 100615 (a)

Faizan, M., Karabulut, F. & Alam, P. et al. (2024). Silicon Nutrition: A New Perspective to Improve Heavy Metal Toxicity Tolerance in Plants for *Sustainable Agriculture. Silicon 16, 3191–3203* https://doi.org/10.1007/s12633-024-02937-6 (b)

Fang, C.; Fernie, A.R.; Luo, J. Exploring the diversity of plant metabolism. Trends Plant Sci. 2019, 24, 83–98.

Feng, X., Zhan, Y., Wang, Q., Yang, X., Yu, C., Wang, H., ... & He, Y. (2020). Hyperspectral imaging combined with machine learning as a tool to obtain high-throughput plant salt-stress phenotyping. *The Plant Journal, 101(6), 1448-1461.*

Ferentinos, K.P. (2018). Deep learning models for plant disease detection and diagnosis. *Computers Electronics Agriculture, 145, 311–318.*

Figueira, E., Matos, D., Cardoso, P., Pires, A., Fernandes, C., Tauler, R. & Bedia, C. A. (2023). Biochemical and Lipidomic Approach to Perceive *Halimione portulacoides* (L.) Response to Mercury: An Environmental Perspective. *Marine Pollution Bulletin, 186, 114393.*

Fukunaga K. & Narendra, P. M. “A Branch and Bound Algorithm for Computing k-Nearest Neighbors”, IEEE Transactions on Computer, c. C-24, sy 7, ss. 750-753, Tem. 1975, doi: 10.1109/T-C.1975.224297.

Galieni, A., D'Ascenzo, N., Stagnari, F., Pagnani, G., Xie, Q., & Pisante, M. (2021). Past and future of plant stress detection: an overview from remote sensing to positron emission tomography. *Frontiers in Plant Science, 11, 609155.*

Gao, L., Jiang, H., Li, M., Wang, D., Xiang, H., Zeng, R., Chen, L., Zhang, X., Zuo, J. & Yang, S.; et al. (2024). Genetic and Lipidomic Analyses Reveal the Key Role of Lipid Metabolism for

Cold Tolerance in Maize. *Journal of Genetics and Genomics, 51, 326–337.*

Gerhards, M., Schlerf, M., Mallick, K., & Udelhoven, T. (2019). Challenges and future perspectives of multi-/hyperspectral thermal infrared remote sensing for crop water-stress detection: a review. *Remote Sensing. 11:1240*. doi: 10.3390/rs11101240.

Ghosal, S., Blystone, D., Singh, A.K., Ganapathysubramanian, B., Singh, A. & Sarkar, S. (2018). An explainable deep machine vision framework for plant stress phenotyping. *Proceedings of the National Academy of Sciences, USA, 115, 4613–4618.*

Golhani, K., Balasundram, S.K., Vadamalai, G. & Pradhan, B. (2019). Selection of a Spectral Index for Detection of Orange Spotting Disease in Oil Palm (*Elaeis guineensis* Jacq.) Using Red Edge and Neural Network Techniques. *Journal Indian Society of Remote Sensing, 47, 639–646.*

Han, X. & Gross, R.W. (2022). The Foundations and Development of Lipidomics. *J. Lipid Res., 63,* 100164.

Harrington, P. (2012). Machine Learning in Action; Manning Publications: Greenwich, CT, USA,; p. 384.

Hassan, M.J., Qi, H., Cheng, B., Hussain, S., Peng, Y., Liu,W., Feng, G., Zhao, J. & Li, Z. (2022). Enhanced Adaptability to Limited Water Supply Regulated by Diethyl Aminoethyl Hexanoate (DA-6) Associated With Lipidomic Reprogramming in Two White Clover Genotypes. *Frontiers in Plant Science, 13, 879331.*

Heavisides, E., Rouger, C., Reichel, A., Ulrich, C. Wenzel-Storjohann, A., Sebens, S. & Tasdemir, D. (2018). Seasonal variations in the metabolome and bioactivity profile of Fucus vesiculosus extracted by an optimized, pressurized liquid extraction protocol. *Marine Drugs 16, 503.*

Helm, L.T., Shi, H., Lerdau, M.T. & Yang, X. (2020). Solar-Induced Chlorophyll Fluorescence and Short-Term Photosynthetic Response to Drought. *Ecological Applications, 30, e02101.*

Henschel, J.M., Dias, T.J., de Moura, V.S., de Oliveira Silva, A.M., Lopes, A.S., da Silva Gomes, D., Araujo, D.J., Silva, J.B.M.; da Cruz, O.N., & Batista, D.S. (2024). Hydrogen Peroxide and Salt Stress in Radish: Effects on Growth, Physiology, and Root Quality. Physiology and Molecular *Biology of Plants.*

Higashi, Y. & Saito, K. (2019). Lipidomic Studies of Membrane Glycerolipids in Plant Leaves under Heat Stress. Progress in Lipid Research, 75, 100990.

Honda, S., Yamazaki, Y., Mukada, T., Cheng, W., Chuba, M., Okazaki, Y., Saito, K., Oikawa, A.; Maruyama, H. & Wasaki, J.; et al. (2023). Lipidome Profiling of Phosphorus Deficiency-Tolerant Rice Cultivars Reveals Remodeling of Membrane Lipids as a Mechanism of Low P Tolerance. *Plants, 12, 1365.*

Humpal, J., McCarthy, C., Percy, C., & Thomasson, J.A. Detection of crown rot in wheat utilising near-infrared spectroscopy: Towards remote and robotic sensing. In Proceedings of the Autonomous Air and Ground Sensing Systems for Agricultural Optimization and Phenotyping V, Online, 27 April–8 May 2020.

Inc, S. 2007. SPSS Clementine 12.0 Algorithms Guide, SPSS Inc, Chicago.

Jin, K.H., McCann, M.T., Froustey, E. & Unser, M. (2017). Deep Convolutional Neural Network for Inverse Problems in Imaging. IEEE Transactions on Image Processing, *26, 4509–4522.*

Jin, M. & Deng, W. (2018). “Predication of different stages of Alzheimer’s disease using neighborhood component analysis and ensemble decision tree”, *Journal of Neuroscience Methods, c. 302, ss. 35-41*, doi: 10.1016/j.jneumeth.2018.02.014.

Jones, H. & Schofield, P. (2008). Thermal and other remote sensing of plant stress. *General and Applied Plant Physiology, 34, 19–32*.

Kang, M., Shin, S., Zhang, G., Jung, J. & Kim, Y. T. (2021). “Mental Stress Classification Based on a Support Vector Machine and Naive Bayes Using Electrocardiogram Signals”, *Sensors, c. 21, sy 23, Art. sy 23*, doi: 10.3390/s21237916.

Karabulut N. & Ozmen Koca, G. (2020). “Wave height prediction with single input parameter by using regression methods”, *Energy Sources Part Recovery Utillization Environmental Effcet, c. 42, sy 24, ss. 2972-2989*, doi: 10.1080/15567036.2020.1733711.

Kargiotidou, A., Deli, D., Galanopoulou, D. Tsaftaris, A. & Farmaki, T. (2008). Low Temperature and Light Regulate Delta 12 Fatty Acid Desaturases (FAD2) at a Transcriptional Level in Cotton (*Gossypium hirsutum*). *Journal Experimental Botany*, *59, 2043–2056*.

Kehelpannala, C., Rupasinghe, T., Hennessy, T., Bradley, D., Ebert, B. & Roessner, U. (2021). The State of the Art in Plant Lipidomics. *Molecular Omics, 17, 894–910*.

Kim, J.S., Kidokoro, S., Yamaguchi-Shinozaki, K., Shinozaki, K. (2024). Regulatory Networks in Plant Responses to Drought and Cold Stress. *Plant Physiology, 195, 170–189*.

Kior, A., Sukhov, V. & Sukhova, E. (2021). Application of reflectance indices for remote sensing of plants and revealing actions of stressors. *Photonics, 8, 582*.

Kollist, H., Zandalinas, S. I., Sengupta, S., Nuhkat, M., Kangasjarvi, J., & Mittler, R. (2019). Rapid responses to abiotic stress: Priming the landscape for the signal transduction network. *Trends Plant Science, 24, 25–37*. doi: 10.1016/j.tplants.2018.10.003.

Korte, P., Unzner, A., Damm, T., Berger, S., Krischke, M. & Mueller, M.J. (2023). High Triacylglycerol Turnover Is Required for Efficient Opening of Stomata during Heat Stress in Arabidopsis. *Plant Journal, 115, 81–96*.

Koza, J.R., Bennett, F.H., Andre, D. & Keane, M.A. (1996). Automated design of both the topology and sizing of analog electrical circuits using genetic programming. In Artificial Intelligence in Design'96; Springer: Dordrecht, *The Netherlands,; pp. 151–170.*

Krenker, A., Bester, J. & Kos, A. (2011). Introduction to the Artificial Neural Networks. In Artificial Neural Networks—Methodological Advances and Biomedical Applications; Suzuki, K., Ed.; InTech: Rijeka, Croatia,; ISBN 978-953-307-243-2.

Kulak, M. (2020). Recurrent Drought Stress Effects on Essential Oil Profile of Lamiaceae Plants: An Approach Regarding Stress Memory. *Industrial Crops and Products, 154, 112695*.

Kuska, M. T., & Mahlein, A. K. (2018). Aiming at decision making in plant disease protection and phenotyping by the use of optical sensors. *European Journal of Plant Pathology, 154, 987–992*. doi: 10.1007/s10658-018-1464-1.

Lavergne, F.D., Broeckling, C.D., Cockrell, D.M., Haley, S.D., Peairs, F.B., Jahn, C.E. & Heuberger, A.L. (2018). GC-MS Metabolomics to Evaluate the Composition of Plant Cuticular Waxes for Four Triticum aestivum Cultivars. *International journal of molecular sciences, 9, 249*.

LeCun, Y., Bengio, Y. & Hinton, G. (2015). Deep learning. *Nature, 521, 436–444*.

Lei, L., Yang, Q., Yang, L. et al. (2024). Deep learning implementation of image segmentation in agricultural applications:

a comprehensive review. *Artificial Intelligence Review, 57, 149* https://doi.org/10.1007/s10462-024-10775-6.

Lever, J., Krzywinski, M. & Altman, N. (2017). Principal component analysis. *Nature Methods, 14, 641–642*.

Li, H., Zhao, R. & Wang, X. (2014). Highly efficient forward and backward propagation of convolutional neural networks for pixelwise classification. arXiv, arXiv:1412.4526.

Li, S., Liu, X., Yin, L., Wang, S. & Deng, X. (2024). Alteration in Lipid Metabolism Is Involved in Nitrogen Deficiency Response in Wheat Seedlings. *Plant Physiology and Biochemistry, 214, 108883*.

Li, Z., Cheng, B., Zhao, Y., Luo, L., Zhang, Y., Feng, G., Han, L., Peng, Y. & Zhang, X. (2022). Metabolic Regulation and Lipidomic Remodeling in Relation to Spermidine-Induced Stress Tolerance to High Temperature in Plants. *International Journal of Molecular Sciences, 23, 12247*.

Liang, Y., Huang, Y., Liu, C., Chen, K. & Li, M. (2023). Functions and Interaction of Plant Lipid Signalling under Abiotic Stresses. *Plant Biology, 25,* 361–378.

Littlejohn, G.R., Breen, S., Smirnoff, N. & Grant, M. (2021). Chloroplast immunity illuminated. *New Phytologist, 229,* 3088–3107.

Lu, H., Wang, Z., Xu, C., Li, L. & Yang, C. (2021). Multiomics Analysis Provides Insights into Alkali Stress Tolerance of Sunflower (*Helianthus annuus* L.). *Plant Physiology and Biochemistry, 166*, 66–77.

Lu, J., Hu, J., Zhao, G., Mei, F. & Zhang, C. (2017). An in-field automatic wheat disease diagnosis system. *Computer Electronics Agricultre, 142,* 369–379.

Lu, Y., Yi, S., Zeng, N., Liu, Y. & Zhang, Y. (2017). Identification of rice diseases using deep convolutional neural networks. *Neurocomputing, 267,* 378–384.

Ma, J., Du, K., Zheng, F., Zhang, L., Gong, Z. & Sun, Z. A (2018). Recognition method for cucumber diseases using leaf symptom images based on deep convolutional neural network. *Computer Electronic Agricultre, 154*, 18–24.

Mallat, S. (2016). Understanding deep convolutional networks. Philosophical Transactions A Math. Phys. Eng. Sci., 374, 20150203.

Mishra, P., Polder, G., & Vilfan, N. (2020). Close range spectral imaging for disease detection in plants using autonomous platforms: a review on recent studies. *Current Robotic Reports 1,* 43–48. doi: 10.1007/s43154-020-00004-7.

Mohanty, S.P., Hughes, D.P. (2016). Salathé, M. Using Deep Learning for Image-Based Plant Disease Detection. *Frontiers Plant Science, 7, 1419.*

Moradi, P., Mahdavi, A., Khoshkam, M. & Iriti, M. (2017). Lipidomics Unravels the Role of Leaf Lipids in Thyme Plant Response to Drought Stress. *International Journal Molecular Sciences, 18*, 2067.

Moraes, R., Valiati, J. F. and Gavião Neto, W. P. (2013). “Document-level sentiment classification: An empirical comparison between SVM and ANN”, *Expert System Application, c. 40, sy 2, ss.* 621-633, doi: 10.1016/j.eswa.2012.07.059.

Narayan, Y. (2021). “Comparative analysis of SVM and Naive Bayes classifier for the SEMG signal classification”, Materiyals Today Proceedings, c. 37, ss. 3241-3245, doi: 10.1016/j.matpr.2020.09.093.

Nevlud, P., Bures, M., Kapicak, L. & Zdralek, J. (2013). "Anomaly-based Network Intrusion Detection Methods", Advances in *Electrical and Electronic Engineering, c. 11, sy 6, Art. Sy 6,* doi: 10.15598/aeee.v11i6.877.

Nnamoko, N. A., Arshad, F. N., England, D. & Vora, J. (2014). "Meta-classification model for diabetes onset forecast: A proof of concept", IEEE International Conference on Bioinformatics and *Biomedicine (BIBM),* 2014, *ss. 50-56*. doi: 10.1109/BIBM.2014.6999247.

Ojala, T., Pietikainen, M. & Maenpaa, T. (2002). Multiresolution gray-scale and rotation invariant texture classification with local binary patterns. IEEE *Transactions on Pattern Analysis Machine Intelligence, 24,* 971–987.

Omia E, Bae H, Park E, Kim MS, Baek I, Kabenge I, Cho B-K. (2023). Remote Sensing in Field Crop Monitoring: A Comprehensive Review of Sensor Systems, Data Analyses and Recent Advances. *Remote Sensing. 15(2):*354. https://doi.org/10.3390/rs15020354.

Peng, Y., Lou, H., Tan, Z., Ouyang, Z., Zhang, Y., Lu, S., Guo, L. & Yang, B. (2023). Lipidomic and Metabolomic Analyses Reveal Changes of Lipid and Metabolite Profiles in Rapeseed during Nitrogen Deficiency. *Plant Cell Physiology, 65,* 904–915.

Pfaff, J., Denton, A.K., Usadel, B. & Pfaff, C. (2020). Phosphate Starvation Causes Different Stress Responses in the Lipid Metabolism of Tomato Leaves and Roots. *Biochim. Biophys. Acta Molecular and Cell Biology of Lipids, 1865*, 158763.

Pineda, M., Pérez-Bueno, M.L., Paredes, V. & Barón, M. (2017). Use of multicolour fluorescence imaging for diagnosis of bacterial and fungal infection on zucchini by implementing machine learning. *Functional Plant Biology, 44*, 563.

Qayyum, A., Malik, A.S., Saad, N.M. , Iqbal, M., Faris Abdullah, M., Rasheed, W. & Bin Jafaar, M.Y. (2017). Scene classification for aerial images based on CNN using sparse coding technique. *International Journal of Remote Sensing, 38,* 2662–2685.

Rabeh, K., Sbabou, L., Rachidi, F., Ferradouss, A., Laghmari, G., Aasfar, A., El Arroussi, H., Ouajdi, M., El Antry, S., Belkadi, B., et al. (2023). Lipidomic Profiling of *Argania spinosa* L. (Skeels) Following Drought Stress. *Applied Biochemistry and Biotechnology, 195*, 1781–1799.

Raghu, S. & Sriraam, N. (2018). “Classification of focal and non-focal EEG signals using neighborhood component analysis and machine learning algorithms”, Expert *System Application, c. 113*, ss. 18-32, doi: 10.1016/j.eswa.2018.06.031.

Ramos-Giraldo, P., Reberg-Horton, C., Locke, A.M., Mirsky, S. & Lobaton, E. (2020). Drought Stress Detection Using Low-Cost Computer Vision Systems and Machine Learning Techniques. *IT Prof., 22*, 27–29.

Rao, Y., Peng, T. & Xue, S. (2023). Mechanisms of Plant Saline-Alkaline Tolerance. *Journal of Plant Physiology, 281*, 153916.

Rawat, N., Singla-Pareek, S.L. & Pareek, A. (2021). Membrane Dynamics during Individual and Combined Abiotic Stresses in Plants and Tools to Study the Same. *Physiologia Plantarum, 171*, 653–676.

Razzaq, A., Sadia, B., Raza, A., Khalid Hameed, M. & Saleem, F. (2019). Metabolomics: A Way Forward for Crop Improvement. *Metabolites, 9,* 303.

Riyazuddin, R., Nisha, N., Singh, K., Verma, R. & Gupta, R. (2022). Involvement of Dehydrin Proteins in Mitigating the

Negative Effects of Drought Stress in Plants. *Plant Cell Reports, 41*, 519–533.

Rodriguez, M.Z., Comin, C.H., Casanova, D., Bruno, O.M., Amancio, D.R., da Costa, L.F. & Rodrigues, F.A. (2019). Clustering algorithms: A comparative approach. *PLoS ONE*, *14*, e0210236.

Rumpf, T., Mahlein, A.-K., Steiner, U., Oerke, E.-C., Dehne, H.-W. & Plümer, L. (2010). Early detection and classification of plant diseases with Support Vector Machines based on hyperspectral reflectance. *Computers and Electronics in Agriculture, 74*, 91–99.

Saha, D., & Manickavasagan, A. (2021). Machine learning techniques for analysis of hyperspectral images to determine quality of food products: A review. Current Research in Food Science, 4, 28-44.

Salama, K.H.A., Mansour, M.M.F., Ali, F.Z.M., & Abou-Hadid, A.F. (2007). NaCl-Induced Changes in Plasma Membrane Lipids and Proteins of *Zea mays* L. Cultivars Differing in Their Response to Salinity. *Acta Physiologiae Plantarum*, *29*, 351–359.

Saleem, M.H., Potgieter, J. & Mahmood Arif, K. (2019). Plant Disease Detection and Classification by Deep Learning. *Plants, 8*, 468.

Sato, H., Mizoi, J., Shinozaki, K. & Yamaguchi-Shinozaki, K. (2024). Complex Plant Responses to Drought and Heat Stress under Climate Change. *Plant Journal, 117*, 1873–1892.

Shang, R., He, J., Wang, J., Xu, K., Jiao, L. & Stolkin, R. (2020). “Dense connection and depthwise separable convolution based CNN for polarimetric SAR image classification”, *Knowledge-Based Systems, c. 194*, s. 105542, doi: 10.1016/j.knosys.2020.105542.

Shao, D., Yu, C., Chen, Y., Qiu, X., Chen, J., Zhao, H., Chen, K., Wang, X., Chen, P., Gao, G. et al. (2024). Lipids Signaling and

Unsaturation of Fatty Acids Participate in Ramie Response to Submergence Stress and Hypoxia-Responsive Gene Regulation. *International Journal Biological Macromolecules, 263*, 130104.

Sharma, P., Lakra, N., Goyal, A., Ahlawat, Y.K., Zaid, A. & Siddique, K.H.M. (2023). Drought and Heat Stress Mediated Activation of Lipid Signaling in Plants: A Critical Review. *Frontiers in Plant Science, 14*, 1216835.

Shen, J., Wang, S., Sun, L., Wang, Y., Fan, K., Li, C., Wang, H., Bi, C., Zhang, F. & Ding, Z. (2022). Dynamic Changes in Metabolic and Lipidomic Profiles of Tea Plants during Drought Stress and Re-Watering. *Frontiers in Plant Science, 13*, 978531.

Simonyan, K. & Zisserman, A. (2014). Very deep convolutional networks for large-scale image recognition. arXiv, arXiv:1409.1556.

Singh, A., Ganapathysubramanian, B., Singh, A.K. & Sarkar, S. (2016). Machine Learning for High-Throughput Stress Phenotyping in Plants. *Trends Plant Sci*ence, *21*, 110–124.

Sladojevic, S., Arsenovic, M., Anderla, A., Culibrk, D. & Stefanovic, D. (2016). Deep Neural Networks Based Recognition of Plant Diseases by Leaf Image Classification. *Computational Intelligence and Neuroscience*, 2016, 1–11.

Srivastava, N., Mansimov, E. & Salakhudinov, R. Unsupervised learning of video representations using lstms. In Proceedings of the International Conference on Machine Learning, Lille, France, 6–11 July 2015; pp. 843–852.

Sugiyama, M. (2007). Dimensionality reduction of multimodal labeled data by local fisher discriminant analysis. *Journal of machine learning research, 8(5).*

Suthaharan, S. (2016). “Support Vector Machine”, içinde Machine Learning Models and Algorithms for Big Data

Classification: Thinking with Examples for Effective Learning, S. Suthaharan, Ed., içinde Integrated Series in Information Systems. Boston, MA: Springer US, ss. 207-235. doi: 10.1007/978-1-4899-7641-3_9.

Szegedy, C., Vanhoucke, V., Ioffe, S., Shlens, J. & Wojna, Z. Rethinking the inception architecture for computer vision. In Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition, Las Vegas, NV, USA, 26 Jun–1 July 2016; pp. 2818–2826.

Taye MM. (2023). Theoretical Understanding of Convolutional Neural Network: Concepts, Architectures, Applications, Future Directions. *Computation. 11(3):52.* https://doi.org/10.3390/computation11030052.

Terrer, C., Jackson, R.B., Prentice, I.C., Keenan, T.F., Kaiser, C., Vicca, S., Fisher, J.B., Reich, P.B., Stocker, B.D., Hungate, B.A. et al. (2019). Nitrogen and Phosphorus Constrain the CO2 Fertilization of Global Plant Biomass. *Nature. Climate Change, 9*, 684–689.

Thomas, S., Behmann, J., Steier, A., Kraska, T., Muller, O., & Rascher, U. (2018). Quantitative assessment of disease severity and rating of barley cultivars based on hyperspectral imaging in a non-invasive, automated phenotyping platform. *Plant Methods 14, 1–12*. doi: 10.1186/s13007-018-0313-8.

Tran, H. M., Van Nguyen, S., Le, S. T. & Vu, Q. T. (2015) “Fault Data Analytics Using Decision Tree for Fault Detection”, içinde Future Data and Security Engineering, T. K. Dang, R. Wagner, J. Küng, N. Thoai, M. Takizawa, ve E. Neuhold, Ed., içinde Lecture Notes in Computer Science. Cham: Springer International Publishing, ss. 57-71. doi: 10.1007/978-3-319-26135-5_5.

Tsaftaris, S.A., Minervini, M. & Scharr, H. (2016). Machine Learning for Plant Phenotyping Needs Image Processing. *Trends Plant Science, 21*, 989–991.

Tu, J.V. (1996). Advantages and disadvantages of using artificial neural networks versus logistic regression for predicting medical outcomes. *Journal of Clinical Epidemiology, 49*, 1225–1231.

Unver, Ö. & Gamgam, H. (2006). Applied Basic Statistical Methods, Seçkin Publishing, Ankara.

Vatamanu, O.A., Frandes, M., Ionescu, M. & Apostol, S. Content-Based Image Retrieval using Local Binary Pattern, Intensity Histogram and Color Coherence Vector. In Proceedings of the 2013 E-Health and Bioengineering Conference (EHB), Iasi, Romania, 21–23 November 2013; pp. 1–6.

Viant, M.R. & Sommer, U. (2013). Mass spectrometry based environmental metabolomics: A primer and review. *Metabolomics, 9*, 144–158.

Wang, H., Zhao, S., Qi, Z., Yang, C., Ding, D., Xiao, B., Wang, S. & Yang, C. (2024). Regulation of Root Exudation in Wheat Plants in Response to Alkali Stress. *Plants, 13*, 1227.

Wold, S., Esbensen, K. & Geladi, P. (1987). Principal Component Analysis. *Chemometrics and Intelligent Laboratory Systems, 2*, 37–52.

Xie, L.J., Zhou, Y., Chen, Q.F. & Xiao, S. (2021). New Insights into the Role of Lipids in Plant Hypoxia Responses. *Progress in Lipid Research, 81*, 101072.

Xu, L., Pan, R. & Zhang, W. (2020). Membrane Lipids Are Involved in Plant Response to Oxygen Deprivation. *Plant Signaling & Behavior, 15*, 1771938.

Xu, X., Zhang, J., Yan, B., Wei, Y., Ge, S., Li, J., Han, Y., Li, Z., Zhao, C. & Xu, J. (2021). The Adjustment of Membrane Lipid Metabolism Pathways in Maize Roots Under Saline–Alkaline Stress. *Frontiers in Plant Science 12*, 635327.

Yang, C., Hou, B., Ren, B., Hu, Y. & Jiao, L. (2019). "CNN-Based Polarimetric Decomposition Feature Selection for PolSAR Image Classification", *IEEE Transactions on Geoscience and Remote Sensing, 57(11)*, 8796-8812, doi: 10.1109/TGRS.2019.2922978.

Yıldız, O., Tez, M., Bilge, H.Ş., Akcayol, M.A. & Güler, İ. (2012). Gene Selection for Breast Cancer Classification, IEEE 20th Signal Processing and Communication Applications Conference, 18-20 April, Muğla.

You, Z., Zhang, Q., Peng, Z. & Miao, X. (2019). Lipid Droplets Mediate Salt Stress Tolerance in Parachlorella kessleri. *Plant Physiology, 181*, 510–526.

Yue, J., Li, Z., Liu, L. & Fu, Z. (2011). Content-based image retrieval using color and texture fused features. *Mathematical and Computer Modelling, 54*, 1121–1127.

Zhang, F.Q., Wang, Y.S., Lou, Z.P. & Dong, J. De (2007). Effect of Heavy Metal Stress on Antioxidative Enzymes and Lipid Peroxidation in Leaves and Roots of Two Mangrove Plant Seedlings (Kandelia candel and Bruguiera gymnorrhiza). *Chemosphere, 67*, 44–50.

Zhang, K., Wu, Q., Liu, A. & Meng, X. (2018). Can Deep Learning Identify Tomato Leaf Disease? *Advances in Multimedia., 1–10.*

Zhang, X., Gao, Y., Zhuang, L. & Huang, B. (2023). Phosphatidic Acid Priming-Enhanced Heat Tolerance in Tall Fescue (*Festuca arundinacea*) Involves Lipidomic Reprogramming of

Lipids for Membrane Stability and Stress Signaling. *Plant Growth Regulation, 99*, 527–538.

Zhang, X., Ni, Y., Xu, D., Busta, L., Xiao, Y., Jetter, R. & Guo, Y. (2021). Integrative Analysis of the Cuticular Lipidome and Transcriptome of Sorghum Bicolor Reveals Cultivar Differences in Drought Tolerance. *Plant Physiology Biochemistry, 163*, 285–295.

Zhao, S., Zhang, Q., Liu, M., Zhou, H., Ma, C. & Wang, P. (2021). Regulation of Plant Responses to Salt Stress. *International Journal of Molecular Sciences*, *22*, 4609.

Zhao, X., Wei, Y., Zhang, J., Yang, L., Liu, X., Zhang, H.; Shao, W., He, L., Li, Z., Zhang, Y. et al. (2021). Membrane Lipids' Metabolism and Transcriptional Regulation in Maize Roots Under Cold Stress. *Frontiers in Plant Science, 12*, 639132.

Zhao, Y., Li, S., Wu, J., Liu, H., Wang, P. & Xu, L. (2024). Insights into Membrane Lipids Modification in Barley Leaves as an Adaptation Mechanism to Cold Stress. *Plant Growth Regulation, 103*, 369–388.

Zhou, Z.-H. (2018). A brief introduction to weakly supervised learning. *National Science Review, 5*, 44–53.

BÖLÜM 0

TOXIC EFFECTS OF CADMIUM ON HUMAN ERYTHROCYTES AND ANTIOXIDATIVE ROLE OF *HYPERICUM PERFORATUM* OIL

YELİZ ÇAKIR SAHİLLİ[1]

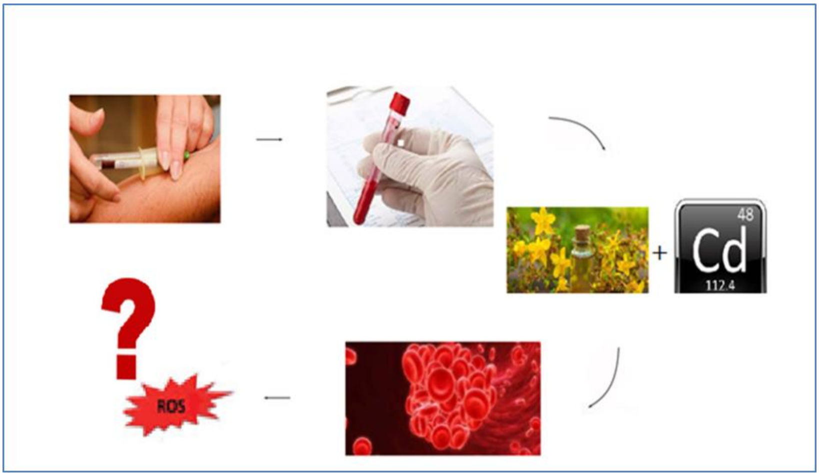
Abstract

Hypericum perforatum L. (St. John's Wort) is a widely used medicinal plant in both traditional and modern phytotherapy, known for its pharmacologically active constituents such as hyperforin, adhyperforin, and hypericin. These compounds exhibit antioxidant, antidepressant, and cytoprotective effects. Cadmium (Cd) is a toxic heavy metal that contributes significantly to environmental pollution and induces oxidative stress in cells by increasing reactive oxygen species (ROS). This study aimed to investigate the potential protective effects of *Hypericum perforatum* oil (HPO) against Cd-induced oxidative stress in human erythrocytes. Erythrocyte suspensions were prepared from blood samples obtained from healthy volunteers and divided into four experimental groups: Control, Cd, HPO, and Cd + HPO. The levels of superoxide dismutase (SOD), malondialdehyde (MDA), and

---

[1] Doç. Dr., Munzur Üniversitesi, Tıbbi Hizmetler ve Teknikler Bölümü, Orcid No: 0000-0003-1905-5506

catalase (CAT) were measured in each group. MDA levels, which increased with Cd exposure, were significantly reduced by HPO treatment (Cd: 64.7 ± 15.2 nmol/gHb; Cd + HPO: 51.9 ± 21.3 nmol/gHb). Furthermore, HPO administration significantly enhanced the activities of SOD and CAT enzymes, which had decreased due to Cd exposure (SOD: Cd: 3142 ± 221.2; Cd + HPO: 3618 ± 253.6 U/gHb; CAT: Cd: 204.2 ± 226.4; Cd + HPO: 278.6 ± 66.2 U/gHb). These results suggest that *Hypericum perforatum* oil may exert protective effects against cadmium-induced oxidative damage by enhancing antioxidant enzyme activity and reducing lipid peroxidation in erythrocytes.



Introduction

Hypericum perforatum L. is a perennial herbaceous plant that grows naturally in temperate regions of Eurasia, Africa, and the Americas. Traditionally, it has been used to treat various ailments using its flowering tops and aerial parts in the form of tea, oil macerates, or alcoholic and hydroalcoholic extracts (Sõukand & Kalle, 2013: 170). One of the most well-documented medicinal uses in scientific literature is its effectiveness in treating mild to moderate depression, particularly in outpatient settings, with minimal side effects. It is also widely used for treating infections, reducing inflammation, and promoting wound healing (Zhang et al., 2020: 254). Preclinical studies have further shown its potential as an antinociceptive (pain-relieving) agent (Boga et al., 2021: 338). Additionally, it is considered a valuable component in cosmeceuticals due to its in vitro anti-inflammatory properties, its ability to inhibit enzymes such as tyrosinase, elastase, and collagenase, and its wound healing potential (Silva et al., 2021: 159).

The most commonly identified bioactive compounds specific to Hypericum species include phloroglucinol derivatives such as hyperforin and adhyperforin, the naphthodianthrone hypericin, the flavonoid hyperoside, and various xanthone derivatives (Zhang et al., 2020: 254). *Hypericum perforatum* oil (HPO), a preparation derived from this species, belongs to the genus Hypericum, family Clusiaceae, and subfamily Hypericoideae. It grows in various regions of Turkey and has been used for centuries in traditional medicine to treat stomach pain, neurological disorders, and wounds. Recently, it has gained increasing attention in both clinical research and modern phytotherapy (Ekren, 2010: 230). Additionally, H. perforatum has demonstrated significant antioxidant activity in vitro (Sánchez-Reus et al., 2007: 610).

While oxygen is essential for life, certain ROS that are by products of cellular metabolism can cause severe damage to cells and tissues (Zhou et al., 2014: 35; Pizzino et al.,2017: 8416763). Compared to molecular oxygen, ROS are chemically more reactive (Nawar, 1996: 228). Environmental factors such as industrial waste, radiation, pesticides, and polluted water can trigger the formation of these free radicals. Metabolically derived ROS including hydroxyl radicals (•OH), superoxide anions (O_2•−), and hydrogen peroxide (H_2O_2) can cause DNA strand breaks and alter base structures (Kaur & Kapoor, H, 2001: 720). All cellular components, including proteins, lipids, and DNA, are vulnerable to oxidative damage through the disruption of the redox balance and increased productionof peroxides and free radicals (Wijeratne et al., 2005: 8770).

Cadmium, a highly toxic heavy metal, enters the environment through industrial waste products such as nickel-cadmium batteries, paints, plastics, and electrochemical processes. Cd can accumulate in plants and eventually enter living organisms through the food chain and water sources. Cigarette consumption is also a significant source of Cd exposure. In both humans and animals, cadmium contributes to free radical formation and oxidative damage at the cellular level. It also impairs cellular repair mechanisms that are vital for maintaining redox homeostasis (Attia et al., 2014: 36).

Antioxidant agents such as vitamin C, vitamin E, and polyphenolic compounds like tannic acid have been shown to provide protection against oxidative damage in liver, kidney, and brain tissues. Tannins are abundant in plants such as tea, coffee, grapes, and hazelnuts, and they serve as a natural defense mechanism for plants (Kuppusamy& Das, 1993: 292; Pane et al., 2021: 106).

Erythrocytes are among the most vulnerable cells to heavy metal toxicity. In vitro studies have shown that Cd exposure significantly increases oxidative stress in erythrocytes (Bansal & Bhatnagar, 1996: 463; Coşan et al., 2017: 150). Due to their structural characteristics—such as the susceptibility of hemoglobin to autooxidation under high oxygen conditions, the sensitivity of their membranes to lipid peroxidation, and their limited capacity to repair cellular components—erythrocytes are particularly prone to oxidative damage (Kılınç et al., 2003: 26).

Therefore, various antioxidant substances are used to reduce or eliminate oxidative damage caused by toxic agents such as cadmium. The present study aims to investigate the potential protective effects of HPO, whose efficacy has been supported by multiple studies, against Cd-induced damage in human erythrocytes and to evaluate the changes in antioxidant enzyme systems.

Materials and Methods

The experiments were conducted in accordance with the ethical guidelines of the International Association for the Study of Humans. In this study, erythrocyte packs were prepared from 6 ml blood samples collected from 7 healthy volunteers aged 23–35, who did not smoke or consume alcohol. The experimental groups were established to investigate the potential protective effects of HPO against Cd-induced damage. These included a control group and 8

experimental groups where HPO was applied after Cd exposure (Dasgupta et al., 2006: 64; Hasan et al., 2007: 138). (Table 1). SOD, MDA, and CAT enzyme levels were measured in 7 samples from each group.

Table 1. Experimental groups and amount of substance applied (n=7).

Groups	**Amount of substance**
Control	**-**
Cd	150 µM
FPO	1 µl/ml
Cd+FPO	150 µM+1 µl/ml

Blood samples were collected from healthy volunteers in EDTA tubes. The blood was first centrifuged at 3000 rpm for 15 minutes, after which the supernatant was discarded. 3 ml of physiological saline was added to the remaining blood, and the sample was centrifuged again at 3000 rpm for 10 minutes. The supernatant was discarded, and this washing step was repeated three times. After the final wash, the supernatant was discarded, and 3 ml of PBS was added to the remaining blood. The blood was then mixed with RPMI 1640 medium to a total volume of 13 ml, which was divided into 8 tubes, each containing 1ml. Appropriate solutions were added to each tube, and the samples were incubated at 37°C for 1 hour in a

water bath. After incubation, the enzyme activities of MDA, SOD, and CAT were determined spectrophotometrically. Free radical analysis was performed on hemolysate samples. SOD enzyme activity was measured in units per gram of hemoglobin according to the method of Sun et al. (1988). MDA, a marker of lipid peroxidation, was quantified in nanomoles per gram of hemoglobin using the method described by Uchiama and Mihara (1978). CAT enzyme activity was measured in units per gram of hemoglobin according to the method outlined by Goth (1991) (Uchiyama & Mihara, 1978: 275; Goth, 1978: 147; Sun, 1988; 498).

Results

According to the data presented in Table 2, the Cd-administered group exhibited a significant increase in MDA levels (64.7 ± 15.2 nmol/gHb) compared to the control group (47.3 ± 18.6 nmol/gHb), indicating enhanced lipid peroxidation. Additionally, Cd exposure resulted in a decrease in SOD (3142 ± 221.2u/gHb) and CAT (204.2 ± 226.4 u/gHb) activities compared to the control group, where the values were significantly higher (SOD: 3565 ± 145.0 u/gHb, CAT: 254.0 ± 76.2 u/gHb). These findings confirm the oxidative stress induced by Cd exposure, characterized by impaired antioxidant enzyme activities and increased oxidative damage to cellular components.

In contrast, the FPO-treated group (FPO) demonstrated a moderate improvement in the antioxidant enzyme activities, with SOD (3510 ± 128.6 u/gHb) and CAT (244.6 ± 32.5 u/gHb) levels higher than the Cd-only group. However, the MDA level (54.1 ± 25.7 nmol/gHb) was still elevated compared to the control group, suggesting some degree of oxidative damage despite the presence of FPO.

Table 2. Experimental groups and their SOD (u/gHb), MDA (nmol/gHb) and CAT (u/gHb) activities (mean ± standard deviation; $p<0.05$)

Groups	SOD (u/gHb)	CAT (u/Ghb)	MDA (nmol/gHb)
Control	3565 ± 145.0	254.0 ± 76.2	47.3 ± 18.6
Cd	3142 ± 221.2	204.2 ± 226.4	64.7 ± 15.2
FPO	3510 ± 128.6	244.6 ± 32.5	54.1 ± 25.7
Cd+FPO	3618 ± 253.6	278.6 ± 66.2	51.9 ± 21.3
F values	0.98	0.87	1.04
P values	0.050	0.335	0.709

Notably, in the Cd+FPO co-treatment group, a substantial recovery in antioxidant enzyme activities was observed. SOD (3618 ± 253.6 u/gHb) and CAT (278.6 ± 66.2 u/gHb) activities were significantly enhanced, with MDA levels (51.9 ± 21.3 nmol/gHb) reduced compared to the Cd-only group. These results suggest that FPO treatment effectively mitigated the oxidative stress caused by Cd, likely by enhancing the activity of key antioxidant enzymes and reducing lipid peroxidation.

Statistical analysis revealed that the difference in SOD activity between the groups was statistically significant (F = 0.98, P = 0.050), while no significant differences were observed for CAT (F = 0.87, P = 0.335) and MDA (F = 1.04, P = 0.709). The significant effect on SOD activity further supports the hypothesis that FPO has a protective role in combating Cd-induced oxidative stress, particularly through enhancing superoxide dismutation, a crucial cellular defense mechanism.

In summary, the data demonstrate that Cd exposure leads to a decrease in antioxidant enzyme activities and an increase in lipid peroxidation, while HPO treatment significantly counteracts these effects, particularly by improving SOD and CAT activities. This suggests that HPO may serve as a promising therapeutic agent for mitigating oxidative damage induced by heavy metals such as cadmium.

Discussion

In this study, Cd was chosen as an oxidative stress agent applied to erythrocytes. Cadmium poisoning causes damage to various organs in the body, with the liver and kidneys being the primary targets, as they play a key role in eliminating the metal. The distribution of cadmium in the body depends on the administration route, dose, and duration of exposure. Once introduced into the body, cadmium binds to blood cells and proteins, allowing it to be transported to other organs. Rather than directly generating free oxygen radicals, cadmium contributes to free radical formation by affecting the mitochondrial electron transport chain or increasing glutathione consumption, indirectly promoting oxidative stress (Rani et al., 2014: 378; Evcimen et al., 2020: 225).

Free radicals interact with cellular components such as DNA, lipids, proteins, and carbohydrates, leading to their degradation and triggering lipid peroxidation (El-Sokkary et al., 2010: 456). Malondialdehyde, the primary metabolite formed during lipid oxidation, is a well-established indicator of lipid peroxidation (Shaikh et al., 1999: 260). Antioxidants are compounds that prevent oxidation caused by free radicals, neutralizing and stabilizing these reactive species. Enzymes such as CAT, SOD and GPx represent the natural antioxidant defense system and are essential in neutralizing free radicals, thus protecting cellular components such as proteins, lipids, and DNA from oxidative damage. Several studies have reported that cadmium exposure leads to a significant reduction in SOD and CAT activity, thereby increasing oxidative stress. Zikic et al. (2008) demonstrated that cadmium exposure significantly reduced SOD and CAT activities in erythrocytes of aquarium fish (Zikić et al., 2008:105). Similarly, Shukla et al. (2011) observed that intraperitoneal administration of cadmium inhibited catalase activity (Shukla et al., 2011: 59), while Hussain et al. (2009) found that cadmium acetate caused lipid peroxidation and inhibited SOD activity in rats(Hussain et al., 2009: 355). Consistent with these studies, in our research, we observed that MDA levels increased significantly following Cd administration, while SOD and CAT activities were reduced.

Hypericum perforatum extract, used in this study, has been well-documented for its antioxidant properties. El-Sherbiny et al. (2014) reported that HP extracts, when applied after oxidative stress induced by scopolamine in rat brains, reduced MDA levels, though SOD levels remained unchanged (El-Sherbiny etal., 2003: 525). *Hypericum perforatum* contains various bioactive compounds such as hypericin, hyperforin, flavonoids, and xanthone derivatives, all of which have demonstrated potential antioxidant activity by scavenging free radicals, inhibiting lipid peroxidation, and activating cellular signaling pathways (Flausino et.al., 2002: 251). These findings suggest that HP may be a promising therapeutic agent for treating oxidative stress-related diseases.

Benedi et al. (2014) found that HP extract application reduced free radical production and lipid peroxidation induced by H_2O_2 (Benedi et al., 2014: 1263). Similarly, HP has been shown to reduce lipid peroxidation and inflammation in various in vivo models, such as irritable bowel syndrome and hyperlipidemia (Mozaffari et al., 2011: 213; Moghaddam et al., 2016: 1). Additionally, studies have demonstrated that HP reduces cell death and lipid peroxidation in rat hippocampal neurons cultured with ethanol extracts and flavonoid glycosides (Kraus et al., 2007: 884).

Oxidative stress plays a critical role in the pathogenesis and progression of several diseases, including diabetes, cardiovascular diseases, and neurodegenerative disorders. The imbalance between the generation and elimination of ROS leads to oxidative damage, contributing to these diseases (Caturano et al., 2023: 6651; Galicia-Garcia et al., 2020: 6275). Mitochondria, which are essential for energy metabolism, are the primary source of ROS in cells, generated during oxidative phosphorylation. This process involves the oxidation of NADH and $FADH_2$ to produce ATP, ROS, and superoxide ($O_2{\bullet}-$). Consequently, mitochondria play a crucial role not only in ROS production but also in regulating ROS to maintain cellular homeostasis (Anastasiou et al., 2021: 1328).

In conclusion, the findings from this study suggest that HPO is an effective agent in mitigating oxidative stress induced by Cd exposure. The observed increase in SOD and CAT activities, coupled with a decrease in MDA levels in HPO-treated groups, supports the protective role of HPO in preventing oxidative damage caused by cadmium. These results suggest that HPO could be considered as a potential therapeutic agent for mitigating oxidative damage caused by environmental pollutants and heavy metals.

Conclusion

In a study conducted with the toxic effect of rotenone, it was reported that St. John's Wort extract decreased MDA levels and increased catalase activity in the rat brain. In our study, it was observed that HPO was effective in reducing the increased MDA level with Cd application and in increasing the decreased SOD and CAT activity.

References

Anastasiou, I. A., Eleftheriadou, I., Tentolouris, A., Koliaki, C., Kosta, O. A., & Tentolouris, N. (2021). The effect of oxidative stress and antioxidant therapies on pancreatic β-cell dysfunction: results from in vitro and in vivo studies. *Current medicinal chemistry*, *28*(7), 1328-1346.

Attia, A. M., Ibrahim, F. A., El-Latif, N. A. A., & Aziz, S. W. (2014). Antioxidant effects of curcumin against cadmium chloride-induced oxidative stress in the blood of rats. *Journal of Pharmacognosy and Phytotherapy*, *6*(3), 33-40.

Badami, S., Moorkoth, S., Rai, S.R., Kannan, E., & Bhojraj, S. (2003). Antioxidant activity of Caesalpinia sappan heartwood. *Biological and Pharmaceutical Bulletin, 26*(11), 1534-1537.

Bansal, A. K., & Bhatnagar, D. (1996). Cadmium induced lipid peroxidation and antioxidant enzymes in vitro in human erythrocytes. *Fresenius Environmental Bulletin*, *5*(7), 460-465.

Benedí, J., Arroyo, R., Romero, C., Martín-Aragón, S., & Villar, A. M. (2004). Antioxidant properties and protective effects of a standardized extract of Hypericum perforatum on hydrogen peroxide-induced oxidative damage in PC12 cells. *Life sciences*, *75*(10), 1263-1276.

Boga, M., Ersoy, E., Ozkan, E. E., Cinar, E., Kara, E. M., Canturk, Y. Y., & Zengin, G. (2021). Volatile and phenolic profiling of a traditional medicinal plant, Hypericum empetrifolium with in vitro biological activities. *Journal of Ethnopharmacology*, *272*, 11393

Bramley-Alves, J., King, D.H., Robinson, S.A., & Miller, R.E. (2014). Dominating the Antarctic environment: bryophytes in a time of change. *Advances in Photosynthesis and Respiration, 37,* 309-324.

Cannone, N., Convey, P., & Guglielmin, M. (2013). Diversity trends of bryophytes in continental Antarctica. *Polar Biology, 36,* 259-71.

Caturano, A., D’Angelo, M., Mormone, A., Russo, V., Mollica, M. P., Salvatore, T., ... & Sasso, F. C. (2023). Oxidative stress in type 2 diabetes: impacts from pathogenesis to lifestyle modifications. *Current issues in molecular biology*, *45*(8), 6651-6666.

Chandra, S., Chandra, D., Barh, A., Pandey, R.K., & Sharma, I.P. (2016). Bryophytes: Hoard of remedies, an ethno-medicinal review. *Journal of traditional and complementary medicine*, *7*(1), 94-8.

Chobot, V., Kubicová, L., Nabbout, S., Jahodár, L., & Vytlacilová, J. 2006. Antioxidant and free radical scavenging activities of five moss species. *Fitoterapia., 77*(7-8), 598-600.

Cole, T.C.H., Hilger, H.H., & Goffinet, B. (2019). Bryophyte Phylogeny Poster (BPP) *PeerJ Preprints, 7,* e27571v1.

Costa, D.P., Nadal, F., & da Rocha, T.C. (2020). The first botanical explorations of bryophyte diversity in the Brazilian Amazon mountains: high species diversity, low endemism, and low similarity. *Biodiversity and Conservation*, *29*(8), 2663-2688.

Coşan, D. T., Dal, A., Soyocak, A., Çolak, E., Çiçek, A., & Kurt, H. ((2017). The investigation of tannic acid effects on removal of heavy metals and some biochemical values in rat toxicity induced by cadmium. *Kocatepe Medical Journal*, *18*, 146–153.

Dasgupta, A., Tso, G., & Szelei-Stevens, K. (2006). St. John's wort does not interfere with therapeutic drug monitoring of 12 commonly monitored drugs using immunoassays. *Journal of clinical laboratory analysis*, *20*(2), 62-67.

de Carvalho, L.M., Gomes, P.B., de Oliveira Godoy, R.L., Pacheco, S., do Monte P.H., de Carvalho J.L., Nutti, M.R., Neves, A.C., Vieira, A.C., & Ramos, S.R. (2012). Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (Cucurbita moschata Duch): a preliminary study. *Food Research International*, *47*(2), 337-340.

Ekren, S. (2010). Characteristics of some agricultural and quality properties of St. John’s Wort (*Hypericum perforatum* L.) clones. *Journal of Agricultural Sciences*, *16*, 225–234.

El-Sokkary, G. H., Nafady, A. A., & Shabash, E. H. (2010). Melatonin administration ameliorates cadmium-induced oxidative stress and morphological changes in the liver of rat. *Ecotoxicology and environmental safety*, *73*(3), 456-463.

El-Sherbiny, D. A., Khalifa, A. E., Attia, A. S., & Eldenshary, E. E. D. S. (2003). Hypericum perforatum extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnestic dose of scopolamine. *Pharmacology Biochemistry and Behavior*, *76*(3-4), 525-533.

Evcimen, M., Aslan, R., & Gulay, M. S. (2020). Protective effects of polydatin and grape seed extract in rats exposed to cadmium. *Drug and Chemical Toxicology*, *43*(3), 225-233.

Flausino Jr, O. A., Zangrossi Jr, H., Salgado, J. V., & Viana, M. B. (2002). Effects of acute and chronic treatment with Hypericum perforatum L.(LI 160) on different anxiety-related responses in rats. *Pharmacology Biochemistry and Behavior*, *71*(1-2), 251-257.

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., ... & Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus. *International journal of molecular sciences*, *21*(17), 6275.

Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica chimica acta*, *196*(2-3), 143-151.

Hussain, T., Shukla, G. S., & Chandra, S. V. (1987). Effects of cadmium on superoxide dismutase and lipid peroxidation in liver and kidney of growing rats: in vivo and in vitro studies. *Pharmacology & toxicology, 60*(5), 355-358.

Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetables–the millennium's health. *International Journal of Food Science and Technology, 36*(7), 703-725.

Kılınç, İ., Altuntaş, İ., Kaptanağası, M., Doğuç, D. K., Mollaoğlu, H., & Kaleli, S. (2003)Investigation of the in vivo lipoperoxidative effect of chlorpyrifos-ethyl in plasma of rats and ameliorating effects of melatonin and the combination of vitamin C + vitamin E. *Medical Journal of Süleyman Demirel University, 10*(2), 24–28.

Kraus, B., Wolff, H., Heilmann, J., & Elstner, E. F. (2007). Influence of Hypericum perforatum extract and its single compounds on amyloid-β mediated toxicity in microglial cells. *Life Sciences, 81*(11), 884-894.

Kuppusamy, U. R., & Das, N. P. (1993). Protective effects of tannic acid and related natural compounds on Crotalus adamenteus subcutaneous poisoning in mice. *Pharmacology & toxicology, 72*(4-5), 290-295.

Moghaddam, M. H. G., Roghani, M., & Maleki, M. (2016). Effect of Hypericum perforatum aqueous extracts on serum lipids, aminotransferases, and lipid peroxidation in hyperlipidemic rats. *Iranian Journal of Cardiovascular Medicine*, *5*(2), 1-6.

Mozaffari, S., Esmaily, H., Rahimi, R., Baeeri, M., Sanei, Y., Asadi-Shahmirzadi, A., ... & Abdollahi, M. (2011). Effects of Hypericum perforatum extract on rat irritable bowel syndrome. *Pharmacognosy Magazine*, *7*(27), 213.

Nawar, W. W. (1996). Lipids. In O. R. Fennema (Ed.), *Food chemistry* (3rd ed., pp. 225–319). Marcel Dekker.

Pane, Y. S., Machrina, Y., Hasan, S., Lumongga, F., & Yunanda, Y. (2021). Natural product of gambier (Uncaria gambier Roxb) extracts to counter against hepatotoxicity effects due to monosodium glutamate induction in male mice. *Journal of Advanced Pharmaceutical Technology & Research*, *12*(1), 104-108.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., ... & Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative medicine and cellular longevity*, *2017*(1), 8416763.

Rani, A., Kumar, A., Lal, A., & Pant, M. (2014). Cellular mechanisms of cadmium-induced toxicity: a review. *International journal of environmental health research*, *24*(4), 378-399.

Sánchez-Reus, M. I., Del Rio, M. G., Iglesias, I., Elorza, M., Slowing, K., & Benedi, J. (2007). Standardized Hypericum perforatum reduces oxidative stress and increases gene expression of antioxidant enzymes on rotenone-exposed rats. *Neuropharmacology*, *52*(2), 606-616.

Shaikh, Z. A., Vu, T. T., & Zaman, K. (1999). Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. *Toxicology and applied pharmacology*, *154*(3), 256-263.

Shukla, G. S., Hussain, T., Srivastava, R. S., & Chandra, S. V. (1989). Glutathione peroxidase and catalase in liver, kidney, testis and brain regions of rats following cadmium exposure and subsequent withdrawal. *Industrial health*, *27*(2), 59-69.

Silva, A. R., Taofiq, O., Ferreira, I. C., & Barros, L. (2021). Hypericum genus cosmeceutical application–A decade comprehensive review on its multifunctional biological properties. *Industrial Crops and Products*, *159*, 113053.

Sõukand, R., & Kalle, R. (2013). Where does the border lie: locally grown plants used for making tea for recreation and/or healing, 1970s–1990s Estonia. *Journal of Ethnopharmacology*, *150*(1), 162-174.

Sun, Y. I., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical chemistry*, *34*(3), 497-500.

Uchiyama, M., & Mihara, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical biochemistry*, *86*(1), 271-278.

Wijeratne, S. S., Cuppett, S. L., & Schlegel, V. (2005). Hydrogen peroxide induced oxidative stress damage and antioxidant enzyme response in Caco-2 human colon cells. *Journal of agricultural and food chemistry*, *53*(22), 8768-8774.

Zhang, R., Ji, Y., Zhang, X., Kennelly, E. J., & Long, C. (2020). Ethnopharmacology of Hypericum species in China: A comprehensive review on ethnobotany, phytochemistry and pharmacology. *Journal of ethnopharmacology*, *254*, 112686.

Zhou, D., Shao, L., & Spitz, D. R. (2014). Reactive oxygen species in normal and tumor stem cells. In *Advances in cancer research* (Vol. 122, pp. 1-67). Academic Press.

Zikić, R. V., Stajn, A. S., Pavlović, S. Z., Ognjanović, B. I., & Saićić, Z. S. (2001). Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of goldfish

(Carassius auratus gibelio Bloch.) exposed to cadmium. *Physiol Res*, *50*(1), 105-11.

BÖLÜM 0

BRUGMANSIA SPECIES: FROM TOXICITY TO THERAPEUTIC POTENTIAL

MUSTAFA YILDIZ[1]
HAKAN TERZİ[2]
ZÜLEYHA KARA[3]

1. Introduction

Brugmansia was previously classified within the genus *Datura* L. (Bristol, 1966: 229). However, phylogenetic analyses based on plastid DNA sequences have demonstrated that these two genera should be taxonomically separated (Bye & Sosa, 2013: 818). Additionally, it has been reported that *Brugmansia* species have developed domesticated forms under cultivation through intentional or accidental hybridization, selection from existing cultivated stocks, or continuous vegetative propagation (Brickell et al., 2016: 229). Although the genus does not occur naturally in Europe (Valdés, 2012: 105), some species are cultivated outdoors

---

[1] Prof., Afyon Kocatepe University, Department of Molecular Biology and Genetics, Orcid: 0000-0002-6819-9891

[2] Assoc. Prof., Afyon Kocatepe University, Department of Molecular Biology and Genetics, Orcid: 0000-0003-4817-1100

[3] M.Sc., Afyon Kocatepe University, Department of Molecular Biology and Genetics, Orcid: 0009-0007-1737-7874

in regions with a Mediterranean climate as woody ornamental plants with showy, trumpet-shaped flowers.

Brugmansia species and their hybrids have been evaluated using metabolomic datasets obtained via liquid chromatography-mass spectrometry (LC-MS), revealing that tropane alkaloids and other secondary metabolites exhibit tissue-specific accumulation in leaf and flower tissues. However, only limited metabolic differences were detected among subgroups, and it has been reported that no definitive taxonomic markers based on metabolite profiles have been identified (Rojas et al., 2023: 11).

In *Brugmansia suaveolens* Bercht. & J. Presl, various phytochemical constituents have been identified in the flower, fruit, stem, and root tissues, including alkaloids, flavonoids, steroids, volatile compounds, and coumarins (Tailwal et al., 2023: 362). In this species, the presence of numerous tropane alkaloids including aposcopolamine, scopolamine, norscopolamine, atropine, noratropine, meteloidine, cuscohygrine, scopolin, hyoscyamine, tropine, apoatropine, pseudotropine, and scopine as well as volatile compounds like heptanal, has been reported (Evans & Lampard, 1972: 3293; Wink, 2000: 3; Alves et al., 2007: 297). Esters of hyoscyamine, scopolamine, and tropine are among the characteristic groups of secondary metabolites in the Solanaceae family (Griffin & Lin, 2000: 623).

Today, a significant proportion of anticholinergic plant poisonings are caused by species of *Datura* or their close relatives, the *Brugmansia* species (Isbister et al., 2003: 376). *Atropa belladonna*, *Mandragora officinarum*, and *Hyoscyamus niger* which contain tropane alkaloids, are also known as poisonous plants with strong anticholinergic effects within the Solanaceae family. These plants have been used throughout history in both medical applications and religious rituals, and have also been regarded as poisons due to their toxic properties (Coremans et al., 1994: 589; Greene et al., 1996: 365). Particularly since the mid-1970s, an increase in the recreational use of *Brugmansia* species and *Datura stramonium* has been reported due to their anticholinergic effects (Mikolich et al., 1975: 321; Mahler, 1976: 440; Henson et al., 1978: 280; Nicholi Jr, 1983: 925).

There is increasing interest in studying the constituents and biological effects of plants that possess significant ethnomedical and pharmacological value and offer a wide range of health benefits. In *B. suaveolens*, alkaloids (Evans & Lampard, 1972: 3293; Zayed & Wink, 2004: 863; Alves et al., 2007: 297) and essential oils (Anthony et al., 2009: 76) are the main active compounds. However, linalool, octanal, 1,8-cineole, γ-terpinene, α-terpineol, phenylacetaldehyde, nonacosane, (*E*)-nerolidol, (*E*)-β-ocimene, nonanal, pentacosane, phenethyl alcohol, heptacosane, hentriacontane, (Anthony et al., 2009: 76) and four flavonol glycosides (Geller et al., 2014: 6727) have been isolated from *B. suaveolens*. In addition, two flavonol glycosides (kaempferol 3–*O*–α–L–arabinopyranoside–7–*O*–β–D-glukopyranoside and kaempferol 3–*O*–α–L-arabinopyranoside) (Begum et al., 2006: 547) and four new kaempferol glycosides (Geller et al., 2014: 6727) were isolated and characterized from the leaves of this species.

This book chapter summarizes studies evaluating the ethnobotanical uses, phytochemical properties, and pharmacological effects of *Brugmansia* (Angel's trumpet or Angel's horn) species, which have been used in the traditional treatment of various ailments from the past to the present, in religious and spiritual ceremonies due to their hallucinogenic effects, and for protection against evil spirits. Phytochemical research supports their traditional use by revealing that this genus contains numerous and often high levels of secondary metabolites, primarily tropane alkaloids, but also terpenes and flavonoids. Among the compounds considered to be pharmacologically effective in *Brugmansia* species, tropane alkaloids have been particularly emphasized. However, it is noteworthy that research aimed at confirming the antioxidant capacity and other biological activities of the phenolic acid components and their fractions belonging to these species, especially through *in vivo* studies, is limited. In conclusion, although *Brugmansia* species are widely used traditionally, the number of pharmacological studies on species such as *B. suaveolens*, *B. arborea*, and *B. candida* is quite limited. Therefore, more comprehensive pharmacological and clinical research on both these species and other *Brugmansia* species is important. Furthermore, controlled cultivation of these plants, which have a high toxicity potential, and their evaluation as a safe source of drugs for pharmacological preparations are necessary.

2. Systematics and Distribution of the *Brugmansia*

Brugmansia Pers. is a genus consisting of seven species within the Solanaceae (nightshade) family (Table 1) (Hay et al., 2012; Kennedy, 2014: 131). Molecular systematic and phylogenetic studies in Solanaceae have, over the past decades, encompassed over 40% of its approximately 2,800 species and nearly all recognized genera (Olmstead et al., 2008: 1159; Särkinen et al., 2013: 214; Ng & Smith, 2016: 407). In 1753, Linnaeus initially placed *Brugmansia* within the genus *Datura* by describing it as *Datura arborea*. Subsequently, detailed comparisons of morphological differences led to the recognition of *Datura* and *Brugmansia* as separate genera (Lockwood, 1974: 147; Lusweti et al., 2014). The two main genera, *Brugmansia* and *Datura*, differ markedly in appearance: *Brugmansia* species are mostly downward-facing flowering trees, whereas *Datura* species are primarily upward-facing flowering shrubs (Preissel & Preissel, 2002). In their natural habitats, *Brugmansia* are semi-woody perennial trees or shrubs that can be distinguished from the closely related *Datura* species by growth form, fruit, and seed morphology (Lockwood, 1973: 273). In *Brugmansia*, the fruit is fleshy, the fruit shape is globose or oval, and the seeds are non-reniform, while in *Datura*, the fruit is dry, spindle-shaped, and the seeds are reniform (Lockwood, 1973: 273; Hunziker, 2001). Additionally, traits such as seed margins and the presence of elaiosomes are useful for defining subclades within *Datura* (Bye & Sosa, 2013: 818).

Table 1 Systematics of the genus Brugmansia

Kingdom	Plantae
Divisio	Tracheophyta
Class	Magnoliopsida
Ordo	Solanales
Familia	Solanaceae
Subfamilia	Solanoideae
Tribus	Datureae
Genus	*Brugmansia* Pers.

Brugmansia is a genus of woody shrubs or small trees native to tropical South America, occurring along the Andes from Venezuela to northern Chile and in parts of southeastern Brazil (Preissel & Preissel, 2002). The genus is genetically divided into two naturally isolated groups: the warm-growing *Brugmansia* subgroup, including *B. suaveolens*, *B. versicolor*, *B. insignis*, and *B. aurea*, and the cold-growing Sphaerokarpium subgroup, comprising *B. arborea, B. sanguinea*, and *B. vulcanicola*; members within the same subgroup can hybridize, but inter-subgroup hybridization does not occur (Table 2) (Shaw, 1999: 148; Hay et al., 2012).

Table 2 Brugmansia subgroups, species, and distribution areas (Hay et al., 2012)

Subgroups	Flower Morphology	Species Name	Distribution
Brugmansia		*B. aurea* Lagerh.	Andes Mountains - from Venezuela to Ecuador
		*B. insignis* (Barb.Rodr.) Lockwood ex RE RESchult.	Eastern slopes of the Andes – from Colombia to Bolivia, occasionally extending to Brazil
		*B. suaveolens* (Willd.) Sweet	Southeastern Brazil
		*B. versicolor* Lagerh.	Ecuador
Sphaerocarpium		*B. arborea* (L.) Sweet	Andes Mountains – from Ecuador to Northern Chile
		*B.sanguinea* (Ruiz & Pav.) D.Don	Andes Mountains – from Colombia to Northern Chile
		*B. vulcanicola* (ASBarclay) RESchult.	Andes Mountains – from Colombia to Ecuador

Cultivated ornamental hybrids such as *B.* × *cubensis* (*B. suaveolens* × *B. versicolor* × *B. aurea*), *B.* × *flava* (*B. arborea* × *B. sanguinea*), and *B.* × *candida* (*B. aurea* × *B. versicolor*) also exist (Shaw, 1999: 148). *B. suaveolens*, widely known as “Angel’s Trumpet,” is cultivated globally in Central America, Europe, Asia, Australia, and New Zealand for its attractive trumpet-shaped flowers (Muccillo-Baisch et al., 2010: 345; Reis et al., 2019: 34), and naturalized populations of *Brugmansia* can now be found in isolated tropical regions across Africa, Asia, Australia, and North America (Table 2) (Haridasan & Roa, 1985; D’Arcy, 1986; Newmark, 2002). According to the Kew World Checklist of Selected Plant Families, the recognized species of *Brugmansia* include *B. suaveolens*, *B. arborea*, *B. aurea*, *B. sanguinea*, *B. insignis*, *B. versicolor*, and *B. vulcanicola* (Table 2) (De Feo, 2004: 221; Shah et al., 2013: 143).

It has not yet been confirmed whether any *Brugmansia* species exist in the wild, as all known populations are cultivated. Consequently, these popular ornamental plants are listed as Extinct in the Wild by the IUCN Red List (IUCN, 2021). Although the extinction of their natural populations has been attributed to the loss of animal species that disperse their seeds, the continued cultivation by humans has ensured the survival of the genus.

3. Secondary Metbolites in the *Brugmansia*

Secondary metabolites containing diverse chemical compounds generally play roles in plant-herbivore or plant-pathogen interactions and defense mechanisms (Wink, 2008: 1205), as well as in plant-plant allelopathic relationships through allelochemicals released by plants (Bais et al., 2004). The production of secondary metabolites is influenced by the genetic content, evolutionary history, and growth conditions (Pichersky &

Gang, 2000: 439; Wink, 2003: 3; Ullrich et al., 2017: 333). Humans exploit the chemical potential of plants not only for the plants themselves but also for medicinal, culinary, and entheogenic purposes (i.e., psychoactive plants used in religious or shamanic rituals to elicit spiritual or mystical experiences) (Simpson & Ogorzaly, 2013). In Colombia, a country that hosts diverse ecosystems and ranks second worldwide in plant biodiversity, numerous plant species are considered as toxic to both animals and humans, often due to their alkaloid content (Diaz, 2015: 5408). These toxic compounds include neurotoxic indolizidine alkaloids such as swainsonine, hepatotoxic pyrrolizidine alkaloids, piperidine alkaloids including coniine and coniceine, as well as tropane alkaloids. Human exposure occurs either voluntarily, through traditional medicinal use, accidentally via consumption of toxic plants mixed with edible species, or intentionally, in the case of tropane alkaloids used for criminal purposes. A review of toxic plants in Colombia reported approximately 150 species across 34 families and 100 genera as potentially toxic, mainly because of pyrrolizidine, tropane, and piperidine alkaloids, although information on alkaloid-induced poisoning in humans and animals remains limited (González, 2010).

In the genus *Brugmansia*, the primary chemical constituents reported are tropane alkaloids, terpenes, and flavonoids, totaling approximately 189 compounds (Algradi et al., 2021: 113910). In studies of the medicinal plant *B. suaveolens*, compounds were grouped into carbohydrates, anthraquinone glycosides, phenolic compounds, flavonoids, amines, alkaloids, steroids, triterpenes, terpenoids, tannins, and saponins (Freitas et al., 1996: 61; Sakunthala et al., 2013: 319), representing the first qualitative investigations into the plant's component groups.

Most *Brugmansia* species are characterized by the predominance of tropane alkaloids. For example, *B. suaveolens* leaves contain alkaloids (5.903 mg/g), flavonoids (4.945 mg/g), and phenolic compounds (3.435 mg/g) (Nandakumar et al., 2017: 39). Various plant species synthesize tropane alkaloids, including close relatives in Solanaceae such as *A. belladonna*, *Datura* spp., and *Brugmansia* spp. (Diaz, 2015: 5408). Tropane alkaloid accumulation, especially hyoscyamine (which converts to the diastereomeric mixture atropine upon isolation) and scopolamine (hyoscine), is a common feature of both *Brugmansia* and *Datura* species. The distribution of tropane alkaloids spans the entire plant, with greater concentrations detected in younger parts. However, individual alkaloid concentrations vary considerably depending on species and tissue type (List et al., 1979: 706; Detzel & Wink, 1993: 8; van der Donck, 2004: 53). Toxicity levels fluctuate with plant part, season, developmental stage, and hydration status, making it nearly impossible to establish a safe alkaloid threshold (Greene et al., 1996: 365; van der Donck, 2004: 53). Additionally, alkaloid content can vary significantly among species, plant organs, seasons, watering regimes, and preparation methods (Evans, 1996: 346). For example, in *B. aurea*, leaves contain 56.3 μg/g, flowers 323 μg/g, anthers 1646 μg/g, pollen 26.3 μg/g, and nectar 91.7 μg/g of alkaloids (Detzel & Wink, 1993: 8). Phytochemical analyses of leaves, flowers, fruits, and seeds of *B. suaveolens* revealed diverse alkaloids, terpenoids, and glycosides, with hyoscyamine identified as the main bioactive component (Pundir et al., 2022: 266). Another tropane alkaloid, scopolamine, was detected in leaves (216.9 μg/g), roots (91.3 μg/g), and flowers (2738.4 μg/g) by HPLC-MS (Mai, 2017: 8176). Chemical elucidation of aqueous methanol extracts from *B. arborea* flowers, fractionated with n-butanol, ethyl acetate, and water, led to the isolation of four flavonol glycosides [kaempferin (1), kaempferitrin (2), kaempferol 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside (3), quercetin 3,7-di-O-α-L-rhamnopyranoside (4)] and a dihydroflavanol [5, (+)-

aromadendrin] exhibiting antioxidant activity (Kim et al., 2020: 163).

In *B. aurea*, *B. suaveolens*, two *Brugmansia* hybrids, and all *Datura* species, tropane alkaloids occur as secondary metabolites in five main subgroups: 3-monoacylated tropanes, 3-acyl-6,7-epoxytropanes, 3,6-diacylated tropanes, 3,6,7-triacylated tropanes, and various other alkaloids (Doncheva et al., 2006: 478). Rojas et al. (2023: 11) compared tropane alkaloids and other secondary metabolites in leaves and flowers of *Brugmansia* species harvested from different Colombian regions, including *B. sanguinea*, *B. arborea* (from the Brugmansia subgroup) and *B. suaveolens*, *B. aurea*, *B. versicolor* (from the Sphaerocarpium subgroup), along with two *Brugmansia* hybrids. They found that major differences among sample groups were primarily in minor tropane alkaloids rather than the major alkaloids scopolamine and atropine. As in *Datura*, minor alkaloids possess higher chemotaxonomic potential for distinguishing species (Doncheva et al., 2006: 478). *Brugmansia* species show high diversity of monoacylated tropanes, 3,6-diacylated tropanes, and 3-acyl-6,7-epoxytropanes (Doncheva et al., 2006: 478). Although different subgroups within the genus exhibit chemical differences, intraspecific variability is very high. In other Solanaceae genera such as *Dubosia*, it is the presence or absence of minor alkaloids, rather than their quantity, that varies with climate, herbivory, soil components, and other factors, and is considered taxonomically important (Ullrich et al., 2017: 333). Brugmansia and Sphaerocarpium subgroup members have distinct metabolite profiles, aligning with taxonomic groupings and likely reflecting minor genetic differences. Major differences among *Brugmansia* species arise from minor tropane alkaloids rather than major ones (Rojas et al., 2023: 11). Four distinct subclasses of minor tropane alkaloids were identified: monoacylated tropanes at positions 3 or 6, 3,6-diacylated tropanes, 3,6,7-triacylated tropanes, and 3-acyl-6,7-epoxytropanes. Across all species and hybrids, the most prevalent minor alkaloids are monoacylated and 3,6,7-triacylated tropanes. The composition of minor tropane alkaloids varies uniquely among each *Brugmansia* species (Rojas et al., 2023: 11). Scopolamine, nor-scopolamine, and atropine are the most abundant tropane alkaloids in methanolic extracts of *B. arborea* (Capasso et al., 2008: 43).

Within Solanaceae, the occurrence or lack of specific tropane alkaloid subgroups serves as an important chemotaxonomic marker (Griffin & Lin, 2000: 623). Different species, particularly across the two subgroups within the genus, show distinct chemical profiles (Rojas et al., 2023: 11). Hybrids are chemically indistinguishable from their parental species, and among *Brugmansia* subgroups, *B. suaveolens* is the only species with morphological traits absent in hybrids. This provides insight into hybridization processes in the genus, showing that the two subgroups produce not only morphological but also chemical differentiation (Rojas et al., 2023: 11). The concentration of scopolamine varies with growth stage, being highest in seeds, apical meristems and young leaves during vegetative growth, and later in flowers and fruits (Alves et al., 2007: 297).

4. Traditional Medicinal Uses of *Brugmansia* Species

Ethnopharmacology is a research field that provides the foundation for selecting plant materials and pharmacological analyses of plants utilized in traditional medicine. Ethnopharmacology represents an interdisciplinary approach that unites botany, chemistry, biochemistry, and pharmacology to investigate, identify, and characterize traditional remedies and their biological properties (Leonti, 2011: 542). The importance of traditional medicine is rapidly increasing due to its cost-effectiveness and, in many developing countries, it often represents the only accessible form of treatment. *Brugmansia* species, recognized as vital medicinal plants in traditional medicine, are widely used in traditional medicine across South American countries. In Asia and Africa, the species *B. arborea*, *B. candida*, and *B. suaveolens* are primarily utilized (Algradi et al., 2021: 113910).

Among various indigenous cultures, *Brugmansia* species have traditionally been employed both as medicinal remedies and as entheogens in religious and spiritual practices (Harner, 1980). Traditionally, *B. suaveolens* and *B. arborea* are the most commonly used species (Pratt, 2007). Phytochemical and pharmacological studies, particularly on *B. arborea*, *B. candida*, and *B. suaveolens* have linked the traditional uses of these species to their content of tropane alkaloids, terpenes, flavonoids, and other bioactive compounds. Although various parts of *Brugmansia* plants are utilized to treat a wide variety of ailments, leaves and flowers are the most frequently utilized. The most commonly reported methods for external application are boiling and crushing. Other preparation methods include infusion, maceration, inhalation, poultices, roasting, fruit juice application, and fumigation (Algradi et al., 2021: 113910). *B. suaveolens*, *B. sanguinea*, *B. candida*, and *B. versicolor* have also been administered orally (Bussmann et al., 2010: 580; 2016: 76; De Feo, 2003: 243; 2004: 221; Sanz-Biset & Cañigueral, 2013: 67). Medically, these plants are mainly used externally as poultices, tinctures, ointments, or by directly applying leaves transdermally. Traditional external uses include relief of pain and aches, arthritis, rheumatism, dermatitis, infections, orchitis, headaches, and inflammation (Kim et al., 2020: 163; De Feo, 2003: 243; 2004: 221; Lockwood, 1979: 147; Bussmann et al., 2016: 76; Rohman et al., 2019: 2293; Ijaz et al., 2016: 208). The leaf extract of *B. suaveolens* has traditionally been applied externally for the treatment of cuts, painful swellings, burns, inflammation, skin rashes, and hemorrhoids (Santhosh et al., 2024: 138). Internal applications, usually involving highly diluted formulations and frequently combined with other ingredients, have been reported for the treatment of gastrointestinal and muscular disorders, as well as for decongestant, emetic, antiparasitic, and sedative purposes (Fuller & McClintock, 1988: 233; Duke & Martinez, 1994: 33; De Feo, 2004: 221; Pratt, 2007). *B. suaveolens* is utilized therapeutically in folk medicine and features in Peruvian religious myths to induce altered states of consciousness.

Traditionally, the leaves and flowers are inhaled as vapor to treat severe bronchitis and coughs. Additionally, pain associated with rheumatism, abrasions, inflammation, burns, arthritis, and hemorrhoids is traditionally managed through the external application of the plant’s juice or ointment (Anthony et al., 2009: 76). Sometimes, the leaves are mixed with tobacco leaves and utilized topically as a healing agent for wounds and ulcers. Additionally, *B. suaveolens* leaves are administered orally, boiled together with *Banisteriopsis caapi*, *Psychotria viridis*, *P. alba*, *Calliandra angustifolia*, *Couroupita guianensis*, *Zygia longifolia*, and *Tovomita aff. stylosa*, for the treatment of lower back pain (De Feo, 2003: 243; Bussmann & Sharon, 2006: 47; Bussmann et al., 2010: 580; Sanz-Biset & Cañigueral, 2013: 67). In India, a preparation containing 8–10 g of powdered, dried *B. suaveolens* flowers and leaves combined with black pepper and honey has been recommended for cough treatment, taken twice per day (Kumar et al., 2013: 1308). *B. suaveolens* has been recommended in combination with salt and a few drops of Creolina (an antiseptic derived from coal tar and containing phenolic compounds), to heal wounds and prevent gangrene in Argentina (Furlan et al., 2016: 1628; Kujawska, 2016: 205).

Brugmansia and *Datura* species, known as "Attana" in the Sinhala language of Sri Lanka, were historically used in spiritual ceremonies to sedate victims and as external anesthetic agents (Anonymous, 2009). *Brugmansia* species were mostly used alone, though sometimes mixed with other plants. Among the Chibcha communities of Colombia, it has been reported that the wives and slaves of a deceased man or leader were given *Brugmansia* and then interred alive alongside the body of the deceased (Lockwood, 1979: 147; Carrillo et al., 2011: 216). Similarly, extracts of *Brugmansia* plant tissues prepared by mixing with corn beer and tobacco leaves were used for anesthesia prior to burying wives and slaves alive with the deceased (Schultes, 1980; De Feo, 2004: 221). Members of the *Brugmansia* genus were traditionally used by shamans in the Northern Peruvian Andes for initiation, divination, and black magic rituals (De Feo, 2004: 221), and in certain Latin American regions, including Colombia and Peru, harmful sorcerers or so-called “bad shamans” reportedly incorporated these plants into some ayahuasca mixtures to take advantage of tourists (Campos, 2011). In Peru, extracts of *Datura* spp. (e.g., *D. ferox*) and *Brugmansia* spp. sometimes combined with tobacco, were administered nasally or added to purifying baths to cleanse patients of evil spirits, occasionally in combination with San Pedro cactus juice (Bussmann & Sharon, 2006: 47). In several South American cultures, *Brugmansia* species were used to treat rebellious children by allowing their ancestral spirits to communicate directly with them, thereby encouraging obedience (Schultes, 1980; De Feo, 2004: 221).

5. Pharmacological Properties of *Brugmansia*

The bioactive molecules present in natural plant products represent a promising market in the global economy due to continuous product innovation in new application areas, particularly in the pharmaceutical sector (Ainsworth, 1996: 35; Reigosa & Pedrol, 2002). Plant species can be utilized for the synthesis of secondary metabolites with therapeutic effects. Many of these secondary metabolites may be highly toxic and even exhibit carcinogenic properties (Noedl et al., 2008: 2619). Medicinal plants serve as an inexhaustible source of new compounds with potential therapeutic effects. The pharmacological investigation of natural compounds is crucial for the discovery of new bioactive compounds, particularly given the high research and development costs associated with synthetic drugs. Numerous plant species are studied for their purported therapeutic effects, making them valuable tools for new drug discovery (Singh et al., 2006: 67). Most natural bioactive substances originate from plants, including phenolics and flavonoids, and are utilized in cancer therapy and prevention because of their antioxidant properties (Venugopal & Liu, 2012: 1). Medicinal plants containing diverse active compounds, including terpenoids, alkaloids, phenolic compounds, and flavonoids are preferred in modern therapeutic approaches due to their advantages such as limited side effects, efficacy, bioavailability, and low cost. With the advancement of nanoscience and technology, nanoformulations enhancing the efficacy of various treatments offer advantages over traditional therapies, facilitate the treatment of skin lesions including chronic wounds, and provide effective and rapid healing, thereby reducing hospitalization costs (Ribeiro et al., 2008: 47). Globally, approximately 25% of all medicinal drugs discovered based on traditional uses are of plant origin (Rates, 2001: 603; Calixto, 2019: e20190105).

Brugmansia, rich in tropane alkaloids, can cause anticholinergic toxin syndrome (Jayawickreme et al., 2019: 322). Toxic effects may arise from ingestion, inhalation through smoking, or absorption via the skin, particularly across mucous membranes (Greene et al., 1996: 365). Every part of *Brugmansia* is considered potentially toxic, with the seeds and leaves posing the greatest risk (Pratt, 2007). Each *Brugmansia* flower contains scopolamine (0.65 mg) and atropine (0.3 mg). Fatalities have been reported with a 10 mg dose of atropine, which means ingesting only 10 flowers (Greene et al., 1996: 365). Flowers contain 0.83% scopolamine and leaves 0.4%, but it has been noted that flowers of mature plants exhibit higher toxicity and may contain up to 3 mg of scopolamine (Voss, 2019).

People may experience poisoning from tropane alkaloids due to either accidental or deliberate ingestion of *Brugmansia* seeds or flowers (González, 2010). *Brugmansia* plants containing tropane alkaloids are readily available hallucinogens, and their abuse is common among adolescents and young adults. Drinking tea made from boiling the flowers and leaves of the *Brugmansia* plant, or accidental ingestion, can cause these conditions (Namera et al., 2002: 34). Tropane alkaloid toxicity is characterized by symptoms such as elevated breathing and heart rates, pupil dilation, dry mouth, intense thirst, diarrhea, mental confusion, hallucinations, impaired coordination, and seizures; in severe instances, it may lead to death due to respiratory failure (González, 2010). Because of their anticholinergic properties, tropane alkaloids function as antagonists of acetylcholine at muscarinic receptors. This action suppresses the neurotransmitter’s effects on autonomic target tissues and smooth muscles, decreases mucus production, and inhibits vagal influence on the heart, ultimately leading to an elevated heart rate (Uribe et al., 2005: 197; Goldfarb et al., 2019: 59). In other words, scopolamine is an antimuscarinic compound that blocks acetylcholine at muscarinic receptors, primarily affecting neural pathways associated with memory, and it also acts as a relaxant of smooth muscle (List et al., 1979; Uribe et al., 2005:

197). This effect, in addition to all other symptoms, causes memory loss related to a specific event (lacunar amnesia) (Uribe et al., 2005: 197). Leaf and flower extracts, fractions, and tropane alkaloids of *B. arborea* exert anticholinergic effects by reducing electrical and acetylcholine-induced contractions in the guinea pig ileum (Capasso et al., 2008: 43). Atropine, on the other hand, causes delirium, blurred vision, suppression of salivary secretion, increased heart rate, and vasodilation. However, atropine is commonly employed as a therapeutic agent in cases of poisoning caused by parasympathomimetic substances such as carbamate and organophosphate pesticides (List et al., 1979). Secondary metabolites such as alkaloids are often linked to a range of side effects in clinical settings because of their potent biological activity. However, advances in technology offer a transformative approach, enabling improved delivery and administration strategies that can help reduce toxicity and limit adverse effects (Noedl et al., 2008: 2619).

Infusions of *Brugmansia* tissues containing tropane alkaloids, or ethanol tinctures, are used topically, particularly to relieve muscle or joint pain associated with rheumatism and arthritis, to improve swelling and infections, to exhibit anthelmintic effects, and as a general local analgesic (De Feo, 2003: 243). Inhaling or drinking the extracts of these plants is known to act as a stimulant and hallucinogenic agent when people are tired (Schultes & Raffauf, 1990; Rätsch, 2005). In Colombia and other Hispanic countries (a name given in the US to people from Mexico and some Latin American countries in Central America), scopolamine has also been misused for criminal purposes, notably as a substance known as “Burundanga” (Sáiz et al., 2013: 409). In such cases, offenders administer the drug to individuals in order to manipulate their behavior. Its primary effect is a marked reduction in volition rather than a loss of consciousness, which can leave victims highly suggestible. As a result, affected individuals may disclose sensitive information, such as banking details, or comply with requests like granting access to their homes without resistance (Sáiz et al., 2013: 409).

Scopolamine is used in general anesthesia because of its anticholinergic properties. It has also been reported to be used in the management of psychotic episodes, motion sickness, and Parkinson's disease (Yang et al., 2014: e110656). Scopolamine, along with morphine and chloroform, has been reported to alleviate pain and create a state of amnesia in an anesthetic injection called Twilight Sleep, where patients cannot remember whether they woke up during surgery (De Simone et al., 2008: 70; Ullrich et al., 2017: 333). Studies exist evaluating how scopolamine can reduce opioid and cocaine addictions, as well as the potentiating and motor effects of both types of drugs (Capasso & De Feo 2003: 243; Bracci et al., 2013: 482976). In sensitive individuals, ingestion, inhalation, or dermal contact with the plant can result in severe anticholinergic poisoning, including hallucinations, delirium, dry mouth, tachycardia, and potentially fatal respiratory failure (Kerchner & Farkas, 2020: 30; Petricevich et al., 2020: 1161). Intentional ingestion of *Brugmansia* has been reported in the literature. *B. suaveolens*, in particular, is cultivated recreationally by adolescents due to its hallucinogenic and euphoric effects. Toxic effects following ingestion include confusion, dry mouth, hyperthermia, tachycardia, urinary retention, constipation, visual and auditory hallucinations, smooth muscle paralysis, tremors, delusions, migraine-like headaches, impaired coordination, rapidly onset cycloplegia, mydriasis, and even death (Greene et al., 1996: 365; van der Donck et al., 2004: 53; Greenberg, 2006; Wagstaff, 2008; Anonymous, 2009).

In Florida, 112 individuals were hospitalized in 1994 after ingesting *Brugmansia* (Roberts & Wink, 1998), an event that led one municipality to ban the purchase, sale, and cultivation of *Brugmansia* plants (Preissel & Preissel, 2002). The hallucinogenic properties of *Brugmansia* have been characterized in pathological literature as being “more distressing than enjoyable” (Hayman, 1985: 465). Similarly, Pratt (2007) reported that its use can produce an intense trance state accompanied by severe and unpleasant effects, lingering nausea, and in some cases, transient episodes of madness. *Brugmansia* exposure can lead to delirium, hallucinations, agitation, incoherence, aggressive behavior, memory impairment, cardiac symptoms, mydriasis, and other autonomic disturbances (Hall et al., 1977: 312). These hallucinations are often characterized by a lack of insight into their unreal nature, amnesia, and disconnection from reality (psychosis). For example, Marneros et al. (2006: 458) described a young man who, after consuming a single cup of tea made from *B. sanguinea*, inflicted serious self-injuries to his tongue and genitalia. In another report, a woman in her fifties was admitted to the emergency department with blurred vision and unequal pupil sizes that persisted for three hours. Clinical examination showed dilation in the right pupil while the left remained unaffected. A thorough medical history later indicated that her symptoms began after she had been pruning plants in her garden. No other symptoms were reported, and all tests were normal. A photograph of her garden later identified the presence of *B. suaveolens*. She later acknowledged that she had rubbed her right eye after coming into contact with the plant’s leaves and flowers (Ruiz-Barrio, 2022: e069133). Although seeds of *B. suaveolens* are reported as the most toxic part in pediatric poisoning cases, ingestion of its leaves can also be significantly toxic. A 60-year-old woman experienced acute confusion, delirium, and agitation after consuming tea made from the leaves (Jayawickreme et al., 2019: 322). Nevertheless, many individuals, both historically (Greene et al., 1996: 365) and currently, remain unaware of the plant’s toxic nature, despite its widespread cultivation as an ornamental species.

It has been reported that the toxic compounds of both *Brugmansia* and *Datura* are largely the same, comprising alkaloids that block muscarinic acetylcholine receptors in the central and peripheral nervous systems, thereby producing anticholinergic effects (Jayawickreme et al., 2019: 322). Clinical manifestations of *Brugmansia* intoxication have been documented in several studies. In an Australian investigation involving adult patients, mydriasis was observed in all cases (100%), followed by delirium in 88% and tachycardia in 33% of individuals (Isbister et al., 2003: 376). Similarly, a hospital-based prospective study conducted in Sri Lanka in 2006 focusing on pediatric plant poisonings identified seven cases attributed to *D. stramonium,* characterized by symptoms such as hyperthermia, dryness of the skin and mucosa, agitation, mydriasis, tachycardia, confusion, hallucinations, and urinary retention. Within this cohort, hyperthermia, mydriasis, and tachycardia were present in all patients (100%), whereas delirium and hallucinations were reported in 71% and 14% of cases, respectively (Lucas, 2006: 111). In more severe instances, intoxication may progress to life-threatening complications, including respiratory failure and cardiovascular collapse (Kurzbaum et al., 2001: 538). Following ingestion, the onset of *Brugmansia* toxicity generally occurs within approximately 30 minutes. Among the observed clinical features, mydriasis may persist for an average of 29 hours, while delirium typically continues for around 18 hours before gradually resolving within approximately 15 hours; pupillary dilation, however, may take up to 24 hours to improve after onset (Isbister et al., 2003: 376).

When *Brugmansia* leaves are added to beverages, particularly those commonly consumed with *Asparagus racemosus* and *Centella asiatica*, an interesting pharmacological interaction may occur. Although *A. racemosus* and *C. asiatica* possess anticholinesterase activity that could theoretically antagonize *Brugmansia*'s anticholinergic effects, the high toxicity of *Brugmansia* and the predominance of its toxic dose reportedly prevent these plants from fully neutralizing its toxic effects (Uddin et al., 2016: 1; Arora et al., 2018: 1344). In a reported case, the patient presented six hours after ingestion, making gastric lavage too late. However, due to delayed gastric emptying caused by the toxin's anticholinergic effects, activated charcoal was administered. Management of acute urinary retention involved catheterization accompanied by adequate fluid replacement. Agitation was effectively controlled with benzodiazepines, while the use of phenothiazines is generally discouraged in cases of agitated delirium due to their anticholinergic effects. In contrast, barbiturates may be considered for seizures that do not respond to benzodiazepine therapy (Kurzbaum et al., 2001: 538). Although physostigmine, a cholinesterase inhibitor, is recognized as a potential antidote for anticholinergic toxicity, its clinical application remains debated despite evidence supporting its safe use. Furthermore, it is relatively contraindicated in patients with cardiac conduction abnormalities. Consequently, physostigmine is typically reserved for severe cases, such as benzodiazepine-resistant agitation or psychosis, refractory seizures, or instances of coma and tachyarrhythmias associated with hemodynamic instability. In the management of this particular case, administration of physostigmine was deemed unnecessary (Kurzbaum et al., 2001: 538; Krenzelok, 2010: 104).

B. suaveolens, which is widespread across Africa, America, Asia, Australia, and Europe, has traditionally been used to treat inflammation, arthritis, wounds, pain, dysmenorrhea, dermatitis, ulcers, fungal infections of the skin, abscesses, and as an antiseptic for vaginal discharges (Reis et al., 2019: 34; De Feo, 2004: 221;

Pundir et al., 2022: 266). Pharmacological studies have showed that *B. suaveolens* possesses antinociceptive (Parker et al., 2007: 234), wound-healing (Schmidt et al., 2009: 523), nematicidal (Nandakumar et al., 2017: 39), and analgesic (Muccillo-Baisch et al., 2010: 345) activities. Hyoscyamine, a tropane alkaloid component of *B. suaveolens*, has been identified as an important bioactive compound with anticancer activity, while also being responsible for severe anticholinergic toxic effects such as delirium, hallucinations, and psychosis (Pundir et al., 2022: 266). Consumption of *B. suaveolens* at high doses can stimulate the central nervous system and suppress peripheral nerves, leading to adverse effects such as hallucinations, disorientation, and panic. In more severe cases, neurological depression accompanied by respiratory and cardiovascular system disturbances may occur, which can be fatal (Anthony et al., 2009: 76; Goldfarb et al., 2019: 59).

Although there are traditional reports indicating that species of the genus *Brugmansia* possess diverse pharmacological properties, research providing experimental evidence to support these traditional uses remains limited (Da Costa et al., 2023a: 2912). Nevertheless, studies have demonstrated that *Brugmansia* species exhibit a range of pharmacological activities, including cytotoxicity (Schmidt et al., 2009: 523; Kim et al., 2018: 687; Kumar et al., 2020: 115552), antiinflammatory effects (Schmidt et al., 2009: 523; Kim et al., 2018: 687), antioxidant activity (Ramos et al., 2003: 241; Kim et al., 2020: 163), antispasmodic effects (Encarnación-Dimayuga et al., 1998: 124), antibacterial activity (San Luis et al., 2014: 56), antiaddictive properties (Bracci et al., 2013: 482976), antiasthmatic effects (Anisa et al., 2016: 1), analgesic activity (Akram et al., 2013: 50), and antiprotozoal effects (Deharo et al., 2001: 91; Gachet et al., 2010: 184; Monzote et al., 2016: 1785).

5.1. Cytotoxicity

Bioactive compounds isolated from medicinal plants have attracted significant interest due to their potential cytotoxic and antiproliferative effects. Two newly identified benzonitrile glycosides, brugmansioside A (compound 180) and brugmansioside B (compound 181), isolated from the ethyl acetate extracts of *B. arborea* flowers, exhibited in vitro cytotoxic activity against human gastric adenocarcinoma (AGS) and hepatocellular carcinoma (HepG2) cell lines. Brugmansioside A exhibited moderate cytotoxic effects at concentrations above 25 µg/mL for AGS cells and 12.5 µg/mL for HepG2 cells, whereas brugmansioside B showed activity at concentrations above 100 µg/mL for AGS and 25 µg/mL for HepG2 (Kim et al., 2018: 687).

Cytotoxic potential was also assessed in lipophilic extracts derived from the leaves, aerial parts, and flowers of *B. suaveolens*. Results indicated that n-hexane and ethanolic extracts exhibited cytotoxicity with inhibition values of 92 and 86% at 100 µg/mL, respectively (Schmidt et al., 2009: 523). However, the specific bioactive compounds responsible for these effects were not definitively identified, highlighting a key limitation and the need for bioactivity-guided fractionation and repeated MTT assays.

According to ISO 10993-5 standards, cell viability below 75% is considered indicative of cytotoxicity (Codevilla et al., 2018: e20170362). Ethanol (70%) leaf extracts of *B. suaveolens* were evaluated across a range of concentrations (5-500 µg/mL) in different cell models. Macrophages (J774) were identified as the most sensitive cell type, while fibroblasts and keratinocytes only exhibited cytotoxicity at the highest concentration (500 µg/mL) (Da Costa et al., 2023b: 3177). The low CC_{50} (29.56 µg/mL) and CC_{90} (174.13 µg/mL) values in macrophages suggest a pronounced biological effect on immune cells, potentially associated with the plant’s tropane alkaloids. Notably, cytotoxicity observed in keratinocytes at 500 μg/mL is relevant for topical applications, as these cells constitute the epidermis and are involved in inflammatory mediator production (Da Costa et al., 2023b: 3177).

Phytochemicals have been established as valuable sources for anticancer drug discovery. Plant-derived anticancer candidates and their derivatives continue to play a central role in drug development (Cragg & Newman, 2013: 3670). The immune system can prevent cancer development through long-term memory and selective targeting of tumor cells without affecting normal cells. Immunological studies provide strong evidence that tumors are recognized and controlled via immunosurveillance mechanisms (Dranoff, 2004: 33). Immunotherapy, which enhances tumor recognition and/or strengthens antitumor immune responses, has been identified as one of the most promising lifelong therapeutic approaches in cancer treatment (Finn, 2008: 2704). *Brugmansia* and *Datura* have demonstrated immunomodulatory activity (Shathish & Guruvayoorappan, 2014: 623; Tiwari et al., 2014: 77; Kumar et al., 2020: 115552). The ethanolic extracts of *B. suaveolens* leaves have been investigated for immune-mediated anticancer activity (Kumar et al., 2020: 115552). A monoterpenoid compound isolated from the extract, SUPH036-022A, increased IL-2 and IFN-γ secretion in human peripheral blood mononuclear cells (PBMCs). SUPH036-022A exposure in PBMCs enhanced cytotoxic effects against MCF7 and A549 cells through ROS generation and mitochondrial membrane destabilization, leading to apoptotic cell death. In the absence of PBMCs, SUPH036-022A displayed only weak cytotoxic activity against cancer cells, indicating that it acts as a lead compound for immune-mediated anticancer activity (Kumar et al., 2020: 115552).

5.2. Antiinflammatory Activity

Inflammation affects a substantial proportion of the global population, underscoring the growing need for alternative therapeutic approaches (Pérez-González et al., 2025: 117972). Despite extensive traditional use, only a limited number of pharmacological investigations have explored the antiinflammatory potential of plant extracts and isolated compounds from certain *Brugmansia* species.

The methanolic extract of *B. arborea* flowers, along with five isolated flavonoids, demonstrated antiinflammatory activity by inhibiting nitric oxide (NO) production in RAW264.7 mouse macrophage cells without significant cytotoxicity (Kim et al., 2020: 163). Furthermore, the isolated flavonoids markedly reduced the lipopolysaccharide-induced increase in cyclooxygenase-2 (COX-2) protein, indicating a mechanistic link between the flavonoid content and the plant’s traditional antiinflammatory potential (Kim et al., 2020: 163). However, these *in vitro* findings require further validation at both *in vitro* and *in vivo* levels (Algradi et al., 2021: 113910). In another study, ethanol extracts (100 µg/mL) of *B. suaveolens* flowers and leaves were assessed for antiinflammatory activity via cellular electrophoretic analyses of NF-κB, p38, TNF-α, and elastase. The results demonstrated inhibition of NF-κB DNA-binding and p38 activity, as well as direct modulation of elastase activity (Schmidt et al., 2009: 523). A noted limitation of such studies is the lack of isolation and identification of the specific bioactive compounds responsible for these effects (Da Costa et al., 2023b: 3177).

Analysis of *in vitro* cultured leaf explants and extracts from natural *B. arborea* plants using gas chromatography–mass spectrometry and thin-layer chromatography identified 18 major metabolites. Acetylated extracts were found to contain acetyl-rutin, whereas hydrolyzed extracts yielded kaempferol (Pérez-González et al., 2025: 117972). By suppressing tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), and nuclear factor kappa B (NF-κB), acetyl-rutin exerts anti-inflammatory effects. Additionally, it impeded leukocyte migration to damaged tissue and strengthened capillary vascular structures (Khorsheed & Raghif, 2024). In the hydrolyzed extract, kaempferol was identified as the main compound with antiinflammatory activity (Pérez-González et al., 2025: 117972). Kaempferol, a bioflavonoid, exhibits multiple antioxidant and antiinflammatory properties (Park et al., 2021: 3079). It suppresses the release of pro-inflammatory mediators associated with oxidative stress, including IL-1β, IL-18, IL-6, and TNF-α, while also acting as a natural antioxidant (Alam et al., 2020: 4073). The inhibitory effect of kaempferol on COX-1 and COX-2 is mediated by its ability to reduce kinase activation induced by UVB exposure (Devi et al., 2015: 1). Moreover, in normal human dermal fibroblasts, kaempferol suppresses intracellular ROS production induced by 12-O-tetradecanoylphorbol-13-acetate (TPA). It also inhibits TPA-induced c-Jun N-terminal kinase (JNK) phosphorylation, NF-κB and IκBα phosphorylation required for cleaved caspase-3 expression and IL-1β secretion. TPA (5 μM) significantly induced cytotoxicity in dermal fibroblasts and elevated mRNA levels of several pro-inflammatory cytokines, particularly IL-1β. Treatment with 100 nM kaempferol significantly attenuated TPA-induced fibroblast cytotoxicity and IL-1β expression. Consequently, kaempferol functions as an effective antiinflammatory agent by disrupting signaling cascades involved in fibroblast-mediated skin inflammation (Park et al., 2021: 3079).

5.3. Antioxidant Activity

Medicinal plants serve as abundant reservoirs of bioactive constituents, including phenolics, flavonoids, alkaloids, and various other antioxidant compounds. To date, research on antioxidant activity within the genus *Brugmansia* has primarily concentrated on *B. arborea*, *B. candida*, and *B. suaveolens*, and has largely been restricted to evaluations of total phenolic content (TPC) together with antioxidant assays such as ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, and FRAP (ferric reducing antioxidant power) (Ramos et al., 2003: 241; Kim et al., 2020: 163; Pérez-González et al., 2025: 117972; Hanumanthappa et al., 2026).

ABTS is a synthetic, water-soluble compound used to assess antioxidant capacity and monitor enzymatic reaction kinetics. Oxidation with potassium persulfate generates a blue-green radical cation ($ABTS^{\bullet+}$), which is decolorized upon reduction by antioxidants (Re et al., 1999: 1231). In *B. arborea* L. flower methanol extracts, n-butanol and ethyl acetate fractions, along with five flavonoid components [kaempferin (1), kaempferitrin (2), kaempferol 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside (3), quercetin 3,7-di-O-α-L-rhamnopyranoside (4), and (+)-aromadendrin (5)], were evaluated for ABTS antioxidant capacity. Butanol fractions contained compounds with higher antioxidant capacity, with flavonoid activity ranking as 5 > 1 > 4 > 3 > 2 (Kim et al., 2020: 163). ABTS-measured antioxidant capacities correlate with the number of –OH groups on the flavonoid core (Kim & Lee, 2004: 253). Aromadendrin, possessing four –OH groups, exhibited the highest antioxidant capacity. Monoglycosidic flavonoid kaempferin showed higher capacity than the diglycosides (kaempferol 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside, kaempferitrin, and quercetin 3,7-di-O-α-L-rhamnopyranoside) due to steric hindrance from sugar moieties. Quercetin 3,7-di-O-α-L-rhamnopyranoside contains a catechol moiety, known to confer high radical scavenging activity, exhibiting greater antioxidant capacity than kaempferol diglycosides (Brand-Williams et al., 1995: 25; Kim & Lee, 2004: 253; Kim et al., 2020: 163).

The DPPH assay is commonly employed to assess antioxidant capacity (Gulcin & Alwasel, 2023: 2248). Among the five flavonoids from *B. arborea* flower methanol fractions, aromadendrin demonstrated the highest DPPH activity (Kim et al., 2020: 163). Quercetin-3-rhamnoside (quercitrin) exhibited roughly half the antioxidant activity of quercetin. DPPH assay evaluates radical-scavenging activity in apolar solvent systems; thus, diglycosides exhibited lower activity than in ABTS assays (Yokozawa et al., 1998: 213; Kim et al., 2002: 3713). Hydroalcoholic leaf and flower extracts of *B. candida* showed DPPH IC_{50} values of 314 µg/mL, indicating antioxidant agents capable of protecting DNA against oxidative stress (Ramos et al., 2003: 241). Encapsulated chitosan nanoparticles of *B. suaveolens* (BSLEE CNPs) demonstrated enhanced antioxidant potential (DPPH, FRAP, TPC) (Hanumanthappa et al., 2026). Conversely, methanol:chloroform extract of *B. arborea* showed weak DPPH activity with an IC_{50} of 199.52 mg/mL (Pérez-González et al., 2025: 117972).

5.4. Antispasmodic Activity

Anticholinergic agents are compounds that inhibit the action of the neurotransmitter acetylcholine (ACh) at synapses within both the central and peripheral nervous systems. These substances selectively inhibit the binding of ACh to its receptors, thereby suppressing parasympathetic nervous system activity. In a study evaluating the spasmolytic (anticholinergic) effects of methanolic extracts from 141 medicinal plant species on electrically stimulated guinea pig ileum, six species—including *B. candida*—exhibited significant spasmolytic activity, highlighting their therapeutic potential (Tortoriello et al., 1995: 57). Among 48 traditionally used medicinal plants, ethanol extracts at concentrations ≤ 300 mg/mL affected rabbit jejunum smooth muscle contractions in 35 species, while 13 species showed no activity. The highest inhibitory activity was observed with the extract of *B. suaveolens*. Under the same experimental conditions, the inhibition profile of the *B. suaveolens* E-337 extract was comparable to that of scopolamine, demonstrating a similar inhibitory and anticholinergic effect (Encarnación-Dimayuga et al., 1998: 124). Tropane alkaloids are known to antagonize the muscarinic effects of acetylcholine, producing atropine-like antimuscarinic activity. Methanolic and aqueous extracts, chromatographic fractions, and three purified alkaloids isolated from *B. arborea* were found to suppress ACh-induced contractions in isolated guinea pig ileum, with relative efficacy ranked as atropine > nor-hyosine > scopolamine (Capasso et al., 2008: 43). The presence of these anticholinergic compounds explains the traditional use of this plant as an antispasmodic (De Feo, 1992: 417). Acetylcholine plays a critical role in the manifestation of opioid withdrawal. Cholinergic agonists are known to intensify withdrawal symptoms, while muscarinic and nicotinic antagonists may relieve some features of the syndrome (Martin & Eades, 1967: 195; Bhargava & Way, 1972: 31). *In vitro* studies have shown that a significant portion of contractions observed during opioid withdrawal depends on ACh release, as these contractions can be blocked by atropine or hyoscyamine (Tsou et al., 1982: 377; Chahl, 1983: 741). The alkaloids atropine, scopolamine, and nor-hyosine from *B. arborea* inhibit both electrically and ACh-induced contractions in the guinea pig ileum (Capasso et al., 1997: 43). Therefore, the anticholinergic effects of these alkaloids likely contribute to the observed antispasmodic activity of *B. arborea* (Capasso & De Feo, 2003: 243).

5.5. Antimicrobial Activity

In antimicrobial studies, organisms associated with human infections and antimicrobial resistance are typically selected as test strains. Hydroalcoholic extracts of *B. suaveolens* flowers and leaves were evaluated against bacteria including *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, as well as yeast strains *Candida albicans* and *Yarrowia lipolytica*. The leaf extract was observed to be more effective against most strains. Notably, *K. pneumoniae* and *P. mirabilis* were the most sensitive microorganisms, exhibiting over 90% growth inhibition in response to the extracts (Da Costa et al., 2023a: 2912). *P. mirabilis*, a common causative agent of urinary, systemic, and ascending infections, is particularly relevant in hospital settings, frequently affecting patients with urinary catheters (Muthusamy et al., 2010: 897). Methanolic extracts of *B. suaveolens* roots, stems, leaves, and flowers, which are rich in secondary metabolites such as flavonoids and tannins, demonstrated antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, *Bacillus cereus*, and *Salmonella typhimurium* (San Luis et al., 2014: 56). In contrast, aqueous extracts of *B. suaveolens* flowers showed no activity against *Bacillus thuringiensis* (Vilani et al., 2017: 1051). Conversely, n-hexane and ethanol extracts of *B. suaveolens* exhibited antibacterial activity against *Bacillus subtilis*, comparable to the standard antibiotic vancomycin (Schmidt et al., 2009: 523). Additionally, *B. suaveolens* encapsulated in chitosan nanoparticles (BSLEE CNPs) demonstrated enhanced antibacterial potential against *E. coli* and *S. aureus* (Hanumanthappa et al., 2026).

5.6. Antiaddictive

Substance addiction is a long-term, relapsing condition marked by a compulsive drive to obtain and use a substance, impaired control over consumption, and the development of negative emotional states when the substance is unavailable (Koob & Le Moal, 1997: 52).

Although a number of pharmacological strategies are approved for managing opioid addiction—including natural poppy-derived alkaloids such as morphine, codeine, and thebaine—their limited effectiveness highlights the need for new therapeutic alternatives (Bracci et al., 2013: 482976). Opioid addiction, involving all substances that bind to opioid receptors and exert morphine-like effects, is among the most common forms of addiction and is associated with high mortality rates (Mattioli et al., 2012: 741925).

Despite the availability of approved pharmacological treatments for opioid addiction, their limited effectiveness highlights the need for new therapeutic strategies. In this context, few pharmacological studies exist on *Brugmansia* species, which are rich in secondary metabolites. *B. arborea* has been shown to reduce the development and expression of morphine tolerance and dependence (Mattioli et al., 2012: 741925). Isolated tropane alkaloids from *B. arborea*, including pure atropine, scopolamine, and nor-hyosin, applied 10 minutes prior to morphine at concentrations of 10^{-7}, 5×10^{-7}, and 10^{-6} M, significantly and concentration-dependently reduced morphine withdrawal *in vitro*. Due to this remarkable pharmacological effect, these tropane alkaloids are considered potential antiaddictive agents (Capasso & De Feo, 2003: 243). Different doses (7.5-60 mg/kg) of methanol extracts from *B. arborea* flowers and leaves did not affect motor activity and showed no effect in the conditioned place preference (CPP) test (Bracci et al., 2013: 482976). The CPP paradigm is widely used to evaluate the conditioned rewarding effects of addictive drugs, as contextual cues paired with a primary reinforcer can acquire secondary reinforcing properties, thereby increasing abuse potential (Tzschentke, 2007: 227). Conversely, the extract partially reduced morphine-induced motor activity (20 and 40 mg/kg) and completely blocked morphine-induced place preference. It also suppressed cocaine-induced hyperactivity but did not affect cocaine-induced place preference. These findings suggest that *B. arborea* may act via dopaminergic and cholinergic pathways, offering potential as an adjunct agent in the treatment of morphine and cocaine addiction (Bracci et al., 2013: 482976). Extracts, chromatographic fractions, and isolated pure compounds from *B. arborea* have demonstrated central nervous system activity (Capasso & De Feo, 2002: 274). The aqueous extract exhibited affinity for serotonergic 5-HT1A, 5-HT2A, and 5-HT2C receptors, which play key roles in addiction-related biological systems (Capasso & De Feo, 2007: 599). Furthermore, *in vitro* studies demonstrated that *B. arborea* extracts interact with central dopaminergic receptors. Radioligand binding assays confirmed the extract's affinity for 5-HT1A, 5-HT2A, 5-HT2C, D1, D2, α1, and α2 receptors. These receptor-binding findings support the plant's traditional and ritual uses (Nencini et al., 2006: 352).

5.7. Antinociceptive (Analgesic) Activity

Pain is a common symptom associated with various diseases, and antinociceptive (analgesic) drugs remain a major focus of pharmaceutical research. Antinociceptive agents are also utilized within the Unani medical system, a traditional medicine framework based on herbal and natural remedies. Originating from Ancient Greek medicine (Greek: *Ionia*), Unani medicine was further developed by Arab and Iranian scholars and is widely practiced in South Asia, particularly in India, Pakistan, and Bangladesh. Although various analgesic drugs are commercially available, they can cause adverse effects such as heartburn and gastric ulcers. Therefore, the development of plant-based preparations with analgesic activity and minimal side effects is gaining importance (Akram et al., 2013: 50). In addition to saponins and flavonoids, tannins and terpenoids are important constituents contributing to analgesic activity (Okokon et al., 2012: 214). Flavonoids mainly target prostaglandin synthesis, which plays a central role in pain perception, whereas alkaloids are well-known for their pain-relieving effects (Salawu et al., 2008: 213; Uche & Aprioku, 2008: 99). *B. suaveolens* infusions have been used for pain management. The aqueous extract of *B. suaveolens* flowers was assessed for antinociceptive activity in mice using acetic acid–induced formalin, tail-flick, writhing, and hot-plate assays. Intraperitoneal administration of the extract at different concentrations significantly inhibited abdominal contractions, increased response latency, or markedly suppressed nociceptive behavior, demonstrating clear antinociceptive effects and supporting the plant’s traditional analgesic use (Parker et al., 2007: 234). Furthermore, methanolic leaf extracts of *B. suaveolens* conjugated with silver nanoparticles (AgNPs) were assessed for analgesic activity using writhing and tail-flick tests. The biosynthesized AgNPs serve as drug carriers, enhancing the delivery of active compounds to target tissues, which likely results in greater efficacy compared to crude extracts. This improved activity may be attributed to the enhanced stability and potency of the conjugated phytochemicals on the surface of AgNPs (Santhosh et al., 2024: 138).

5.8. Antiasthmatic Activity

The antiasthmatic potential of an aqueous extract derived from *B. suaveolens* leaves was evaluated in guinea pigs using the in vivo Vogel method. Prior to oral administration, the aqueous fractions were prepared in distilled water. The findings indicated that a dose of 40 mg/kg body weight produced the most significant effect, outperforming two lower doses and showing comparable efficacy to 0.16 mg/kg of salbutamol sulfate. The activity observed may be attributed to the presence of tropane alkaloids, which could account for both the plant's traditional use and its bronchodilatory effects. These results emphasize the importance of further studies to clarify the underlying mechanisms and isolate the compounds responsible for this activity (Anisa et al., 2016: 1).

5.9. Antimalarial Activity

In traditional medicine, 125 extracts from 122 plant species, including *B. arborea*, were tested *in vitro* for activity against chloroquine-sensitive and resistant strains of *Plasmodium falciparum* and evaluated *in vivo* in *Plasmodium berghei*-infected rodents. Ethanol extracts of *B. arborea* leaves did not demonstrate antimalarial activity (Deharo et al., 2001: 91). In another study, dichloromethane and methanol extracts of *B. suaveolens* leaves exhibited *in vitro* activity against *Leishmania donovani* axenic amastigotes and *P. falciparum* K1 strain (Gachet et al., 2010: 184). Similarly, ethanol-water extracts from different parts of *B. suaveolens* showed *in vitro* activity against *Leishmania amazonensis* promastigotes (Monzote et al., 2016: 1785).

References

Ainsworth, S. J., (1996). Synthesis of exotic soaps in the chemistry laboratory Chem. *Engineering News, 74*, 35.

Akram, M., Asif, H. M., Usmanghani, K., Hamid, A., & Nawaz, A. (2013). Anti-nociceptive activities of medicinal plants: A Review. *RADS Journal of Biological Research & Applied Sciences, 4(1),* 50-58.

Alam, W., Khan, H., Shah, M. A., Cauli, O., & Saso, L. (2020). Kaempferol as a dietary anti-inflammatory agent: current therapeutic standing. *Molecules, 25(18)*, 4073.

Algradi, A.M., Liu, Y., Yang, B.Y., & Kuang, H. X. (2021). Review on the genus *Brugmansia*: Traditional usage, phytochemistry, pharmacology, and toxicity. *Journal of Ethnopharmacology*, *279,* 113910.

Alves, M. N., Sartoratto, A., & Trigo, J.R. (2007). Scopolamine in *Brugmansia suaveolens* (Solanaceae): Defense, allocation, costs, and induced response. *Journal of Chemical Ecology, 33(2),* 297-309.

Anisa, I. N., Soedarmadji, A. A., & Djuliana, D. (2016). Uji efek bronkodilator ekstrak air bunga kecubung gunung (*Brugmansia suaveolens* Bercht & Presl). *Kartika: Jurnal Ilmiah Farmasi*, *4(1)*, 1-4.

Anonymous (2009). Genus: *Brugmansia* Pers. Germplasm Resources Information Network. United States Department of Agriculture. 2009-09-01.

Anthony, S. J., Zuchowski, W., & Setzer, W. N. (2009). Composition of the floral essential oil of *Brugmansia suaveolens*. *Records of Natural Products, 3(2),* 76.

Arora, R., Kumar, R., Agarwal, A., Reeta, K. H., & Gupta, Y. K. (2018). Comparison of three different extracts of *Centella asiatica* for anti-amnesic, antioxidant and anticholinergic activities: *In vitro* and *in vivo* study. *Biomedicine & Pharmacotherapy*, *105,* 1344-1352.

Bais, H. P., Park, S.W., Weir, T. L., Callaway, R. M., & Vivanco, J. M. (2004). How plants communicate using the underground information superhighway. *Trends in Plant Science*, *9(1)*, 26-32.

Begum, M., Gurr, G. M., Wratten, S. D., Hedberg, P. R. & Nicol, H. I. (2006). Using selective food plants to maximize biological control of vineyard pests. *Journal of Applied Ecology, 43*, 547-554.

Bhargava, H. N., & Way, E. L. (1972). Acetylcholinesterase inhibition and morphine effects in morphine tolerant and dependent mice. *The Journal of Pharmacology & Experimental Therapeutics*, *183(1)*, 31-40.

Bracci, A., Daza-Losada, M., Aguilar, M., De Feo, V., Miñarro, J., & Rodríguez-Arias, M. (2013). A methanol extract of *Brugmansia arborea* affects the reinforcing and motor effects of morphine and cocaine in mice. *Evidence-Based Complementary* & *Alternative Medicine*, *2013(1),* 482976.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science* & *Technology*, *28(1)*, 25-30.

Brickell, C. D., Alexander, C., Cubey, J. J., David, J. C., Hoffmann, M. H. A., Leslie, A. C., Malécot, V., & Jin, X. B. (2016). International Code of Nomenclature for Cultivated Plants. Bristol M L, 1966, Notes on The Species of Tree Daturas, Botanical Museum Leaflets, Harvard University 21, 229-248.

Bussmann, R. W., Glenn, A., & Sharon, D. (2010). Healing the body and soul: Traditional remedies for “magical” ailments, nervous system and psychosomatic disorders in Northern Peru. *African Journal of Pharmacy & Pharmacology*, *4(9),* 580-629.

Bussmann, R. W., Zambrana, N. Y. P., Huanca, L. A. M., & Hart, R. (2016). Changing markets-medicinal plants in the markets of La Paz and El Alto, Bolivia. *Journal of Ethnopharmacology*, *193*, 76-95.

Bussmann, R. W., & Sharon, D. (2006). Traditional medicinal plant use in Northern Peru: Tracking two thousand years of healing culture. *Journal of Ethnobiology & Ethnomedicine, 2(1), 47.*

Bye, R., & Sosa, V. (2013). Molecular phylogeny of the jimsonweed genus *Datura* (Solanaceae). *Systematic Botany*, *38(3),* 818-829.

Calixto, J. B. (2019). The role of natural products in modern drug discovery. *Anais da Academia Brasileira de Ciências*, *91*, e20190105.

Campos, D. J. (2011). Journeys to the Sacred Realms: The Shaman and Ayahuasca. Studio City, CA: Divine Arts Media.

Capasso, A., De Feo, V., De Simone, F., & Sorrentino, L. (1997). Activity-directed isolation of spasmolytic (anti-cholinergic) alkaloids from *Brugmansia arborea* (L.) Lagerheim. *International Journal of Pharmacognosy*, *35(1)*, 43-48.

Capasso, A., & Feo, V. D. (2002). Central nervous system pharmacological effects of plants from northern Peruvian Andes: *Valeriana adscendens*, *Iresine herbstii* and *Brugmansia arborea*. *Pharmaceutical Biology*, *40(4)*, 274-293.

Capasso, A., & De Feo, V. (2003). Alkaloids from *Brugmansia arborea* (L.) Lagerhein reduce morphine withdrawal *in vitro*. *Phytotherapy Research*, *17*, 826-829.

Capasso, A., & De Feo, V. (2007). *In vitro* binding receptors study by *Valeriana adscendens*, *Iresine herbstii* and *Brugmansia arborea* extracts. *Medicinal Chemistry, 3(6),* 599-604.

Capasso, A., De Feo, V., De Simone, F., & Sorrentino, L. (1997). Activity-directed isolation of spasmolytic (anti-cholinergic) alkaloids from *Brugmansia arborea* (L.) Lagerheim. *International Journal of Pharmacognosy*, *35(1)*, 43-48.

Capasso, A., De Feo, V., & Sorrentino, L. (2008). Activity-directed isolation of spasmolytic (anticholinergic) alkaloids from

Brugmansia arborea (L.) Lagerheim. *International Journal of Pharmacognosy*, *35(1)*, 43-48.

Carrillo, A. C., Molano, C. E. R., & López, C. O. (2011). *In vitro* insecticidal effect of the ethanolic extract from some plants on the adult fly *Haematobia irritans*. *Revista Cubana de Plantas Medicinales*, *16(3)*, 216-226.

Chahl, L. A. (1983). Contracture of guinea-pig ileum on withdrawal of methionine5-enkephalin is mediated by substance P. *British Journal of Pharmacology*, *80(4)*, 741-749.

Codevilla, C. F., Tischer, B., Gindri, A. L., Nogueira-Librelotto, D. R., Barin, J. S., Silva, C. D. B. D., Rolim, C. M. B., Zepka, L. Q., & Menezes, C. R. D. (2018). Cytotoxicity and antioxidant activity of goldenberry extracts obtained with high intensity ultrasound. *Ciência Rural*, *48(02)*, e20170362.

Coremans, P., Lambrecht, G., Schepens, P., Vanwelden, J., & Verhaegen, H. (1994). Anticholinergic intoxication with commercially available thorn apple tea. *Journal of Toxicology: Clinical Toxicology*, *32(5)*, 589-592.

Cragg, G. M., & Newman, D. J. (2013). Natural products: A continuing source of novel drug leads. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1830(6)*, 3670-3695.

Da Costa, S. P., Schuenck-Rodrigues, R. A., Cardoso, V. D. S., Valverde, S. S., Vermelho, A. B., & Ricci-Júnior, E. (2023a). Therapeutic Potential of Bioactive Compounds from *Brugmansia suaveolens* Bercht. & J. Presl. *Nutrients*, *15(13)*, 2912.

Da Costa, S. P., Schuenck-Rodrigues, R. A., Cardoso, V. D. S., Valverde, S. S., Beatriz Vermelho, A., & Ricci-Júnior, E. (2023b). Phytochemical analysis of *Brugmansia suaveolens* Bercht. & J. Presl and its therapeutic potential for topical use. *Natural Product Research*, *37(18)*, 3177-3183.

D'Arcy, W. G. (Ed.). (1986). Solanaceae, Biology and Systematics. Columbia University Press.

De Feo, V. (1992). Medicinal and magical plants in the Northern Peruvian Andes. *Fitoterapia-Milano*, *63*, 417-417.

De Feo, V. (2003). Ethnomedical field study in Northern Peruvian Andes with particular reference to divination practices. *Journal of Ethnopharmacology*, *85(2-3)*, 243-256.

De Feo, V. (2004). The ritual use of *Brugmansia* species in traditional Andean medicine in Northern Peru. *Economic Botany*, *58(1)*, S221-S229.

De Melo, G. O., Malvar, D. D. C., Vanderlinde, F. A., Rocha, F. F., Pires, P. A., Costa, E. A., Matos, L. G. Kaiser, C. R., & Costa, S. S. (2009). Antinociceptive and anti-inflammatory kaempferol glycosides from Sedum dendroideum. *Journal of Ethnopharmacology*, *124(2)*, 228-232.

De Simone, R., Margarucci, L., & De Feo, V. (2008). Tropane alkaloids: An overview. *Pharmacologyonline*, *1*, 70-89.

Deharo, E., Bourdy, G., Quenevo, C., Munoz, V., Ruiz, G., & Sauvain, M. (2001). A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. *Journal of Ethnopharmacology*, *77(1)*, 91-98.

Detzel, A., & Wink, M. (1993). Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. *Chemoecology*, *4(1)*, 8-18.

Devi, K. P., Malar, D. S., Nabavi, S. F., Sureda, A., Xiao, J., Nabavi, S. M., & Daglia, M. (2015). Kaempferol and inflammation: From chemistry to medicine. *Pharmacological Research*, *99*, 1-10.

Diaz, G. J. (2015). Toxicosis by plant alkaloids in humans and animals in Colombia. *Toxins*, *7(12)*, 5408-5416.

Doncheva, T., Berkov, S., & Philipov, S. (2006). Comparative study of the alkaloids in tribe Datureae and their chemosystematic significance. *Biochemical Systematics & Ecology*, *34(6)*, 478-488.

Dranoff, G. (2004). Cytokines in cancer pathogenesis and cancer therapy. *Nature Reviews Cancer*, *4(1)*, 11-22.

Duke, J. A., Martinez, R. V. (1994). Amazonian Ethnobotanical Dictionary. CRC Press. p. 33, ISBN 9780849336645.

Encarnación-Dimayuga, R., Altamirano, L., & Maki, K. A. (1998). Screening of medicinal plants from Baja California Sur (Mexico) by their effects on smooth muscle contractility. *Pharmaceutical Biology*, *36(2)*, 124-130.

Evans, W. C. (1996). Tropane alkaloids. Trease and Evans Pharmacognosy. London, UK: Saunders Company Ltd, 346-358.

Evans, W. C., & Lampard, J. F. (1972). Alkaloids of *Datura suaveolens*. *Phytochemistry, 11(11)*, 3293-3298.

Finn, O. J., (2008). Cancer immunology. *New England Journal of Medicine*, *358(25)*, 2704-2715.

Freitas, A. V., Trigo, J. R., Brown Jr, K. S., Witte, L., Hartmann, T., & Barata, L. E. (1996). Tropane and pyrrolizidine alkaloids in the ithomiines *Placidula euryanassa* and *Miraleria cymothoe* (Lepidoptera: Nymphalidae). *Chemoecology*, *7(2)*, 61-67.

Fuller, T. C., & McClintock, E. M. (1986). Poisonous Plants of California (Vol. 53). University of California Press, pp. 233-235, ISBN 9780520055698.

Furlan, V., Kujawska, M., Hilgert, N. I., & Pochettino, M. L. (2016). To what extent are medicinal plants shared between country home gardens and urban ones? A case study from Misiones, Argentina. *Pharmaceutical Biology, 54(9)*, 1628-1640.

Gachet, M. S., Lecaro, J. S., Kaiser, M., Brun, R., Navarrete, H., Muñoz, R. A., Bauer, R., & Schühly, W. (2010). Assessment of anti-protozoal activity of plants traditionally used in Ecuador in the treatment of leishmaniasis. *Journal of Ethnopharmacology, 128(1)*, 184-197.

Geller, F., Murillo, R., Steinhauser, L., Heinzmann, B., Albert, K., Merfort, I., & Laufer, S. (2014). Four new flavonol glycosides from the leaves of *Brugmansia suaveolens*. *Molecules*, *19(5)*, 6727-6736.

Goldfarb, J., Pesin, N., & Margolin, E. (2019). Gardening and dilated pupils: An interesting case of anisocoria from *Brugmansia versicolor. Canadian Journal of Ophthalmology*, *54(2)*, 59-61.

González, G. J. D. (2010). Plantas Tóxicas de Importancia en Salud y Producción Animal en Colombia. Universidad Nacional de Colombia.

Greenberg, M. I. (2006). Disaster!: A Compendium of Terrorist, Natural, and Man-Made Catastrophes. Jones & Bartlett Learning.

Greene, G. S., Patterson, S. G., & Warner, E. (1996). Ingestion of Angel's trumpet: An increasingly common source of toxicity. *Southern Medical Journal*, *89(4)*, 365-369.

Griffin, W. J., & Lin, G. D. (2000). Chemotaxonomy and geographical distribution of tropane alkaloids. *Phytochemistry*, *53(6)*, 623-637.

Gulcin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. *Processes*, *11(8)*, 2248.

Hall, R. C., Popkin, M. K., & McHenry, L. E. (1977). Angel’s Trumpet psychosis: a central nervous system anticholinergic syndrome. *American Journal of Psychiatry*, *134(3)*, 312-314.

Hanumanthappa, S. K., Chandrashekar, A. A., Sanganabassapa, R. B., Pathappa, N., Kathayat, G., Rokaya, D., & Hanumanthappa, M. (2026). Enhanced antioxidant and anti-bacterial potential of *Brugmansia suaveolens* conjugated chitosan nanoparticles. *Current Medicinal Chemistry*.

Haridasan, K., & Rao, R. R. (1985). Forest Flora of Meghalaya. Dehra Dun India.

Harner, M. (1980). The Way of the Shaman. Harper & Row.

Hay, A., Gottschalk, M., & Holguín, A. (2012). Huanduj: *Brugmansia*. Royal Botanic Gardens, Kew.

Hayman, J. (1985). *Datura* poisoning-The Angel's trumpet. *Pathology, 17,* 465-466.

Henson, R. W., Miller, L. P., & Herron, J. T. (1978). Abuse of thorn apple. *Medical Journal of Australia*, *1(5),* 280-280.

Hunziker, A. T. (2001). Genera Solanacearum: The genera of Solanaceae Illustrated, Arranged According to A New System. A Collection of Papers Prepared for Health Canada Ottawa.

Ijaz, F., Iqbal, Z., Rahman, I. U., Alam, J., Khan, S. M., Shah, G. M., Khan, K., & Afzal, A. (2016). Investigation of traditional medicinal floral knowledge of Sarban Hills, Abbottabad, KP, Pakistan. *Journal of Ethnopharmacology*, *179*, 208-233.

Isbister, G. K., Oakley, P., Dawson, A. H., & Whyte, I. M. (2003). Presumed Angel's trumpet (*Brugmansia*) poisoning: Clinical effects and epidemiology. *Emergency Medicine*, *15(4),* 376-382.

IUCN. (2021). The IUCN Red List of Threatened Species (Version 2021-1).

Jayawickreme, K. P., Janaka, K. V. C., & Subasinghe, S. A. S. P. (2019). Unknowing ingestion of *Brugmansia suaveolens* leaves presenting with signs of anticholinergic toxicity: A case report. *Journal of Medical Case Reports*, *13(1)*, 322.

Kennedy David, O. (2014). The Deliriants-The Nightshade (Solanaceae) Family. Plants and the Human Brain, 131-137.

Kerchner, A., & Farkas, Á. (2020). Worldwide poisoning potential of *Brugmansia* and *Datura*. *Forensic Toxicology*, *38(1),* 30-41.

Khorsheed, S. M., & Raghif, A. R. A. (2024). Anti-proliferative, anti-oxidant and anti-inflammatory effects of topical rutin on imiquimod-induced psoriasis in Mice. *Pakistan Journal of Life and Social Sciences, 22(1)*, 1962-1976.

Kim, D. O., & Lee, C. Y. (2004). Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. *Critical Reviews in Food Science & Nutrition*, *44(4),* 253-273.

Kim, H. G., Ko, J. H., Oh, H. J., Song, H. S., Kwon, J. H., Lee, Y. G., Lee, D. Y., Kang, S. C., & Baek, N. I. (2018). New cytotoxic benzonitrile glycosides from *Brugmansia arborea* flowers. *Bulletin of the Korean Chemical Society, 39*(5), 687-690.

Kim, D. O., Lee, K. W., Lee, H. J., & Lee, C. Y. (2002). Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *Journal of Agricultural & Food Chemistry*, *50(13)*, 3713-3717.

Kim, H. G., Jang, D., Jung, Y. S., Oh, H. J., Oh, S. M., Lee, Y. G., Kang, S. C., Kim, D. O., Lee, D. Y., & Baek, N. I. (2020). Anti-inflammatory effect of flavonoids from *Brugmansia arborea* L. flowers. *Journal of Microbiology & Biotechnology, 30(2)*, 163.

Koob, G. F., & Le Moal, M. (1997). Drug abuse: Hedonic homeostatic dysregulation. *Science*, *278(5335)*, 52-58.

Krenzelok, E. P. (2010). Aspects of *Datura* poisoning and treatment. *Clinical Toxicology, 48*, 104-110.

Kujawska, M. (2016). Forms of medical pluralism among the Polish community in Misiones, Argentina. *Anthropology & Medicine, 23*, 205-219.

Kumar, S., Chand, G., Sankhyan, P., Chaudhari Manojkumar, V., Gupta, V., Keshari, B. B., & Sahu, M. (2013). Herbal folk remedies for curing various ailments in Lug Valley of district Kullu, Himachal Pradesh (NW Himalaya). *International Journal of Ayurvedic & Herbal Medicine, 3(5)*, 1308-1314.

Kumar, S., Gupta, A., Saini, R. V., Kumar, A., Dhar, K. L., & Mahindroo, N. (2020). Immunomodulation-mediated anticancer activity of a novel compound from *Brugmansia suaveolens* leaves. *Bioorganic & Medicinal Chemistry*, *28(12)*, 115552.

Kumar, S., Saini, R. V., & Mahindroo, N. (2017). Recent advances in cancer immunology and immunology-based anticancer therapies. *Biomedicine & Pharmacotherapy, 96,* 1491-1500.

Kurzbaum, A., Simsolo, C., Kvasha, L., & Blum, A. (2001). Toxic delirium due to *Datura stramonium*. *The Israel Medical Association Journal, 3(7),* 538-539.

Leonti, M. (2011). The future is written: Impact of scripts on the cognition, selection, knowledge and transmission of medicinal plant use and its implications for ethnobotany and ethnopharmacology. *Journal of Ethnopharmacology, 134(3),* 542-555.

List, G. R., Spencer, G. F., & Hunt, W. H. (1979). Toxic weed seed contaminants in soybean processing. *Journal of the American Oil Chemists' Society, 56(8),* 706-710.

Lockwood, T. E. (1973). Generic recognition of *Brugmansia*. Botanical Museum Leaflets, Harvard University. *23*, 273-284.

Lockwood, T. E. (1974). A Taxonomic Revision of Brugmansia (Solanaceae) (Doctoral dissertation, Harvard University).

Lockwood, T. E. (1979). The ethnobotany of *Brugmansia*. *Journal of Ethnopharmacology, 1*, 147-164.

Lucas, G. N. (2008). Plant poisoning in Sri Lankan children: A hospital based prospective study. *Sri Lanka Journal of Child Health, 35,* 111-124

Lusweti, A., Wabuyele, E., Ssegawa, P., & Mauremootoo, J. R. (2011). Invasive Plants of East Africa (Kenya, Uganda and Tanzania), Lucid v. 3.5 Key and Fact sheets. National Museums of Kenya, Makerere University, BioNET-EAFRINET, CABI & The University of Queensland.

Mahler, D. A. (1976). Anticholinergic poisoning from Jimson weed. *Journal of the American College of Emergency Physicians*, *5(6)*, 440-442.

Mai, N. T. (2017). Quantitative analysis of scopolamine in *Brugmansia suaveolens* by HPLC-MS method. *Journal of Multidisciplinary Engineering Science and Technology*, *4*, 8176-8179.

Marneros, A., Gutmann, P., & Uhlmann, F. (2006). Self-amputation of penis and tongue after use of Angel's Trumpet. *European Archives of Psychiatry & Clinical Neuroscience*, *256(7)*, 458-459.

Martin, W. R., & Eades, C. G. (1967). Pharmacological studies of spinal cord adrenergic and cholinergic mechanisms and their relation to physical dependence on morphine. *Psychopharmacologia*, *11(3)*, 195-223.

Mattioli, L., Bracci, A., Titomanlio, F., Perfumi, M., & De Feo, V. (2012). Effects of *Brugmansia arborea* extract and its secondary metabolites on morphine tolerance and dependence in mice. *Evidence-Based Complementary & Alternative Medicine*, *2012(1)*, 741925.

Mikolich, J. R., Paulson, G. W., & Cross, C. J. (1975). Acute anticholinergic syndrome due to jimson seed ingestion: Clinical and laboratory observation in six cases. *Annals of Internal Medicine*, *83(3),* 321-325.

Monzote, L., Jiménez, J., Cuesta-Rubio, O., Márquez, I., Gutiérrez, Y., da Rocha, C. Q., Marchi, M., Setzer. W. N. Setzer, & Vilegas, W. (2016). *In vitro* assessment of plants growing in Cuba belonging to Solanaceae family against *Leishmania amazonensis*. *Phytotherapy Research*, *30(11),* 1785-1793.

Muccillo-Baisch, A. L., Parker, A. G., Cardoso, G. P., Cezar-Vaz, M. R., & Flores Soares, M. C. (2010). Evaluation of the analgesic effect of aqueous extract of *Brugmansia suaveolens* flower in mice: Possible mechanism involved. *Biological Research for Nursing*, *11(4),* 345-350.

Muthusamy, P., Nivedhitha, M., & Jayshree, N. (2010). Analgesic and anti-inflammatory activities of *Datura metel* Linn. root in experimental animal models. *Research Journal of Pharmacy & Technology*, *3(3),* 897-899.

Namera, A., Yashiki, M., Hirose, Y., Yamaji, S., Tani, T., & Kojima, T. (2002). Quantitative analysis of tropane alkaloids in biological materials by gas chromatography-mass spectrometry. *Forensic Science International*, *130(1),* 34-43.

Nandakumar, A., Vaganan, M. M., Sundararaju, P., & Udayakumar, R. (2017). Nematicidal activity of aqueous leaf extracts of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens*. *American Journal of Entomology*, *1(2),* 39-45.

Nencini, C., Cavallo, F., Bruni, G., Capasso, A., De Feo, V., De Martino, L., & Micheli, L. (2006). Affinity of *Iresine herbstii* and *Brugmansia arborea* extracts on different cerebral receptors. *Journal of Ethnopharmacology*, *105(3),* 352-357.

Newmark, W. D. (2002). Conserving biodiversity in East African forests: a study of the Eastern Arc Mountains (Vol. 155). Springer Science & Business Media.

Ng, J., & Smith, S. D. (2016). Widespread flower color convergence in Solanaceae via alternate biochemical pathways. *New Phytologist*, *209(1),* 407-417.

Nicholi Jr, A. M. (1983). The nontherapeutic use of psychoactive drugs: A modern epidemic. *New England Journal of Medicine*, *308(16),* 925-933.

Noedl, H., Se, Y., Schaecher, K., Smith, B. L., Socheat, D., & Fukuda, M. M. (2008). Evidence of artemisinin-resistant malaria in western Cambodia. *New England Journal of Medicine*, *359(24),* 2619-2620.

Okokon, J. E., Etebong, E. O., Udobang, J. A., & Essien, G. E. (2012). Antiplasmodial and analgesic activities of *Clausena anisata*. *Asian Pacific Journal of Tropical Medicine*, *5(3),* 214-219.

Olmstead, R. G., Bohs, L., Migid, H. A., Santiago-Valentin, E., Garcia, V. F., & Collier, S. M. (2008). A molecular phylogeny of the Solanaceae. *Taxon*, *57(4),* 1159-1181.

Park, S. J., Kim, D. W., Lim, S. R., Sung, J., Kim, T. H., Min, I. S., & Lee, S. J. (2021). Kaempferol blocks the skin fibroblastic interleukin 1β expression and cytotoxicity induced by 12-O-tetradecanoylphorbol-13-acetate by suppressing c-Jun N-terminal kinase. *Nutrients, 13(9),* 3079.

Parker, A. G., Peraza, G. G., Sena, J., Silva, E. S., Soares, M. C. F., Vaz, M. R. C., Furlong. E. B., & Muccillo-Baisch, A. L. (2007). Antinociceptive effects of the aqueous extract of *Brugmansia suaveolens* flowers in mice. *Biological Research for Nursing*, *8(3),* 234-239.

Pérez-González, M. Z., del Carmen Juárez-Vázquez, M., Sánchez-Ramos, M., Moreno-Villalba, L., & Jiménez-Arellanes, M. A. (2025). Anti-inflammatory, antioxidant, and acute toxicity of *Brugmansia arborea* extracts from wild plants and shoots obtained by indirect organogenesis. A thermographic assay to anti-inflammatory evaluation. *Biomedicine & Pharmacotherapy*, *186*, 117972.

Petricevich, V. L., Salinas-Sánchez, D. O., Avilés-Montes, D., Sotelo-Leyva, C., & Abarca-Vargas, R. (2020). Chemical compounds, pharmacological and toxicological activity of *Brugmansia suaveolens*: A review. *Plants, 9(9),* 1161.

Pichersky, E., & Gang, D. R. (2000). Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends in Plant Science*, *5(10)*, 439-445.

Pratt, C. (2007). An Encyclopedia of Shamanism Volume 1 (Vol. 1). The Rosen Publishing Group, Inc.

Preissel, U., & Preissel, H. G. (2002). *Brugmansia* and *Datura*: Angel's Trumpets and Thorn Apples. Firefly Books.

Pundir, S., Shukla, M. K., Singh, A., Chauhan, R., Lal, U. R., Ali, A., & Kumar, D. (2022). A comprehensive review on Angel's trumpet (*Brugmansia suaveolens*). *South African Journal of Botany*, *151*, 266-274.

Ramos, A., Visozo, A., Piloto, J., Garcıa, A., Rodrıguez, C. A., & Rivero, R. (2003). Screening of antimutagenicity via antioxidant activity in Cuban medicinal plants. *Journal of Ethnopharmacology*, *87(2-3),* 241-246.

Rates, S. M. K. (2001). Plants as source of drugs. *Toxicon*, *39(5),* 603-613.

Rätsch, C. (2005). The Encyclopedia of Psychoactive Plants: Ethnopharmacology and Its Applications. Simon and Schuster.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26(10)*, 1231-1237.

Reigosa, M., & Pedrol, N. (2002). Allelopathy: From Molecules to Ecosystems. Science Publishers.

Reis, R. B., Bragagnolo, F. S., Gianeti, T. M., Rodrigues, S. A., Funari, C. S., Gonçalves, G. G., & Ming, L. C. (2019).

Brugmansia suaveolens leaf productivity and alkaloid contents under different doses of organic fertilizer. *Journal of Agricultural* Sciences, *11(3)*, 34.

Ribeiro, B., Lopes, R., Andrade, P. B., Seabra, R. M., Gonçalves, R. F., Baptista, P., & Quelhas, I. (2008). Comparative study of phytochemicals and antioxidant potential of wild edible mushroom caps and stipes. *Food chemistry, 110(1),* 47-56.

Roberts, M. F. (Ed.). (2013). Alkaloids: Biochemistry, Ecology, and Medicinal Applications. Springer Science & Business Media.

Rohman, F., Juma, Y., Utomo, D. H., Lestari, S. R., Arifah, S. N., & Putra, W. E. (2019). Plants diversity as a medicinal plants by the tengger tribe, bromo tengger Semeru National Park, East Java, Indonesia. *EurAsian Journal of BioSciences*, *13(2),* 2293-2298.

Rojas, S., Madriñan, S., Stahl, M., & Harter, K. (2023). Cultural use of tropane alkaloids of *Brugmansia* species and cultivars in Colombia depends on local plant growth environment rather than genetic diversity. *BioRxiv*, 2023-11.

Ruiz-Barrio, I., Guisado-Alonso, D., Bulnes-González, V., & Green, B. T. (2022). Isolated dilated pupil. *British Medical Journal*, *376*, e069133.

Sáiz, J., Mai, T. D., López, M. L., Bartolome, C., Hauser, P. C., & Garcia-Ruiz, C. (2013). Rapid determination of scopolamine in evidence of recreational and predatory use. *Science & Justice*, *53(4)*, 409-414.

Sakunthala, P., Charles, A., Kesavan, D., & Ramani, V. A. (2013). Phytochemical screening and adsorption studies of *Brugmansia suaveolens*. *Chemical Science Review & Letters, 29,* 319-322.

Salawu, O. A., Chindo, B. A., Tijani, A. Y., & Adzu, B. (2008). Analgesic, anti-inflammatory, antipyretic and antiplasmodial effects of the methanolic extract of *Crossopteryx febrifuga*. *Journal of Medicinal Plants Research*, *2(8)*, 213-218.

San Luis, G. D., Balangcod, T. D., Abucay, J. B., Wong, F. M., Balangcod, K. D., Afifi, N. I. G., & Apostol, O. G. (2014). Phytochemical and antimicrobial screening of indigenous species that have potential for revegetation of landslides in Atok, Benguet, Philippines. *Indian Journal of Traditional Knowledge*, *13*, 56-62.

Santhosh, K., Manjunatha, H., Ravindranath, B., Swapna, S., & Deepthi, M. (2024). Evaluation of analgesic property of bio-synthesized AgNPs using leaf methanol extract of *Brugmansia suaveolens*. *Rasayan Journal of Chemistry*, *17(1),* 138.

Sanz-Biset, J., & Cañigueral, S. (2013). Plants as medicinal stressors, the case of depurative practices in Chazuta valley (Peruvian Amazonia). *Journal of Ethnopharmacology*, *145(1),* 67-76.

Särkinen, T., Bohs, L., Olmstead, R. G., & Knapp, S. (2013). A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): A dated 1000-tip tree. *BMC Evolutionary Biology*, *13(1),* 214.

Schmidt, C., Fronza, M., Goettert, M., Geller, F., Luik, S., Flores, E. M. M., Bittencourt, C. F., Zanetti, G. D., Heinzmann, B. M., Laufer, S., & Merfort, I. (2009). Biological studies on Brazilian plants used in wound healing. *Journal of Ethnopharmacology*, *122(3),* 523-532.

Schultes, R. E. (1980). The Botany and Chemistry of Hallucinogens. Harvard University Press.

Schultes, R. E., & Raffauf, R. F. (1990). The Healing Forest: Medicinal and Toxic Plants of the Northwest Amazonia. Dioscorides Press.

Shah, V. V., Shah, N. D., & Patrekar, P. V. (2013). Medicinal plants from Solanaceae family. *Research Journal of Pharmacy and Technology*, *6*, 143-151.

Shathish, K., & Guruvayoorappan, C. (2014). *Solanum muricatum* Ait. inhibits inflammation and cancer by modulating the immune system. *Journal of Cancer Research and Therapeutics*, *10(3)*, 623-630.

Shaw, J. M. H. (1999). Nomenclature notes on *Brugmansia*. *Royal Horticultural Society, 6*, 148-151.

Simpson, B. B., & Conner-Ogorzaly, M. (2013). Plants in our world: Economic botany. McGraw-Hill Education.

Singh, M., Govindarajan, R., Nath, V., Rawat, A. K. S., & Mehrotra, S. (2006). Antimicrobial, wound healing and antioxidant activity of *Plagiochasma appendiculatum* Lehm. et Lind. *Journal of Ethnopharmacology, 107(1),* 67-72.

Tailwal, A., Negi, A., & Chauhan, V. A. (2023). Wild traditional medicinal plant Angel's trumpet (*Brugmansia suaveolens*): An overview. *International Journal of Pharmacognosy, 10,* 362-367.

Tiwari, R., Chakraborty, S., Saminathan, M., Dhama, K., & Singh, S. V. (2014). Ashwagandha (*Withania somnifera*): Role in safeguarding health, immunomodulatory effects, combating infections and therapeutic applications: A review. *Journal of Biological Sciences, 14(2),* 77.

Tortoriello, J., Meckes-Fischer, M., Villarreal, M. L., Berlin, B., & Berlin, E. (1995). Spasmolytic activity of medicinal plants used to treat gastrointestinal and respiratory diseases in the Highland of Chiapas. *Phytomedicine, 2(1),* 57-66.

Tsou, K., Louie, G., & Way, E. L. (1982). Manifestations of gut opiate withdrawal contracture and its blockade by capsaicin. *European Journal of Pharmacology, 81(3),* 377-383.

Tzschentke, T. M. (2007). Review on CPP: Measuring reward with the conditioned place preference (CPP) paradigm: Update of the last decade. *Addiction Biology, 12(3-4),* 227-462.

Uche, F. I., & Aprioku, J. S. (2008). The phytochemical constituents, analgesic and anti-inflammatory effects of methanol extract of *Jatropha curcas* leaves in mice and Wister albino rats.

Journal of Applied Sciences and Environmental Management, 12(4). 99-102

Uddin, M. S., Asaduzzaman, M., Mamun, A. A., Iqbal, M. A., Wahid, F., & Rony, R. K. (2016). Neuroprotective activity of *Asparagus racemosus* Linn. against ethanol-induced cognitive impairment and oxidative stress in rats brain: auspicious for controlling the risk of Alzheimer's disease. *Journal of Alzheimers Disease & Parkinsonism*, *6(4),* 1-10.

Ullrich, S. F., Hagels, H., & Kayser, O. (2017). Scopolamine: A journey from the field to clinics. *Phytochemistry Reviews*, *16(2),* 333-353.

Uribe, M., Moreno, C. L., Zamora, A., & Acosta, P. J. (2005). Perfil epidemiológico de la intoxicación con burundanga en la Clínica Uribe Cualla S.A. de Bogotá, D.C. *Acta Neurológica Colombiana*, *21*, 197-201.

Valdés, B. (2012). Solanaceae. In Euro+Med Plantbase- The information resource for Euro-Mediterranean plant diversit, *Solanum carense* Dunal in Candolle, *Prodr. 13*, 105.

Van der Donck, I., Mulliez, E., & Blanckaert, J. (2004). Angel's Trumpet (*Brugmansia arborea*) and mydriasis in a child. *Bulletin de la Societe Belge d'Ophtalmol*, *292,* 53-56.

Venugopal, R., & Liu, R. H. (2012). Phytochemicals in diets for breast cancer prevention: The importance of resveratrol and ursolic acid. *Food Science and Human Wellness*, *1(1),* 1-13.

Vilani, A., Lozano, E. R., Potrich, M., Alves, L. F. A., Maia, F. M. C., de Lima, J. D. A., & de Gouvea, A. (2017). Activity of plant aqueous extracts on *Bacillus thuringiensis* and their interactions on *Anticarsia gemmatalis* (Lepidoptera: Erebinae). *Semina: Ciências Agrárias*, *38(2),* 1051-1057.

Voss, J. L. (2019). Guide to Poisonous Plants. College of Veterinary Medicine & Biomedical Sciences, Colorado State University.

Wagstaff, D. J. (2008). International Poisonous Plants Checklist: An evidence-Based Reference. CRC Press.

Wink, M. (2000). Interference of Alkaloids with Neuroreceptors and Ion Channels. Studies in Natural Products Chemistry, *21*, 3-122.

Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry*, *64(1),* 3-19.

Wink, M. (2008). Plant secondary metabolism: Diversity, function and its evolution. *Natural Product Communications*, *3(8),* 1205-1216.

Yang, Y., Yuanye, D., Qing, L., Jinjian, L., Xiwen, L., & Yitao, W. (2014). Complete chloroplast genome sequence of poisonous and medicinal plant *Datura stramonium*: Organizations and implications for genetic engineering. *PloS One*, *9(11),* e110656.

Yokozawa, T., Chen, C. P., Dong, E., Tanaka, T., Nonaka, G. I., & Nishioka, I. (1998). Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2-picrylhydrazyl radical. *Biochemical Pharmacology*, *56(2),* 213-222.

Zayed, R., & Wink, M. (2004). Induction of tropane alkaloid formation in transformed root cultures of *Brugmansia suaveolens* (Solanaceae). *Zeitschrift für Naturforschung C*, *59(11-12),* 863-867.

BÖLÜM 0

ANTIMICROBIAL POTENTIAL OF FRESHWATER MACROALGAE AND THEIR IMPORTANCE IN MEDICAL MICROBIOLOGY

OSMAN UÇ[1]
KÖKSAL PABUÇCU[2]

Introduction

Antimicrobial resistance is widely recognized as one of the most significant public health challenges of the twenty-first century. The increasing prevalence of resistance mechanisms among bacteria, fungi, and other pathogenic microorganisms has complicated the treatment of infectious diseases and reduced the effectiveness of existing antimicrobial agents (Murray et al., 2022, p. 629-655; Ventola, 2015, p. 277-283). The World Health Organization has identified antimicrobial resistance as a global threat capable of affecting millions of individuals in the coming decades and has emphasized the discovery of novel antimicrobial compounds as a major research priority (WHO, 2023).

---

[1] Dr., Dicle Üniversitesi, Mikrobiyoloji Laboratuvarı, Orcid: 0000-0002-3738-9836

[2] Prof. Dr., Erzincan Binali Yıldırım Üniversitesi, Eczacılık Fakültesi, Meslek Bilimleri Bölümü, Farmakognozi Anabilim Dalı, Orcid: 0000-0002-4473-8002

Furthermore, the rapid emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) microorganisms has intensified the need for alternative therapeutic approaches and the exploration of new natural sources of antimicrobial compounds.

Natural products have historically played a crucial role in drug discovery. Many antimicrobial agents currently used in clinical practice have been derived directly from natural compounds or developed through structural modification of naturally occurring molecules (Newman & Cragg, 2020, p. 770-803). However, the extensive use of antimicrobial drugs with similar mechanisms of action has accelerated the emergence and spread of resistant microorganisms (Atanasov et al., 2021, p. 200-216). Consequently, increasing attention has been directed toward previously underexplored biological resources with the potential to provide novel bioactive compounds. In addition to their structural diversity, natural products frequently possess unique molecular scaffolds that may interact with microbial targets through mechanisms distinct from those of conventional antimicrobial agents.

Algae have emerged as promising organisms in natural product research during recent decades. Although marine algae have been extensively investigated and have generated a substantial body of scientific literature, freshwater macroalgae remain comparatively understudied (Pereira, 2018, p. 1-560; Shannon & Abu-Ghannam, 2016, p. 1-23). Nevertheless, recent studies have demonstrated that freshwater macroalgae produce a variety of metabolites exhibiting significant biological activities, including antimicrobial properties (Cotas et al., 2024, p. 797; Pabuçcu & Demiriz Yücer, 2022, p. 927-932). Unlike marine environments, freshwater ecosystems are characterized by highly dynamic physicochemical conditions, including fluctuations in nutrient availability, temperature, light intensity, and microbial community composition. These environmental pressures may contribute to the synthesis of unique secondary metabolites with potential pharmaceutical relevance.

Freshwater macroalgae are macroscopic photosynthetic organisms inhabiting rivers, lakes, reservoirs, and various inland aquatic ecosystems (Wehr & Sheath, 2015, p. 13-74). Many of the secondary metabolites synthesized by these organisms as adaptive responses to environmental conditions have been associated with antimicrobial activity (Abd El Baky & El Baroty, 2020, p. 1439-1457; Gupta & Abu-Ghannam, 2011, p. 315-326). These metabolites are believed to function primarily as ecological defense compounds that protect algal cells against microbial colonization, biofilm formation, epiphytic growth, grazing pressure, and interspecific competition. Recent investigations have reported inhibitory effects of extracts obtained from different freshwater macroalgal species against both bacterial and fungal microorganisms (Pabuçcu & Demiriz Yücer, 2022, p. 927-932; Uç & Pabuçcu, 2024, p. 12-20). Furthermore, elevated phenolic content, considerable antioxidant capacity, and biologically relevant fatty acid profiles have been identified in several freshwater macroalgal taxa (Demiriz Yücer & Pabuçcu, 2023, p. 73-79; Demiriz Yücer & Pabuçcu, 2024, p. 87-95; Uç & Pabuçcu, 2026).

One of the primary reasons for the growing scientific interest in freshwater macroalgae is their remarkable chemical diversity, which may serve as a valuable source of novel antimicrobial molecules (Atanasov et al., 2021, p. 200-216; Pereira, 2018, p. 1-560). Existing studies also suggest that compounds derived from these organisms may influence biological processes involved in microbial pathogenesis beyond merely inhibiting microbial growth (Cotas et al., 2024, p. 797). For example, certain algal metabolites have been reported to interfere with quorum sensing systems, microbial adhesion, virulence factor production, and biofilm development, all of which play crucial roles in the establishment and persistence of infectious diseases. Therefore, freshwater macroalgae have become increasingly important research subjects in both natural product chemistry and medical microbiology.

Despite the promising findings reported to date, the antimicrobial potential of freshwater macroalgae remains far from fully explored. The vast majority of freshwater algal taxa have not yet been systematically screened for antimicrobial activity, and the chemical structures and mechanisms of action of many bioactive metabolites remain poorly characterized. Consequently, interdisciplinary studies integrating phycology, microbiology, natural product chemistry, metabolomics, and pharmaceutical sciences will be essential for fully elucidating the biomedical potential of freshwater macroalgae.

Freshwater Macroalgae and the Chemical Basis of Their Antimicrobial Potential

Freshwater macroalgae are capable of surviving under a wide range of ecological conditions and exhibit considerable adaptability to environmental fluctuations (Wehr & Sheath, 2015, p. 13-74). This adaptability is reflected not only in their morphology but also in their metabolic capabilities (Pereira, 2018, p. 1-560). The biological activities observed in many freshwater macroalgae are largely attributed to the diverse metabolites synthesized in response to environmental stressors and ecological pressures (Gupta & Abu-Ghannam, 2011, p. 315-326).

Freshwater environments are characterized by continuous interactions among algae, bacteria, fungi, protozoans, invertebrate grazers, and competing phototrophic organisms. Consequently, many secondary metabolites produced by freshwater macroalgae are believed to function as ecological defense compounds that enhance survival, reduce microbial colonization, and improve competitive fitness within aquatic ecosystems.

The metabolic profiles of freshwater macroalgae are influenced by numerous physical and chemical characteristics of their habitats. Environmental factors such as light intensity, temperature, nutrient availability, water movement, and dissolved oxygen concentration can significantly affect metabolite production (Pereira, 2018, p. 1-560; Stevenson et al., 1996, p. 3-30). Consequently, specimens belonging to the same species but collected from different geographical locations may exhibit considerable variation in their chemical composition. Such environmental variability is considered one of the major factors contributing to differences in biological activity reported across studies (Cotas et al., 2024, p. 797; Pabuçcu & Demiriz Yücer, 2022, p. 927-932).

In addition to environmental factors, taxonomic diversity also contributes significantly to chemical variability. Freshwater macroalgae belonging to Chlorophyta, Charophyta, Rhodophyta, and Xanthophyceae often differ substantially in their biosynthetic capacities and metabolite composition, resulting in distinct biological activity profiles.

Among the compound groups most frequently associated with antimicrobial activity in freshwater macroalgae are phenolic compounds. These molecules contain one or more hydroxyl groups attached to aromatic rings and represent an important class of natural products (Daglia, 2012, p. 174-181). Phenolic compounds play protective roles against environmental stress and occur in varying concentrations among algal species (Pereira, 2018, p. 1-560). Recent investigations have reported elevated levels of phenolic compounds in several freshwater macroalgae, suggesting a possible relationship between phenolic content and biological activity (Uç & Pabuçcu, 2024, p. 12-20; Uç & Pabuçcu, 2026).

Phenolic compounds may exert antimicrobial effects through multiple mechanisms, including disruption of microbial cell membranes, inhibition of extracellular enzymes, interference with energy metabolism, and induction of oxidative stress within microbial cells.

Flavonoids, a subgroup of polyphenolic compounds, have also attracted considerable attention in antimicrobial research. These compounds are widely distributed in nature and have been associated with numerous biological activities (Cushnie & Lamb, 2011, p. 99-107).

Although flavonoid composition in freshwater macroalgae remains less extensively studied than in terrestrial plants, recent investigations have suggested the occurrence of flavonoid-like compounds and flavonoid derivatives in certain freshwater algal taxa. However, their biosynthetic pathways, ecological functions, and taxonomic distribution remain incompletely understood.

Fatty acids constitute another important component of freshwater macroalgal chemistry. In particular, unsaturated fatty acids have attracted interest due to their biological and pharmaceutical relevance (Desbois & Smith, 2010, p. 1629-1642). Chemical analyses have identified compounds such as palmitic acid, oleic acid, linoleic acid, and stearic acid in various freshwater algal species (Demiriz Yücer, 2024, p. 1026-1031; Demiriz Yücer & Pabuçcu, 2023, p. 73-79; Demiriz Yücer & Pabuçcu, 2024, p. 87-95). Differences in fatty acid composition among species may contribute to variations in biological activity profiles (Desbois & Smith, 2010, p. 1629-1642).

Several fatty acids have been reported to inhibit microbial growth through membrane destabilization, disruption of electron transport processes, and alteration of membrane permeability, thereby contributing to the antimicrobial properties of algal extracts.

Polysaccharides are also major structural and functional components of freshwater macroalgae. Recent studies have demonstrated that certain polysaccharides possess biological functions extending beyond their structural roles within algal cells (Cotas et al., 2024, p. 797). Sulfated polysaccharides, in particular, have attracted interest because of their potential pharmaceutical applications and diverse biological activities (Cotas et al., 2024, p. 797).

In addition to antimicrobial effects, sulfated polysaccharides have been reported to exhibit antiviral, anticoagulant, immunomodulatory, anti-inflammatory, and antioxidant properties. These multifunctional biological activities considerably increase their potential value for pharmaceutical and biomedical applications.

Pigments represent another characteristic group of metabolites found in freshwater macroalgae. Chlorophylls and carotenoids are not only essential for photosynthesis but are also associated with strong antioxidant properties (Abd El Baky & El Baroty, 2020, p. 1439-1457). These compounds contribute to cellular protection under environmental stress conditions and constitute an important component of the biochemical diversity observed in freshwater macroalgae (Shannon & Abu-Ghannam, 2016, p. 1-23).

Certain carotenoids, including β-carotene, lutein, and zeaxanthin, have also been associated with anti-inflammatory and protective biological effects that may indirectly contribute to host defense mechanisms.

The extraction and characterization of bioactive compounds are critical steps in evaluating the antimicrobial potential of freshwater macroalgae. Different solvents, including methanol, ethanol, acetone, hexane, and water, differ in their ability to extract compounds with varying polarities. Consequently, extracts obtained from the same algal material may exhibit substantial differences in both chemical composition and biological activity depending on the extraction procedure employed (Pabuçcu & Demiriz Yücer, 2022, p. 927-932; Plaza et al., 2009, p. 7159-7170).

Extraction efficiency may also be influenced by algal developmental stage, seasonal variation, environmental conditions, drying methods, and storage procedures, all of which can affect the recovery of bioactive metabolites.

Advanced analytical techniques have become increasingly important in contemporary algal research. Methods such as gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC), and liquid chromatography–mass spectrometry (LC–MS/MS) are widely used for the identification and characterization of bioactive metabolites (Atanasov et al., 2021, p. 200-216; Plaza et al., 2009, p. 7159-7170).

More recently, metabolomics-based approaches, high-resolution mass spectrometry (HRMS), molecular networking, and bioinformatics-assisted dereplication strategies have emerged as powerful tools for the rapid discovery and characterization of novel algal metabolites.

Overall, freshwater macroalgae possess a highly complex chemical composition that cannot be attributed to a single group of compounds. The coexistence of phenolic compounds, flavonoids, fatty acids, polysaccharides, pigments, and other secondary metabolites provides a biochemical foundation for the diverse biological activities reported in the literature (Atanasov et al., 2021, p. 200-216; Gupta & Abu-Ghannam, 2011, p. 315-326).

Furthermore, the biological activities of freshwater macroalgal extracts are likely to result from synergistic interactions among multiple metabolite classes rather than from the action of individual compounds alone. Understanding these interactions represents an important challenge and opportunity for future antimicrobial drug discovery efforts.

This chemical diversity forms the basis for the antimicrobial potential of freshwater macroalgae and justifies continued investigation of these organisms as promising sources of novel bioactive compounds.

Antibacterial Findings

A significant proportion of antibacterial studies involving freshwater macroalgae have focused on filamentous green algae. Among these, species belonging to the genera Spirogyra, Cladophora, Mougeotia, Pithophora, and Oedogonium have received particular attention due to their reported biological activities (Gupta & Abu-Ghannam, 2011, p. 315-326; Pereira, 2018, p. 1-560).

Most available studies have employed crude extracts rather than purified compounds. Consequently, the reported antibacterial activities likely result from the combined action of multiple metabolites, including phenolic compounds, fatty acids, pigments, and other secondary metabolites acting synergistically.

Among freshwater macroalgae, Spirogyra species represent one of the most extensively investigated groups in antimicrobial research. Pabuçcu & Demiriz Yücer (2022, p. 927-932) reported that extracts obtained from *Spirogyra aequinoctialis* using different solvent systems exhibited inhibitory effects against various bacterial and fungal microorganisms. The observed variation in antimicrobial activity among extracts highlighted the importance of extraction methodology and suggested that different classes of metabolites contribute differently to biological activity. These findings demonstrate that antimicrobial potential should not be evaluated solely at the species level but also in relation to extraction strategies and chemical composition.

In addition, differences in solvent polarity may substantially influence the extraction efficiency of bioactive compounds, thereby affecting both the qualitative and quantitative antimicrobial profiles of algal extracts.

The genus Cladophora has likewise emerged as a promising source of biologically active compounds. Chemical investigations have revealed that different Cladophora species contain substantial amounts of phenolic compounds, fatty acids, and photosynthetic pigments (Demiriz Yücer & Pabuçcu, 2024, p. 87-95; Pereira, 2018, p. 1-560). Studies on *Cladophora fracta var. intricata* have demonstrated considerable antioxidant capacity and the presence of fatty acid profiles associated with biological activity (Demiriz Yücer & Pabuçcu, 2024, p. 87-95). Although antioxidant activity cannot be directly equated with antibacterial efficacy, several studies suggest that these properties may be linked through common biochemical mechanisms involving oxidative stress regulation and cellular protection (Shannon & Abu-Ghannam, 2016, p. 1-23).

Several classes of algal metabolites have been proposed to exert antibacterial effects through disruption of bacterial membranes, alteration of membrane permeability, inhibition of essential enzymes, interference with nutrient uptake, and induction of oxidative damage within microbial cells. However, these mechanisms remain insufficiently characterized in most freshwater macroalgal species.

Compared with Spirogyra and Cladophora, the genus Mougeotia has received less scientific attention. Nevertheless, available studies indicate that species belonging to this genus contain phenolic compounds and other secondary metabolites that may contribute to antimicrobial activity (Pereira, 2018, p. 1-560). The limited number of investigations currently available prevents definitive conclusions regarding their pharmaceutical potential; however, existing findings suggest that further exploration of this group is warranted.

Species of Pithophora and Oedogonium have also demonstrated antibacterial properties in several studies (Gupta & Abu-Ghannam, 2011, p. 315-326). Although inhibitory effects against various bacterial species have been reported, detailed chemical characterization and mechanistic investigations remain relatively scarce. Consequently, the compounds responsible for the observed biological activities have not yet been fully identified. Another noteworthy group is represented by the genus Vaucheria.

Although frequently associated with filamentous green algae because of morphological similarities, Vaucheria belongs to the class Xanthophyceae (yellow-green algae). This taxonomic distinction may contribute to its unique metabolic profile and biological activities.

Recent investigations involving *Vaucheria frigida* and *Vaucheria aversa* have demonstrated both antioxidant and antimicrobial activities, accompanied by significant concentrations of phenolic compounds and biologically relevant metabolites (Uç & Pabuçcu, 2024, p. 12-20; Uç & Pabuçcu, 2026). These findings indicate that *Vaucheria* species may represent promising candidates for future natural product research and antimicrobial screening programs.

Beyond direct growth inhibition, future studies should evaluate whether freshwater macroalgal metabolites influence bacterial virulence traits such as biofilm formation, quorum sensing, motility, and adhesion. Targeting these processes may provide alternative antimicrobial strategies that exert reduced selective pressure for resistance development.

When the available literature is evaluated collectively, the most consistent antibacterial findings appear to be associated with Spirogyra, Cladophora, and Vaucheria. However, the majority of freshwater macroalgal diversity remains unexplored from an antimicrobial perspective.

Antifungal Findings

Compared with antibacterial investigations, studies addressing the antifungal properties of freshwater macroalgae remain relatively limited. Nevertheless, available evidence suggests that extracts derived from several freshwater macroalgal species can inhibit the growth of yeasts and filamentous fungi (Gupta & Abu-Ghannam, 2011, p. 315-326; Shannon & Abu-Ghannam, 2016, p. 1-23).

Antifungal activity is particularly important because fungal pathogens are eukaryotic organisms and therefore share greater cellular similarity with human hosts than bacteria. This biological complexity often limits the number of selective antifungal targets available for drug development.

One notable example is the study conducted by Pabuçcu & Demiriz Yücer (2022, p. 927-932), which evaluated the antifungal activity of *Spirogyra aequinoctialis* extracts prepared using different solvent systems. The results demonstrated inhibitory effects against selected fungal microorganisms and suggested that freshwater macroalgae may produce metabolites capable of affecting eukaryotic pathogens in addition to bacteria.

Among fungal pathogens, *Candida albicans* and various *Aspergillus species* have been the most frequently used targets in antimicrobial screening studies due to their clinical significance (Fisher et al., 2022, p. 557-571; Perfect, 2017, p. 603-616). Opportunistic infections caused by these fungi are particularly problematic in immunocompromised individuals and continue to pose major challenges in healthcare settings (Fisher et al., 2022, p. 557-571).

Several natural products are known to exert antifungal effects through disruption of fungal cell membranes, inhibition of ergosterol biosynthesis, interference with cell wall assembly, induction of oxidative stress, or impairment of mitochondrial function. Whether freshwater macroalgal metabolites act through similar mechanisms remains largely unknown and warrants further investigation.

Despite encouraging preliminary findings, most investigations involving freshwater macroalgae have focused on screening rather than mechanistic analysis.

Furthermore, only a limited number of studies have evaluated potential synergistic interactions between freshwater macroalgal extracts and clinically used antifungal agents. Such combination approaches may represent an important strategy for overcoming antifungal resistance and improving therapeutic efficacy in the future.

As a result, the molecular pathways underlying antifungal activity remain poorly understood. Future studies should therefore prioritize the identification of active compounds, elucidation of mechanisms of action, and evaluation of potential synergistic interactions with existing antifungal drugs.

Findings on Biofilm Formation and Virulence

Modern microbiology recognizes that successful infection is influenced not only by microbial growth but also by virulence-associated traits such as biofilm formation, adhesion, communication systems, and stress tolerance (Flemming et al., 2016, p. 563-575). Consequently, the evaluation of potential antimicrobial agents increasingly includes their effects on these processes rather than focusing exclusively on growth inhibition.

This shift has contributed to the emergence of anti-virulence strategies, which aim to attenuate pathogenicity without necessarily inhibiting microbial growth. Such approaches may reduce the selective pressure responsible for the development of antimicrobial resistance.

Biofilms are structured microbial communities embedded within self-produced extracellular matrices. These structures protect microorganisms from environmental stress and contribute substantially to antimicrobial tolerance (Hall & Mah, 2017, p. 276-301).

The extracellular polymeric substances (EPS) that constitute the biofilm matrix typically contain polysaccharides, proteins, extracellular DNA, and lipids. This matrix acts as a physical and chemical barrier that limits antimicrobial penetration and enhances microbial survival.

Microorganisms growing within biofilms often exhibit significantly greater resistance to antimicrobial agents than their planktonic counterparts, making biofilm-associated infections particularly difficult to eradicate.

Although biofilm-focused studies involving freshwater macroalgae remain limited, several reports suggest that algal metabolites, particularly phenolic compounds and polysaccharides, may interfere with microbial adhesion and biofilm development (Cotas et al., 2024, p. 797).

Inhibition of initial microbial attachment is considered especially important because prevention of biofilm establishment is generally more effective than the removal of mature biofilms, which often exhibit substantial tolerance to antimicrobial treatment.

In addition to biofilm inhibition, recent research in natural product microbiology has increasingly emphasized the importance of targeting microbial virulence factors. Rather than directly killing microorganisms, certain bioactive compounds may reduce pathogenicity by interfering with processes such as quorum sensing, toxin production, motility, or host colonization.

Quorum sensing is a cell-density-dependent communication system that regulates collective microbial behaviors through signaling molecules such as acyl-homoserine lactones in Gram-negative bacteria and autoinducing peptides in Gram-positive bacteria. Interference with quorum sensing pathways may suppress virulence factor production and biofilm formation without exerting strong selective pressure for resistance development.

Although evidence involving freshwater macroalgae remains limited, this represents a promising direction for future investigation.

The Role of Freshwater Macroalgae in Medical Microbiology

The significance of freshwater macroalgae in medical microbiology extends beyond their potential as sources of novel antimicrobial compounds.

Freshwater macroalgae may also provide valuable chemical diversity for the discovery of anti-virulence agents, quorum sensing inhibitors, anti-biofilm compounds, and immunomodulatory molecules that could complement conventional antimicrobial therapies.

Among the microorganisms currently considered critical threats to public health are methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum β-lactamase (ESBL)-producing Enterobacterales, and carbapenem-resistant Gram-negative bacteria (Murray et al., 2022, p. 629-655; WHO, 2023).

Many of these organisms are included among the so-called ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter species*), which are recognized for their ability to evade existing antimicrobial therapies and cause severe healthcare-associated infections.

The antimicrobial activities reported for freshwater macroalgal extracts suggest that these organisms may serve as valuable reservoirs of chemically diverse metabolites with pharmaceutical potential (Gupta & Abu-Ghannam, 2011, p. 315-326; Pabuçcu & Demiriz Yücer, 2022, p. 927-932).

In many cases, the observed biological activities are likely attributable to synergistic interactions among multiple metabolites rather than to the action of a single compound. Understanding these interactions may represent an important future direction in freshwater macroalgal research.

From a drug discovery perspective, freshwater macroalgae should be regarded primarily as sources of lead compounds rather than direct therapeutic agents.

The identification of a lead compound represents only an early stage in the drug development pipeline. Subsequent phases typically involve structural characterization, optimization of biological activity, toxicity assessment, formulation studies, pharmacokinetic evaluation, and preclinical testing.

Another area in which freshwater macroalgae may have particular relevance is the management of biofilm-associated infections.

Beyond direct antimicrobial activity, compounds capable of disrupting biofilm architecture or preventing microbial adhesion may substantially improve the effectiveness of existing antimicrobial therapies when used in combination approaches.

Recent advances in metabolomics, genomics, transcriptomics, and mass spectrometry-based analytical techniques offer new opportunities for exploring freshwater macroalgae at unprecedented levels of detail (Atanasov et al., 2021, p. 200-216).

In particular, high-resolution mass spectrometry, molecular networking platforms, bioinformatics-assisted dereplication, and integrated omics approaches have greatly accelerated the discovery of previously uncharacterized natural products from biological organisms.

These technologies may facilitate the identification of previously unknown compounds and improve our understanding of the molecular mechanisms underlying their biological activities.

The integration of phycology, microbiology, metabolomics, natural product chemistry, and pharmaceutical sciences will likely play a central role in transforming freshwater macroalgal metabolites from laboratory observations into clinically relevant therapeutic candidates.

Overall, freshwater macroalgae occupy a unique position at the intersection of natural product chemistry, microbiology, and pharmaceutical sciences.

Conclusion

Antimicrobial resistance continues to undermine the effectiveness of existing therapeutic strategies and represents one of the most urgent challenges facing global healthcare systems (Murray et al., 2022, p. 629-655; WHO, 2023). In response to this growing threat, the identification of novel antimicrobial resources has become a major priority in both microbiological and pharmaceutical research. Freshwater macroalgae have emerged as promising candidates in this context due to their remarkable chemical diversity and the increasing number of studies reporting biologically active metabolites with antimicrobial potential (Atanasov et al., 2021, p. 200-216; Gupta & Abu-Ghannam, 2011, p. 315-326).

The literature reviewed in this chapter demonstrates that freshwater macroalgae contain a broad range of bioactive compounds, including phenolic compounds, flavonoids, fatty acids, polysaccharides, and pigments. These metabolites constitute the biochemical basis of the antibacterial, antifungal, and anti-biofilm activities reported for various freshwater macroalgal species. Among the genera investigated to date, Spirogyra, Cladophora, and Vaucheria appear to be among the most promising candidates, although many freshwater taxa remain largely unexplored (Pabuçcu & Demiriz Yücer, 2022, p. 927-932; Uç & Pabuçcu, 2024, p. 12-20; Uç & Pabuçcu, 2026).

Importantly, current knowledge is based on only a small fraction of the known freshwater macroalgal diversity. Numerous taxa belonging to Chlorophyta, Charophyta, Rhodophyta, and Xanthophyceae have not yet been systematically investigated for antimicrobial properties, suggesting that substantial undiscovered biochemical diversity remains available for future exploration.

A critical evaluation of the available literature indicates that current research has focused predominantly on antibacterial activity, whereas studies investigating antifungal mechanisms, virulence inhibition, and biofilm-related effects remain relatively limited (Cotas et al., 2024, p. 797; Fisher et al., 2022, p. 557-571).

In addition, substantial methodological differences among studies complicate direct comparisons and highlight the need for standardized research protocols.

Furthermore, the majority of published investigations have relied on crude extract screening, whereas relatively few studies have progressed toward bioassay-guided fractionation, compound isolation, structural elucidation, and mechanistic characterization. Addressing these limitations will be essential for transforming preliminary biological observations into pharmacologically meaningful discoveries.

At present, the available evidence does not support the direct clinical application of freshwater macroalgal extracts as antimicrobial therapeutics. Nevertheless, these organisms should not be evaluated solely on the basis of immediate clinical applicability. Their greatest value lies in their potential to provide novel lead compounds, unique molecular scaffolds, and previously unrecognized biochemical pathways that may contribute to future antimicrobial drug development (Atanasov et al., 2021, p. 200-216; Newman & Cragg, 2020, p. 770-803).

Beyond conventional antimicrobial activity, freshwater macroalgal metabolites may also contribute to the development of anti-biofilm, anti-virulence, and quorum sensing inhibitory strategies. Such approaches are increasingly recognized as promising alternatives or complements to traditional antimicrobial therapies because they may reduce pathogenicity while exerting lower selective pressure for resistance development.

Future research should prioritize the exploration of underinvestigated freshwater macroalgal taxa, the identification of active metabolites, the elucidation of mechanisms of action, and the evaluation of toxicity and pharmacokinetic properties. The integration of advanced analytical technologies with microbiological and pharmaceutical approaches will be essential for unlocking the full potential of these organisms.

In particular, metabolomics, high-resolution mass spectrometry, molecular networking, genomics, transcriptomics, and other integrated omics technologies are expected to accelerate the discovery of novel bioactive metabolites and improve our understanding of their ecological and biomedical functions.

In conclusion, freshwater macroalgae represent a promising yet still underexplored biological resource at the interface of natural product chemistry and medical microbiology. Continued interdisciplinary research is likely to expand our understanding of their antimicrobial potential and may ultimately contribute to the development of innovative strategies for combating antimicrobial resistance and infectious diseases.

As the search for new antimicrobial solutions becomes increasingly urgent, freshwater macroalgae should be regarded not merely as alternative biological resources but as valuable reservoirs of chemical innovation with the potential to contribute to the next generation of antimicrobial, anti-biofilm, and anti-virulence therapeutics.

References

Abd El Baky, H. H., & El Baroty, G. S. (2020). Optimization cultivation of Chlamydomonas reinhardtii in a tubular photobioreactor (2000 Liter) for biomass and green bioenergy (biodiesel) production. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *48*(3), 1439–1457. https://doi.org/10.15835/NBHA48311803

Atanasov, A. G., Zotchev, p. B., Dirsch, V. M., Orhan, I. E., Banach, M., Rollinger, J. M., Barreca, D., Weckwerth, W., Bauer, R., Bayer, E. A., Majeed, M., Bishayee, A., Bochkov, V., Bonn, G. K., Braidy, N., Bucar, F., Cifuentes, A., D'Onofrio, G., Bodkin, M., … Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug Discovery 2021 20:3*, *20*(3), 200–216. https://doi.org/10.1038/s41573-020-00114-z

Balouiri, M., Sadiki, M., & Ibnsouda, p. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/J.JPHA.2015.11.005

Cotas, J., Lomartire, S., Gonçalves, A. M. M., & Pereira, L. (2024). From Ocean to Medicine: Harnessing Seaweed's Potential for Drug Development. *International Journal of Molecular Sciences*, *25*(2). https://doi.org/10.3390/IJMS25020797

Cushnie, T. P. T., & Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*, *38*(2), 99–107. https://doi.org/10.1016/j.ijantimicag.2011.02.014

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology*, *23*(2), 174–181. https://doi.org/10.1016/j.copbio.2011.08.007

Demiriz Yücer, T. (2024). Investigation (In-Vitro) of Antiproliferative Properties of *Pseudopediastrum boryanum* (Turpin) E. Hegewald Extracts in Various Cancer Cell Lines. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *27*(5), 1026–1031. https://doi.org/10.18016/ksutarimdoga.vi.1420650

Demiriz Yücer, T., & Pabuçcu, K. (2023). Contents of Fatty Acid in *Gomphonema olivaceum*. *Journal of Gaziosmanpasa Scientific Research*, *12*(2), 73–79. https://dergipark.org.tr/en/pub/gbad/article/1289262

Demiriz Yücer, T., & Pabuçcu, K. (2024). Fatty acids, Vitamins and Antioxidant Properties of Cladophora fracta var. intricata. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *14*(1), 87–95. https://doi.org/10.21597/JIST.1362003

Desbois, A. P., & Smith, V. J. (2010). Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*, *85*(6), 1629–1642. https://doi.org/10.1007/s00253-009-2355-3

Fisher, M. C., Alastruey-Izquierdo, A., Berman, J., Bicanic, T., Bignell, E. M., Bowyer, P., Bromley, M., Brüggemann, R., Garber, G., Cornely, O. A., Gurr, Sarah. J., Harrison, T. S., Kuijper, E., Rhodes, J., Sheppard, D. C., Warris, A., White, P. L., Xu, J., Zwaan, B., & Verweij, P. E. (2022). Tackling the emerging threat of antifungal resistance to human health. *Nature Reviews Microbiology*, *20*(9), 557–571. https://doi.org/10.1038/s41579-022-00720-1

Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, p. A., & Kjelleberg, p. (2016). Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology*, *14*(9), 563–575. https://doi.org/10.1038/nrmicro.2016.94

Gupta, S., & Abu-Ghannam, N. (2011). Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science & Technology*, *22*(6), 315–326. https://doi.org/10.1016/J.TIFS.2011.03.011

Hall, C. W., & Mah, T.-F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews*, *41*(3), 276–301. https://doi.org/10.1093/femsre/fux010

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, p. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, *399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Newman, D. J., & Cragg, G. M. (2020). Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *Journal of Natural Products*, *83*(3), 770–803. https://doi.org/10.1021/ACS.JNATPROD.9B01285

Pabuçcu, K., & Demiriz Yücer, T. (2022). *Spirogyra aequinoctialis*'in Farklı Çözgenler Ile Hazırlanan Ekstraktlarının Antibakteriyal ve Antifungal Etkileri. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *25*(5), 927–932. https://doi.org/10.18016/KSUTARIMDOGA.VI.979361

Pereira, L. (2018). Therapeutic and Nutritional Uses of Algae. CRC Press, Taylor & Francis Group. ISBN: 9781498755382. https://doi.org/10.1201/9781315152844

Perfect, J. R. (2017). The antifungal pipeline: a reality check. *Nature Reviews Drug Discovery 2017 16:9*, *16*(9), 603–616. https://doi.org/10.1038/nrd.2017.46

Plaza, M., Herrero, M., Alejandro Cifuentes, A., & Ibáñez, E. (2009). Innovative natural functional ingredients from microalgae. *Journal of Agricultural and Food Chemistry*, *57*(16), 7159–7170. https://doi.org/10.1021/jf901070g

Shannon, E., & Abu-Ghannam, N. (2016). Antibacterial Derivatives of Marine Algae: An Overview of Pharmacological Mechanisms and Applications. *Marine Drugs*, *14*(4). https://doi.org/10.3390/MD14040081

Stevenson, J., Bothwell, M. L., & Lowe, R. L. (1996). Algal Ecology. In *Algal Ecology*. Elsevier. https://doi.org/10.1016/B978-0-12-668450-6.X5027-9

Uç, O., & Pabuçcu, K. (2024). *Vaucheria frigida* (Roth) C. Agardh'ın Antioksidan ve Antibakteriyel Özellikleri. Gaziosmanpaşa Bilimsel Araştırma Dergisi, 13(2), 12–20. https://dergipark.org.tr/tr/pub/gbad/issue/88124/1495261

Uç, O., & Pabuçcu, K. (2026). Antioxidant and antimicrobial activities and phenolic composition of *Vaucheria aversa*. *Journal Of Agriculture and Nature*, (Advanced Online Publication). https://doi.org/10.18016/ksutarimdoga.vi.1681866

Ventola, C. L. (2015). The Antibiotic Resistance Crisis: Part 1: Causes and Threats. P & T, 40(4), 277–283. PMID: 25859123.

PMCID: PMC4378521.Wehr, J. D., & Sheath, R. G. (2015). Habitats of Freshwater Algae. *Freshwater Algae of North America: Ecology and Classification*, 13–74. https://doi.org/10.1016/B978-0-12-385876-4.00002-5

WHO. (2023, November 20). *Antimicrobial resistance*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

BÖLÜM 0

PLANT GROWTH REGULATOR: KARRIKINS

HAKAN TERZİ[1]
MUSTAFA YILDIZ[2]
SALİHA AYDIN[3]

Introduction

Plant responses to changing environmental stress factors are among the most critical questions for plant biologists. Drought, temperature, and salinity stress are among the most common and significant environmental stresses affecting plant growth (Wani et al., 2016:162). In order to tolerate these adverse conditions, plants have developed various adaptation strategies at morphological, physiological, and molecular levels (Xiong et al., 2002:165; Suzuki et al., 2014:32). Nevertheless, because stress tolerance is governed by complex characteristics, traditional breeding methods often remain insufficiently effective, highlighting the need for innovative

---

[1] Assoc. Prof., Afyon Kocatepe University, Department of Molecular Biology and Genetics, Orcid: 0000-0003-4817-1100

[2] Prof., Afyon Kocatepe University, Department of Molecular Biology and Genetics, Orcid: 0000-0002-6819-9891

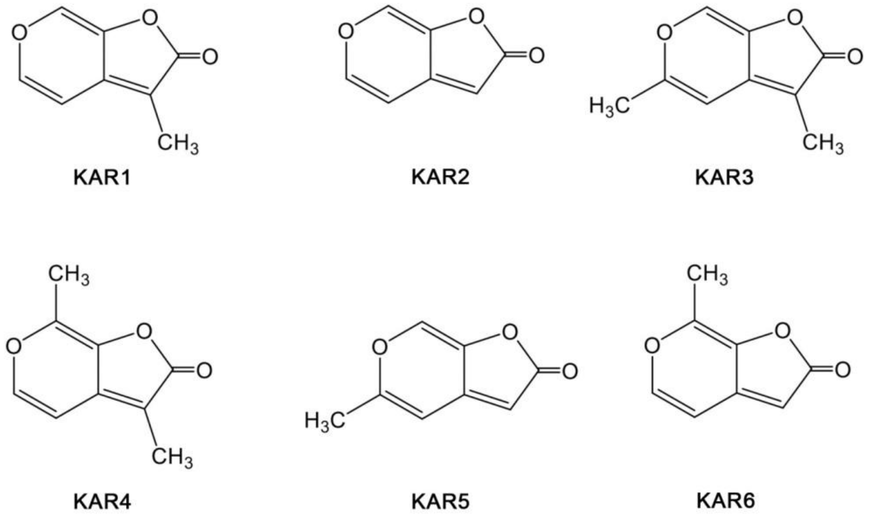
[3] Res. Asist., Afyon Kocatepe University, Department of Molecular Biology and Genetics, Orcid: 0000-0002-7830-0535

strategies to reduce the disparity between worldwide food production and increasing demand. In this context, phytohormones represent one of the feasible options for cultivating environmentally stress-tolerant and high-yielding plants (Zheng et al., 2023:1095363). Plant hormones are important organic compounds that modulate plant's responses to environmental stress conditions. These signaling molecules, which are synthesized within the plant and are also referred to as plant growth regulators, play roles in stress responses through the coordination of different signal transduction pathways (Samanta and Roychoudhury, 2025:62). Various phytohormones not only promote plant development but also establish signaling networks that regulate adaptation to stressful conditions (Das et al., 2025:329). Novel regulatory compounds such as polyamines, nitric oxide (NO), and strigolactones (SLs) are also involved in these regulatory processes (Gray, 2004:311). Following the discovery of SLs, karrikins (KARs), which exhibit structural similarity to SLs and play roles in post-fire ecosystems, have also taken their place among plant growth regulators (Flematti et al., 2004a:977).

KARs are named after the term “karrik,” which means “smoke” in the Noongar language spoken by the Indigenous people of Australia. They constitute a group of water-soluble plant growth regulators composed of different butenolide derivatives (Ghebrehiwot et al., 2008:270; Dixon et al., 2009:155; Flematti et al., 2009:9475; Nelson et al., 2012:107; Smith and Li, 2014:23; Sami et al., 2021:1257). Although KARs have naturally been present for a long time, their discovery originated from findings that smoke and the combustion of woody vegetation after fires were capable of promoting the germination of post-fire plant species (Keeley and Pizzorno, 1986:1289; Baldwin et al., 1994:2345; Van Staden et al., 2004:654). It was demonstrated that burned wood enhanced the germination of *Emmenanthe* *penduliflora*, a species that develops after fire events (Wicklow, 1977:201). Similarly, smoke generated during plant combustion was reported to stimulate seed germination in *Audouinia capitata* (De Lange and Boucher, 1990:700). Once the germination-stimulating effect of smoke water obtained from cellulose combustion was understood, this active compound was isolated and purified from charred filter paper composed of 99% cellulose (Flematti et al., 2004a:977; Flematti et al., 2004b:1). This compound, capable of breaking dormancy in the seeds of many smoke-responsive species, was subsequently classified into the butenolide group and named KAR1. KAR1 possesses the structure of 3-methyl-2H-furo[2,3-c]pyran-2-one and is also known as karrikinolide (Flematti et al., 2005:5719). It has been proposed that the precursor of this compound could be a pyranose sugar formed during the combustion of pure xylose, glucose, or cellulose (Flematti et al., 2011:1195). In addition, five related analogs, designated KAR2 through KAR6, have been identified and isolated from smoke and fire-derived plant residues (Figure 1) (Flematti et al., 2009:9475; Fang et al., 2023:100185). An important characteristic of KARs is their thermostability. Their structures do not degrade at room temperature, and they can easily dissolve in aqueous solutions. This property greatly facilitates both their use in laboratory experiments and their transfer to field applications in agriculture (Waters and Nelson, 2023:1525).

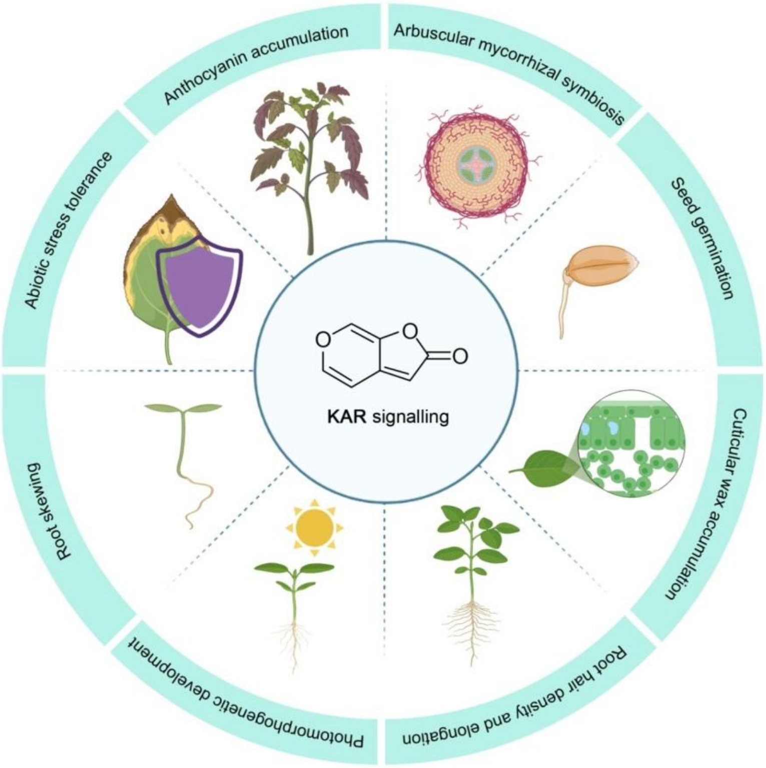
The effects of KARs are not restricted solely to seed germination or to species adapted to fire-prone habitats. The application of plant-derived smoke has been shown to improve several biochemical attributes, including photosynthetic pigment levels, total nitrogen content, soluble protein concentration, and photosynthetic activity (Jamil et al., 2014:159). Moreover, under phosphorus-deficient conditions, smoke water was found to promote root development by enhancing meristematic activity and stimulating cell elongation (Ariyakulkiat et al., 2026:12). KAR1 not only enhances germination but also improves seedling vigor and tolerance in many crops (Antala et al., 2019:43; Wang et al., 2025a:110800). In plants, the KAR signaling pathway is involved not only in germination but also in the regulation of diverse developmental and physiological processes, including mesocotyl elongation under dark conditions, anthocyanin and cuticular wax accumulation, photomorphogenic seedling growth, tolerance to abiotic stresses, leaf shape formation, root hair density and elongation, root skewing, and interactions with arbuscular mycorrhizal fungi (Figure 2) (Deng et al., 2025:2775). In light of the current knowledge, plant-derived smoke appears to be a potential plant growth regulator that both promotes plant growth and mitigates the detrimental effects of environmental stresses.

Figure 1 Chemical structures of karrikinolide (KAR1) and its structural analogues (KAR2–KAR6) isolated from smoke and burned plant-derived materials



Reference: Adapted from Fang et al., 2023:100185

Figure 2 Multifaceted effects of the KAR signaling pathway on plant development



This section provides a holistic assessment of current knowledge regarding the chemical structures, synthesis, and signal transduction mechanisms of KARs, which are plant growth regulators, as well as their roles in germination processes and environmental stress responses.

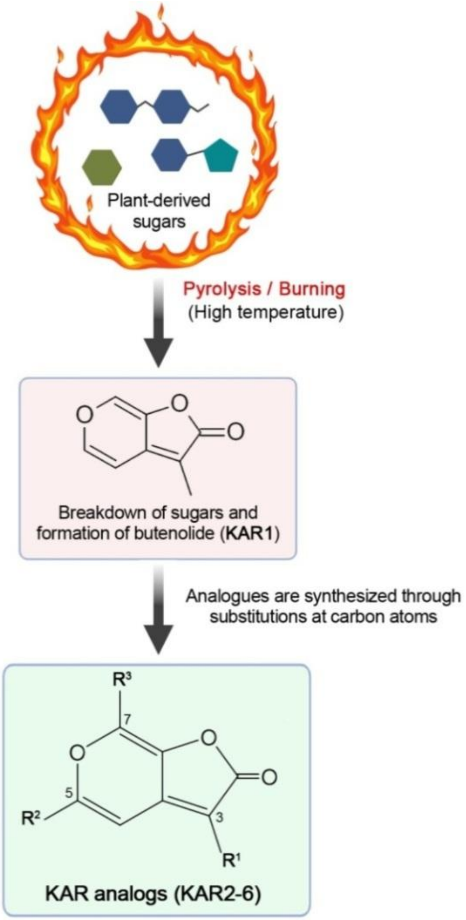
Chemical Structures and Synthesis

In studies conducted to identify the germination-stimulating compound in smoke, a compound containing a butenolide fused with a pyran ring, formed during the combustion of plant material or cellulose, was isolated. The chemical structure of the compound was confirmed as 3-methyl-2H-furo[2,3-c]pyran-2-one through chemical synthesis (Flematti et al., 2004a:977). Initially named butenolide, this compound was later designated as karrikinolide or karrikin 1 (KAR1) (Flematti et al., 2004a:977; Van Staden et al., 2004:654). KARs are smoke-derived small molecules that are not synthesized within the plant. KARs are produced during the combustion of cellulose, glucose, or other simple sugars. During the pyrolysis process, a molecular chain containing butenolide is formed (Figure 3) (Fichtner et al., 2024:900). The bicyclic structures of KARs contain only carbon, hydrogen, and oxygen atoms (Flematti et al., 2015:108). Various analogs of KARs are synthesized through substitutions at the 3rd, 5th, and 7th carbon atoms (Flematti et al., 2005:5719; Goddard-Borger et al., 2007:3925; Flematti et al., 2010:8612). The biological activities of these analogs, designated from KAR1 to KAR6, have been evaluated in smoke-sensitive species such as lettuce, and it has been demonstrated that plant responses to different analogs vary considerably (Flematti et al., 2007:2189; Waters et al., 2014:623).

Effects of KARs on Seed Germination

KARs were identified by their ability to break seed dormancy in smoke-affected ecosystems, and this property is still regarded as their most prominent physiological function. KARs, particularly KAR1 and KAR2, stimulate germination through various processes (Nakamura et al., 2013:2613). Smoke or smoke water promotes germination in various plants, including many crop species (Ullah et al., 2023:2604). KAR1 stimulates germination at very low concentrations (approximately 10^{-9} M), indicating its significant potential for seed priming (Kępczyński and Kępczyńska, 2023:2378). Smoke released during forest fires contains potent bioactive compounds such as KAR1, trimethylbutenolide, and smoke water. Particularly in post-fire environments, KARs regulate the mobilization of storage reserves such as lipids, proteins, carbohydrates, and starch by increasing hydrolase activity during the early stages of seed germination (Lin et al., 2009:1512; Nelson et al., 2011:8897).

Figure 3 Karrikin synthesis



The germination-promoting effect of plant-derived smoke was initially demonstrated through studies conducted on *Audouinia capitata* (Bruniaceae), an endangered plant species. Following the recognition that the seeds of this species germinated naturally after fires, experimental studies revealed that germination was initiated by chemical factors present in smoke derived from burned plant material (De Lange and Boucher, 1990:700). Investigations into the mechanisms promoting germination and breaking dormancy demonstrated that plant-derived smoke increased amylase activity in dormant oat caryopses before radicle emergence and also enhanced DNA replication and β-tubulin accumulation. These processes contribute to the breaking of seed dormancy and the initiation of germination (Cembrowska-Lech and Kępczyński, 2017:39). Similarly, in lettuce seeds, smoke water and KAR1 applications induced dormancy release and germination initiation by decreasing ABA levels and enhancing the activity of hydrolytic enzymes (Gupta et al., 2019:458). It has also been demonstrated that KAR1 and smoke water applications possess the potential to serve as alternatives to GA3 in overcoming dormancy, which represents a major problem in the cultivation of medicinal and aromatic plants (Rolando et al., 2026:865).

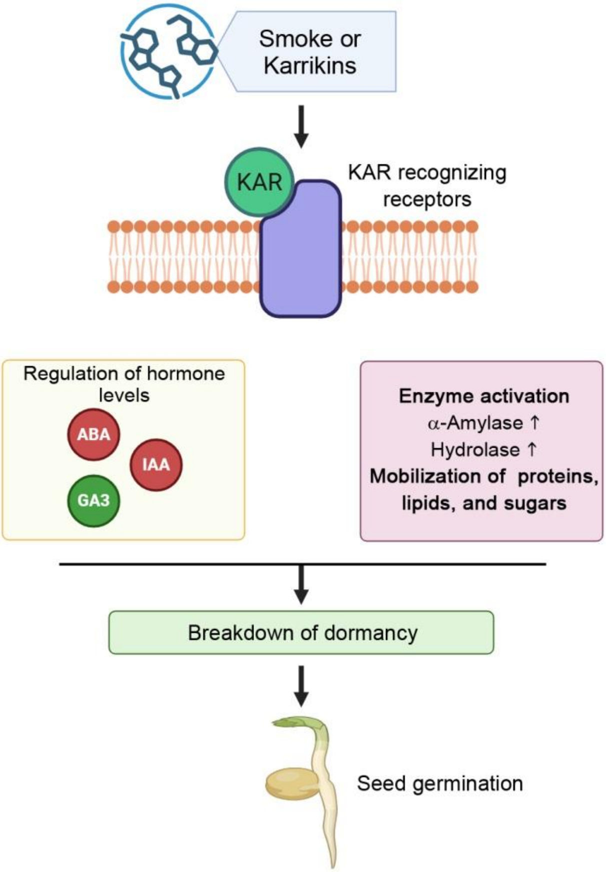
At the molecular level, KAR1 application in lettuce seeds enhanced germination by suppressing transcripts associated with ABA and dormancy. In contrast, trimethylbutenolide inhibited germination by increasing ABA content and decreasing cytokinin levels (Soós et al., 2012:1060; Toh et al., 2012:107). KAR1 application enhanced germination and accelerated the germination process in *Boopis gracilis* and *Nicotiana linearis*. However, smoke water did not promote germination and exhibited inhibitory effects at high concentrations, particularly in *N. linearis*. These findings indicate that KAR1 is a reliable germination stimulant, whereas smoke water exerts more limited and species-dependent effects due to variability in its composition (Gonzalez et al., 2025:1550692). In *Solidago* species, KAR1 was shown to promote seed germination at levels comparable to light and GA treatments (Bączek-Kwinta et al., 2026:1419). In lettuce seeds exposed to high temperatures, KAR1 application effectively alleviated thermoinhibition. This response was associated with an increased GA3/ABA ratio, enhanced activities of antioxidant and hydrolytic enzymes, and preservation of membrane integrity, collectively contributing to more rapid and uniform germination under high-temperature stress conditions (Wang et al., 2026:1).

Responses to KARs vary among plant species. For example, Although KAR2 exhibits the highest activity among karrikins in *Arabidopsis thaliana*, its effectiveness is lower than that of KAR1 in several other plant species (Flematti et al., 2007:2189; Nelson et al., 2009:863). While KAR4 is inactive in Arabidopsis, it can promote germination at higher concentrations in lettuce and other smoke-sensitive species (Flematti et al., 2007:2189). At low KAR1 concentrations (<1 ppb), strong germination activity has been observed in lettuce seeds and in species such as *Conostylis aculeata*, *Stylidium affine* (Australia), *Syncarpha vestita* (Africa), and *Nicotiana attenuata* and *Emmenanthe penduliflora* (North America) (Flematti et al., 2004a:977; Flematti et al., 2015:108). Nearly 1200 plant species encompassing all categories of seed dormancy have been reported to exhibit positive responses to smoke treatment (Dixon et al., 2009:155; Kępczyński, 2018:87).

Light and hormone interactions also regulate the effects of KARs on germination. KAR1 enhances the biosynthesis of GA by regulating *GA3ox* and *YUCCA* genes through the phytochrome system and decreases IAA and ABA levels (Gupta et al., 2019:458; Xu et al., 2023:112740). Additionally, KAR1 may interact with NO to promote germination of wild oat by inducing ethylene biosynthesis. This promotive effect is mediated by the activation of

ACC synthase and ACC oxidase, two key enzymes participating in ethylene biosynthesis (Ruduś et al., 2019:719). KARs may also delay germination under shaded conditions by affecting GA and ABA levels; however, this effect is not observed under white light or in darkness (Figure 4) (Stirnberg et al., 2002:1131; Nelson et al., 2010:7095).

Figure 4 Proposed mechanism of karrikin-mediated seed germination



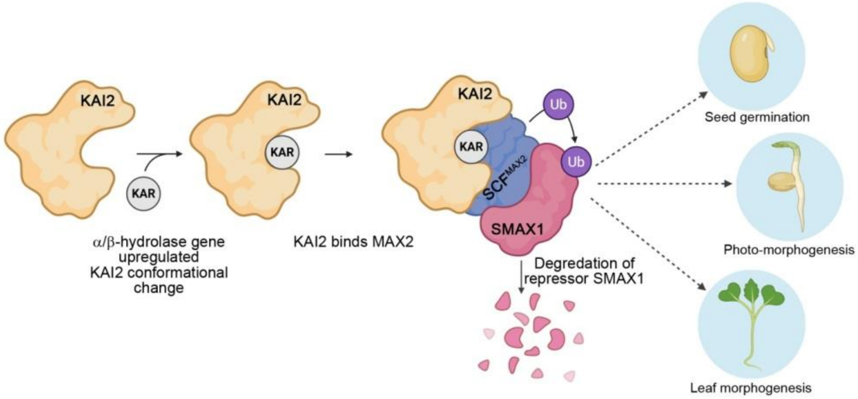
In conclusion, the capacity of KARs to promote germination offers significant potential for agricultural applications. In particular, KARs may be employed to enhance seed performance in plant species characterized by strong dormancy or reduced germination capacity under stressful conditions (Deng et al., 2025:2775).

KAR Signal Transduction Mechanisms

The karrikin (KAR) signal transduction pathway occurs through three main stages involving ligand perception, receptor complex formation, and the degradation of repressor proteins (Figure 5) (Wang et al., 2020a:2251; Wang et al., 2020b:277; Waters and Nelson, 2023:1525). In the first stage, KAR compounds are recognized by the receptor protein KAI2 (KARRIKIN INSENSITIVE 2), a member of the α/β-hydrolase protein family. In the second stage, activated KAI2 interacts with the F-box protein MAX2 (MORE AXILLARY GROWTH 2) to form an SCF^{MAX2} (Skp–Cullin–F-box) ubiquitin ligase complex. This complex triggers the ubiquitination and subsequent degradation of repressor proteins such as SMAX1 and SMXL2 through the 26S proteasome. Consequently, the suppression of downstream target genes is removed, leading to the regulation of various plant growth and developmental processes (Figure 5) (Yao and Waters, 2020:1774; Varshney and Gutjahr, 2023:984).

KAI2 was first identified as the receptor of KARs in Arabidopsis (Guo et al., 2013:8284; Kagiyama et al., 2013:147). Structurally homologous to the SL receptor D14, KAI2 is an α/β-hydrolase enzyme capable of recognizing KARs (Deng et al., 2025:2775). A recent study have demonstrated that KAR1 directly interacts with KAI2 and triggers a signal transduction cascade similar to the SL signaling pathway (Wang et al., 2022:450). KAR binding induces a conformational alteration in KAI2, and this alteration initiates the interaction of KAI2 with downstream signaling components. Upon activation by KAR binding, KAI2 interacts with MAX2 and promotes the proteasomal degradation of SUPPRESSOR OF MAX2-LIKE (SMXL) family repressor proteins, specifically SMAX1 and SMXL2 in Arabidopsis (Khosla et al., 2020:2639; Wang et al., 2020a:2251; Zheng et al., 2020:2780; Park et al., 2022:2671). This interaction subsequently leads to the formation of the SCF^{MAX2} (Skp–Cullin–F-box) ubiquitin ligase complex. During this process, SMAX1 and SMXL2 proteins undergo polyubiquitination and are subsequently degraded by the 26S proteasome, which in turn activates the expression of KAR-responsive genes (Yao and Waters, 2020:1774; Li et al., 2023:1043).

Figure 5 Karrikin signaling pathway and physiological responses



KAI2 receptors are capable of sensing not only KARs and SLs, but also sesquiterpene lactone molecules (Takei et al., 2023:996; Stirling et al., 2024:1318). For instance, in petunia, the PhKAI2ia receptor specifically detects (−)-germacrene D, a sesquiterpene produced by PhTPS1 in flowers, thereby triggering a

KAI2-dependent signaling mechanism essential for plant fitness (Stirling et al., 2024:1318). These findings demonstrate that KAI2 may function not only in the perception of KAR-like compounds but also in the detection of diverse volatile signaling molecules, emphasizing the potential importance of this receptor in plant biology and agricultural applications (Deng et al., 2025:2775).

A recent study has shown that SMAX1 is not only a repressor protein but also functions in transcriptional regulation by binding to its own promoter as well as to the promoters of *GA3ox1* and *GA3ox2* (Xu et al., 2023:112740). As a result of the degradation of repressor proteins, numerous physiological processes, including seed germination, photomorphogenesis, hypocotyl elongation, seedling vigor, and abiotic stress tolerance, are regulated (Guo et al., 2013:8284; Bürger and Chory, 2020:395).

KAI2 and D14 receptors utilize similar mechanisms through their α/β-hydrolase structures and Ser-His-Asp catalytic triads. In the D14–SL axis, the SL ligand binds and repressor proteins SMXL6/7/8 are degraded via MAX2, resulting in alterations in shoot branching and root architecture. In the KAI2–KAR axis, degradation of SMXL2 and SMAX1 triggers germination and seedling development. Therefore, the KAI2–MAX2–SMAX1/SMXL2 and D14–MAX2–SMXL6/7/8 complexes are considered core signaling modules involved in plant development (Waters et al., 2012:1285; Morffy et al., 2016:176; Mizuno et al., 2021:2395; Basso et al., 2024:1; Guillory et al., 2024:1655). Although both pathways share MAX2 as a common component, their biological outputs differ due to distinct receptors and repressor proteins (Mitra et al., 2021:1591). Nevertheless, researches have revealed that SMXL proteins are not restricted to a single signaling pathway but also participate in cross-regulation between KAR and SL signaling pathways (Wang et al., 2020a:2251; Wang et al., 2020b:277; Li et al., 2022:100303).

Crystallographic studies and ligand-binding experiments investigating the molecular basis of the KAR signaling pathway have demonstrated that specific regions of the KAI2–KAR1 complex form an appropriate binding surface for signal transduction. Hydrophobic amino acids connecting the polar edge of KAR1 with the helical domain insertion direct the interaction of KAI2 with downstream components (Guo et al., 2013:8284). Furthermore, *kai2* mutants do not respond to KAR1 application, clearly demonstrating that KAI2 is essential for KAR perception (Nelson et al., 2011:8897; Waters et al., 2012:1285).

The molecular constituents of the KAR signaling pathway are also conserved in plant species other than Arabidopsis, and the roles of MAX2- and SMAX1-like proteins have been confirmed in rice and *Lotus japonicus* (Waters et al., 2014:623; Carbonnel et al., 2020:21757). Phylogenetic analyses have revealed that KAI2 possesses multiple copies in parasitic plants such as those belonging to the Orobanchaceae family, with some copies perceiving KARs while others showing no response in Arabidopsis (Conn et al., 2015:540; Toh et al., 2015:203). In addition, a KAR-specific receptor named KAI2iB has been identified in *Striga hermonthica*, and positional differences at the entrance of the ligand-binding pocket were shown to confer KAR-specific selectivity (Xu et al., 2016:31386). These findings highlight both the evolutionary relationship and the specialized functions of KAR and SL signaling pathways.

Roles of KARs in Environmental Stress Responses

Abiotic stresses, including salinity, high temperature conditions, drought, and heavy metal exposure, influence plants at both the morphological and molecular scales, ultimately causing declines in plant growth and productivity (Zhang et al., 2022:104).

Plants exhibit various cellular and molecular responses to reduce stress-induced damage and maintain viability (Fahad et al., 2015:391). To maintain growth and productivity under stressful environments, plants employ various physiological, biochemical, morphological, and developmental strategies. Adaptation to these stresses involves complex responses, including the accumulation of various organic solutes, maintenance of cellular mechanisms and ion balance, scavenging of free radicals, increased expression of certain proteins and genes, and induction of phytohormones (Saleem et al., 2024:753). In particular, phytohormones have emerged as an environmentally friendly approach that helps enhance abiotic stress tolerance and regulates plant growth, development, and responses under stressful conditions (Das et al., 2025:329). KARs have gained attention as important exogenous molecules that strengthen plant resilience to abiotic stresses (Shah et al., 2020:216; Kamran et al., 2024:1174). KARs enhance cold tolerance in tomato (Liu et al., 2023:111720), promote leaf expansion (Baldrianová et al., 2015:7), and stimulate seed germination in various species (Sami et al., 2021:1257). Additionally, stress tolerance-enhancing effects of KARs have been strongly confirmed through examples such as alleviation of salt stress in maize and rice (Jamil et al., 2014:159; Waheed et al., 2016:1763), reduction of heat stress in tomato (Jain et al., 2006:263), and enhancement of drought tolerance in soybean (Li et al., 2018:238). Activation of the KAR signaling pathway in plants under abiotic stress conditions such as drought suggests that KARs may play a potential role in the regulation of abiotic stress tolerance (Bai et al., 2017:41700). This characteristic demonstrates the biostimulant nature of KARs.

KARs contribute to stress tolerance by regulating the expression of stress-related genes, sustaining cellular redox equilibrium, and increasing the accumulation of protective metabolites at both the molecular and metabolic levels. These effects further strengthen abiotic stress tolerance in plants (Li et al., 2020:1789321; Shah et al., 2020:216). It has been proposed that KAI2 and MAX2 may mediate KAR-mediated drought tolerance by regulating ABA sensitivity and the accumulation of protective metabolites. KAR-induced tolerance and its related signaling pathways are believed to involve the stimulation of antioxidant defense mechanisms together with the alleviation of damage caused by lipid peroxidation (Shah et al., 2020:216).

Exogenous application of KAR1 increased seed germination rate, shoot length, radicle length, and seedling vigor in *Trachyspermum copticum* and *Foeniculum vulgare* under water deficient stress (MousaviNik et al., 2016:57). However, the mechanisms underlying this tolerance remain only partially understood. The reduction of KAR1-induced lipid peroxidation and increases in the antioxidant defense system has been attributed to drought tolerance (Sunmonu et al., 2016:4). The KAI2 receptor plays an important role in plant responses to drought stress. *kai2* mutants exhibited hypersensitivity to water-deficit conditions, increased stomatal opening due to ABA insensitivity, and uncontrolled water loss. In addition, membrane damage increased while anthocyanin content decreased in the mutants (Li et al., 2017:1007076; Wang et al., 2018:605).

KAR signaling is also effective in enhancing resilience to high temperatures. In tomato, KAR1 improved seed germination and seedling vigor under high-temperature conditions above 25 °C (Jain and Van Staden, 2007:175). Similarly, treatment of *Eragrostis tef* seeds with 10 nM KAR1 resulted in greater seedling length under both control and heat treatments (Ghebrehiwot et al., 2008:270). The involvement of KAR signaling in plant tolerance to temperature-related stress was initially identified through studies on the *kai2* mutant, which exhibited reduced germination capacity and increased susceptibility to abiotic stress compared with wild-type plants (Wang et al., 2018:605). Under temperature stress conditions, KAR signaling regulates heat- and cold-shock proteins (Shah et al., 2021a:921; Liu et al., 2023:111720). Transcriptome-based studies on kai2 mutants demonstrated that KAI2 contributes to thermotolerance in Arabidopsis through the regulation of heat shock factors and heat shock proteins. Transcription factors such as WRKY and NAC were proposed to be involved in this process (Abdelrahman et al., 2022:1914). In tomato, silencing of KAI2 and MAX1 through virus-induced gene silencing showed that KAR enhanced growth and yield through interaction with ABA signaling, reinforcement of C-repeat binding factor transcriptional activation, and reduction of ROS levels (Liu et al., 2023:111720). Comparative transcriptomic evaluation of cold-stressed *Chorispora bungeana* seedlings and *A. thaliana* indicated that biological processes associated with KAR responses were significantly enriched in both species under low-temperature conditions (Zhao et al., 2012:222). KARs also exhibited ameliorative effects in tea plants subjected to cold stress (Zhao et al., 2020:362). Exposure to abiotic stresses, particularly cold treatment at 4 °C, triggered the expression of the *SsKAI2* homolog gene in *Sapium sebiferum* (Shah et al., 2021b:657960). In addition, transgenic Arabidopsis plants with elevated *SsKAI2* expression exhibited lower ROS accumulation, greater ABA sensitivity during germination and stomatal movement, and upregulated transcripts of cold-responsive proteins and C-repeat binding factors (Shah et al., 2021b:657960). Application of KAR1 improved heat stress tolerance in *Agrostis stolonifera* by preserving leaf water status, limiting membrane injury, enhancing antioxidant enzyme activities, and upregulating the expression of stress- and heat-responsive genes, ultimately strengthening plant resistance to elevated temperatures (Wang et al., 2025b:016).

In *Sapium sebiferum* treated with KAR1 under high salinity and osmotic stress conditions, increases in germination, seedling development, and antioxidant enzyme activities were associated with the upregulation of genes involved in ABA signaling and stress response processes, including *WRKY33, DREB2A, ERF6,* and *SOS1* (Shah et al., 2020:216). In *Nigella sativa*, supplementation of 10 μM KAR1 pre-treatment with calcium application resulted in a salt-tolerant phenotype characterized by higher gas exchange capacity, enhanced ascorbate–glutathione cycling, and induced total unsaturated fatty acid content (Sharifi and Bidabadi, 2020:112460). In wheat seeds subjected to salinity, KAR1 application increased the activities of superoxide dismutase, ascorbate peroxidase, peroxidase, and catalase, as well as glutathione levels, thereby counteracting ROS accumulation and oxidative stress (Shah et al., 2021a:921). Investigations of salt stress responses in *kai2* mutants demonstrated that these plants exhibited pronounced phenotypic changes, reduced biomass accumulation, enhanced water loss, and higher oxidative damage, while NaCl exposure also caused excessive Na^+ accumulation together with a decreased K^+/Na^+ ratio relative to wild-type seedlings, reflecting increased ion toxicity (Mostofa et al., 2022:1927). Under NaCl stress conditions, KAR1 treatment enhanced relative leaf water content, shoot biomass, proline accumulation, chlorophyll levels, and antioxidant enzyme activities in *Agrostis stolonifera*, while simultaneously lowering hydrogen peroxide and malondialdehyde accumulation as well as electrolyte leakage, thereby mitigating the detrimental effects of salinity stress (Wang et al., 2025a:110800).

The protective role of KAR1 against heavy metals and persistent organic pollutants has also been demonstrated. In *Brassica alboglabra* subjected to combined Cd, BDE-28 (2,4,4′-tribromodiphenyl ether), and heat stress treatments, KAR1 exerted protective and regulatory influences on plant physiological functions. KAR1 application significantly decreased Cd accumulation, metal tolerance index, and translocation coefficient, indicating its potential to reduce Cd-induced toxicity and promote the restoration of metabolic homeostasis in plants (Ahmad et al., 2021:112047). Nanomolar KAR application alleviated Cd stress in *Triticum aestivum*. KAR reduced Cd uptake and consequently decreased Cd accumulation in plants while simultaneously increasing biomass, protecting the photosynthetic system, and strengthening antioxidant defense. Furthermore, it enhanced stress tolerance by regulating genes associated with metal transport, ROS balance, and hormone signaling. These results indicate that KAR possesses potential as an environmentally friendly application for heavy metal-contaminated agricultural lands (Zhou et al., 2025:139568).

Shaded environments constitute a major type of low-light stress affecting plant growth and development. Vegetation layers positioned above the canopy limit light penetration by decreasing both light intensity and the red to far-red light ratio, which consequently impairs seed germination, plant establishment, and early seedling development. In addition, plants cultivated under shaded conditions generally become more vulnerable to both abiotic and biotic stress factors (Casal, 2013:403). Previous studies have shown that KARs can function as external regulatory compounds that mitigate shade avoidance responses by stimulating hypocotyl growth and enhancing chlorophyll accumulation in plants (Nelson et al., 2010:7095).

In conclusion, the survival, growth, and maintenance of productivity in plants under abiotic stress conditions require the coordination of complex molecular and physiological mechanisms. KARs, as small biologically active components of plant-derived smoke, play an important role in strengthening these adaptive responses. Under stress conditions, KARs have been demonstrated to positively influence seed germination, seedling vigor, growth, and antioxidant systems, while activating signaling pathways through KAI2 and MAX2 and enhancing the expression of stress-protective genes. In addition, KARs strengthen plant stress tolerance through the reduction of lipid peroxidation, maintenance of cellular redox balance, and hormonal regulation. These findings suggest that KARs can be considered not only as signals regulating germination and seedling development but also as potential biostimulants protecting plants against environmental stresses. In the future, more detailed investigations into the mechanisms by which KARs enhance abiotic stress tolerance in different species will provide new opportunities for developing sustainable stress management strategies in agricultural applications.

References

Abdelrahman, M., Mostofa, M. G., Tran, C. D., El-Sayed, M., Li, W., Sulieman, S., ... & Tran, L. S. P. (2022). The karrikin receptor KARRIKIN INSENSITIVE2 positively regulates heat stress tolerance in *Arabidopsis thaliana*. *Plant and Cell Physiology*, *63*(12), 1914-1926.

Ahmad, A., Shahzadi, I., Mubeen, S., Yasin, N. A., Akram, W., Khan, W. U., & Wu, T. (2021). Karrikinolide alleviates BDE-28, heat and Cd stressors in *Brassica alboglabra* by correlating and modulating biochemical attributes, antioxidative machinery and osmoregulators. *Ecotoxicology and Environmental Safety*, *213*, 112047.

Antala, M., Sytar, O., Rastogi, A., & Brestic, M. (2019). Potential of karrikins as novel plant growth regulators in agriculture. *Plants*, *9*(1), 43.

Ariyakulkiat, L., Pinit, S., & Chaiwanon, J. (2026). Smoke water promotes root meristem activity and cell elongation but inhibits root hair growth under phosphorus deficiency in rice. *South African Journal of Botany*, *191*, 12-19.

Bączek-Kwinta, R., Grabowska-Joachimiak, A., Baran, A., & Rizwana Jamal, A. (2026). Karrikin 1 Modulates Germination and Growth of Invasive *Solidago gigantea*: Potential for Ecological Management and Photoblastism Research. *Applied Sciences*, *16*(3), 1419.

Bai, Z. Y., Wang, T., Wu, Y. H., Wang, K., Liang, Q. Y., Pan, Y. Z., ... & Liu, Q. L. (2017). Whole-transcriptome sequence analysis of differentially expressed genes in *Phormium tenax* under drought stress. *Scientific Reports*, *7*(1), 41700.

Baldrianová, J., Černý, M., Novák, J., Jedelský, P. L., Divíšková, E., & Brzobohatý, B. (2015). Arabidopsis proteome responses to the smoke-derived growth regulator karrikin. *Journal of Proteomics*, *120*, 7-20.

Baldwin, I. T., Staszak-Kozinski, L., & Davidson, R. (1994). Up in smoke: I. Smoke-derived germination cues for postfire annual, Nicotiana attenuata torr. Ex. Watson. *Journal of Chemical Ecology*, *20*(9), 2345-2371.

Basso, M. F., Contaldi, F., Lo Celso, F., Baratto, C. M., Grossi-de-Sa, M. F., Barone, G., ... & Martinelli, F. (2024). Identification and expression profile of the *SMAX/SMXL* family genes in chickpea and lentil provide important players of biotechnological interest involved in plant branching. *Planta*, *259*(1), 1.

Bürger, M., & Chory, J. (2020). The many models of strigolactone signaling. *Trends in Plant Science*, *25*(4), 395-405.

Carbonnel, S., Das, D., Varshney, K., Kolodziej, M. C., Villaécija-Aguilar, J. A., & Gutjahr, C. (2020). The karrikin signaling regulator SMAX1 controls *Lotus japonicus* root and root hair development by suppressing ethylene biosynthesis.

Proceedings of the National Academy of Sciences, *117*(35), 21757-21765.

Casal, J. J. (2013). Photoreceptor signaling networks in plant responses to shade. *Annual Review of Plant Biology*, *64*, 403–427.

Cembrowska-Lech, D., & Kępczyński, J. (2017). Plant-derived smoke induced activity of amylases, DNA replication and β-tubulin accumulation before radicle protrusion of dormant *Avena fatua* L. caryopses. *Acta Physiologiae Plantarum*, *39*(1), 39.

Conn, C. E., Bythell-Douglas, R., Neumann, D., Yoshida, S., Whittington, B., Westwood, J. H., ... & Nelson, D. C. (2015). Convergent evolution of strigolactone perception enabled host detection in parasitic plants. *Science*, *349*(6247), 540-543.

Das, S., Shil, S., Rime, J., Alice, A. K., Yumkhaibam, T., Mounika, V., ... & Singh, S. (2025). Phytohormonal signaling in plant resilience: advances and strategies for enhancing abiotic stress tolerance. *Plant Growth Regulation*, *105*(2), 329-360.

De Lange, J. H., & Boucher, C. (1990). Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. *South African Journal of Botany*, *56*(6), 700-703.

Deng, Q., Wang, H., Qiu, Y., Wang, D., Xia, Y., Zhang, Y., ... & Zhang, H. (2025). The multifaceted impact of Karrikin signaling in plants. *International Journal of Molecular Sciences*, *26*(6), 2775.

Dixon, K. W., Merritt, D. J., Flematti, G. R., & Ghisalberti, E. L. (2009). Karrikinolide–a phytoreactive compound derived from smoke with applications in horticulture, ecological restoration and agriculture. *Acta Horticulturae*, *813*(2009), 155-170.

Fahad, S., Hussain, S., Matloob, A., Khan, F. A., Khaliq, A., Saud, S., ... & Huang, J. (2015). Phytohormones and plant responses to salinity stress: a review. *Plant Growth Regulation*, *75*(2), 391-404.

Fang, P., Hu, Y., Guo, Q., Li, L., & Xu, P. (2023). Karrikins, redox and plant abiotic stress tolerance: a focal review. *Plant Stress*, *9*, 100185.

Fichtner, F., Humphreys, J. L., Barbier, F. F., Feil, R., Westhoff, P., Moseler, A., ... & Beveridge, C. A. (2024). Strigolactone signalling inhibits trehalose 6-phosphate signalling independently of BRC1 to suppress shoot branching. *New Phytologist*, *244*(3), 900-913.

Flematti, G. R., Ghisalberti, E. L., Dixon, K. W., & Trengove, R. D. (2004a). A compound from smoke that promotes seed germination. *Science*, *305*(5686), 977-977.

Flematti, G. R., Ghisalberti, E. L., Dixon, K. W., & Trengove, R. D. (2004b). Molecular weight of a germination-enhancing compound in smoke. *Plant Soil*, *263*(1), 1–4.

Flematti, G. R., Ghisalberti, E. L., Dixon, K. W., & Trengove, R. D. (2005). Synthesis of the seed germination stimulant 3-methyl-2H-furo [2, 3-c] pyran-2-one. *Tetrahedron Letters*, *46*(34), 5719-5721.

Flematti, G. R., Goddard-Borger, E. D., Merritt, D. J., Ghisalberti, E. L., Dixon, K. W., & Trengove, R. D. (2007). Preparation of 2 H-furo [2, 3-c] pyran-2-one derivatives and evaluation of their germination-promoting activity. *Journal of Agricultural and Food Chemistry*, *55*(6), 2189-2194.

Flematti, G. R., Ghisalberti, E. L., Dixon, K. W., & Trengove, R. D. (2009). Identification of alkyl substituted 2 H-furo [2, 3-c] pyran-2-ones as germination stimulants present in smoke. *Journal of Agricultural and Food Chemistry*, *57*(20), 9475-9480.

Flematti, G. R., Scaffidi, A., Goddard-Borger, E. D., Heath, C. H., Nelson, D. C., Commander, L. E., ... & Ghisalberti, E. L. (2010). Structure− activity relationship of karrikin germination stimulants. *Journal of Agricultural and Food Chemistry*, *58*(15), 8612-8617.

Flematti, G. R., Scaffidi, A., Dixon, K. W., Smith, S. M., & Ghisalberti, E. L. (2011). Production of the seed germination stimulant karrikinolide from combustion of simple carbohydrates. *Journal of Agricultural and Food Chemistry*, *59*(4), 1195-1198.

Flematti, G. R., Dixon, K. W., & Smith, S. M. (2015). What are karrikins and how were they ‘discovered’by plants?. *BMC Biology*, *13*(1), 108.

Ghebrehiwot, H. M., Kulkarni, M. G., Kirkman, K. P., & Van Staden, J. (2008). Smoke-water and a smoke-isolated butenolide improve germination and seedling vigour of Eragrostis Tef (Zucc.)

Trotter under high temperature and low osmotic potential. J*ournal of Agronomy and Crop Science*, *194*(4), 270-277.

Goddard-Borger, E. D., Ghisalberti, E. L., & Stick, R. V. (2007). Synthesis of the Germination Stimulant 3-Methyl-2H-furo [2, 3-c] pyran-2-one and Analogous Compounds from Carbohydrates. *European Journal of Organic Chemistry*, *2007*, 3925–3934.

Gonzalez, S., Franzese, J., & Ghermandi, L. (2025). Germination responses of native fire ephemerals of *Patagonian grasslands* to smoke water and karrikinolide. *Frontiers in Plant Science*, *16*, 1550692.

Gray, W. M. (2004). Hormonal regulation of plant growth and development. *PLoS Biology*, *2*(9), e311.

Guillory, A., Lopez-Obando, M., Bouchenine, K., Le Bris, P., Lécureuil, A., Pillot, J. P., ... & Bonhomme, S. (2024). SUPPRESSOR OF MAX2 1-LIKE (SMXL) homologs are MAX2-dependent repressors of *Physcomitrium patens* growth. *The Plant Cell*, *36*(5), 1655-1672.

Guo, Y., Zheng, Z., La Clair, J. J., Chory, J., & Noel, J. P. (2013). Smoke-derived karrikin perception by the α/β-hydrolase KAI2 from Arabidopsis. *Proceedings of the National Academy of Sciences*, *110*(20), 8284-8289.

Gupta, S., Plačková, L., Kulkarni, M. G., Doležal, K., & Van Staden, J. (2019). Role of smoke stimulatory and inhibitory biomolecules in phytochrome-regulated seed germination of *Lactuca sativa*. *Plant Physiology*, *181*(2), 458-470.

Jain, N., Kulkarni, M. G., & Van Staden, J. (2006). A butenolide, isolated from smoke, can overcome the detrimental effects of extreme temperatures during tomato seed germination. *Plant Growth Regulation*, *49*(2), 263-267.

Jain, N., & Van Staden, J. (2007). The potential of the smoke-derived compound 3-methyl-2H-furo [2, 3-c] pyran-2-one as a priming agent for tomato seeds. *Seed Science Research*, *17*(3), 175-181.

Jamil, M., Kanwal, M., Aslam, M. M., Khan, S. U., Malook, I., Tu, J., & ur Rehman, S. (2014). Effect of plant-derived smoke priming on physiological and biochemical characteristics of rice under salt stress condition. *Australian Journal of Crop Science*, *8*(2), 159-170.

Kagiyama, M., Hirano, Y., Mori, T., Kim, S. Y., Kyozuka, J., Seto, Y., ... & Hakoshima, T. (2013). Structures of D 14 and D 14 L in the strigolactone and karrikin signaling pathways. *Genes to Cells*, *18*(2), 147-160.

Kamran, M., Melville, K. T., & Waters, M. T. (2024). Karrikin signalling: impacts on plant development and abiotic stress tolerance. *Journal of Experimental Botany*, *75*(4), 1174-1186.

Keeley, S. C., & Pizzorno, M. (1986). Charred wood stimulated germination of two fire-following herbs of the

California chaparral and the role of hemicellulose. *American Journal of Botany*, *73*(9), 1289-1297.

Kępczyński, J. (2018). Induction of agricultural weed seed germination by smoke and smoke-derived karrikin (KAR1), with a particular reference to *Avena fatua* L. *Acta Physiologiae Plantarum*, *40*(5), 87.

Kępczyński, J., & Kępczyńska, E. (2023). Plant-derived smoke and karrikin 1 in seed priming and seed biotechnology. *Plants*, *12*(12), 2378.

Khosla, A., Morffy, N., Li, Q., Faure, L., Chang, S. H., Yao, J., ... & Nelson, D. C. (2020). Structure–function analysis of SMAX1 reveals domains that mediate its karrikin-induced proteolysis and interaction with the receptor KAI2. *Plant Cell*, *32*(8), 2639-2659.

Li, W., Nguyen, K. H., Chu, H. D., Ha, C. V., Watanabe, Y., Osakabe, Y., ... & Tran, L. S. P. (2017). The karrikin receptor KAI2 promotes drought resistance in *Arabidopsis thaliana*. *PLoS Genetics*, *13*(11), e1007076.

Li, X., ur Rehman, S., Yamaguchi, H., Hitachi, K., Tsuchida, K., Yamaguchi, T., ... & Komatsu, S. (2018). Proteomic analysis of the effect of plant-derived smoke on soybean during recovery from flooding stress. *Journal of Proteomics*, *181*, 238-248.

Li, W., Gupta, A., Tian, H., Nguyen, K. H., Tran, C. D., Watanabe, Y., ...& Phan Tran, L. S. (2020). Different strategies of strigolactone and karrikin signals in regulating the resistance of

Arabidopsis thaliana to water-deficit stress. *Plant Signaling & Behavior*, *15*(9), 1789321.

Li, Q., Martín-Fontecha, E. S., Khosla, A., White, A. R., Chang, S., Cubas, P., & Nelson, D. C. (2022). The strigolactone receptor D14 targets SMAX1 for degradation in response to GR24 treatment and osmotic stress. *Plant Communications*, *3*(2), 100303.

Li, C., Lu, X., Liu, Y., Xu, J., & Yu, W. (2023). Strigolactone alleviates the adverse effects of salt stress on seed germination in cucumber by enhancing antioxidant capacity. *Antioxidants*, *12*(5), 1043.

Lin, H., Wang, R., Qian, Q., Yan, M., Meng, X., Fu, Z., ... & Wang, Y. (2009). DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. *The Plant Cell*, *21*(5), 1512-1525.

Liu, M., Shan, Q., Ding, E., Gu, T., & Gong, B. (2023). Karrikin increases tomato cold tolerance via strigolactone and the abscisic acid signaling network. *Plant Science, 332*, 111720.

Mitra, D., BE, G. S., Khoshru, B., De Los Santos Villalobos, S., Belz, C., Chaudhary, P., ... & Mohapatra, P. K. D. (2021). Impacts of arbuscular mycorrhizal fungi on rice growth, development, and stress management with a particular emphasis on strigolactone effects on root development. *Communications in Soil Science and Plant Analysis*, *52*(14), 1591-1621.

Mizuno, Y., Komatsu, A., Shimazaki, S., Naramoto, S., Inoue, K., Xie, X., ... & Kyozuka, J. (2021). Major components of the

KARRIKIN INSENSITIVE2-dependent signaling pathway are conserved in the liverwort *Marchantia polymorpha*. *The Plant Cell*, *33*(7), 2395-2411.

Morffy, N., Faure, L., & Nelson, D. C. (2016). Smoke and hormone mirrors: action and evolution of karrikin and strigolactone signaling. *Trends in Genetics*, *32*(3), 176-188.

Mostofa, M. G., Abdelrahman, M., Rahman, M. M., Tran, C. D., Nguyen, K. H., Watanabe, Y., ... & Tran, L. S. P. (2022). Karrikin receptor KAI2 coordinates salt tolerance mechanisms in *Arabidopsis thaliana*. *Plant and Cell Physiology*, *63*(12), 1927-1942.

MousaviNik, M., Jowkar, A., & RahimianBoogar, A. (2016). Positive effects of karrikin on seed germination of three medicinal herbs under drought stress. *Iran Agricultural Research*, *35*(2), 57-64.

Nakamura, H., Xue, Y. L., Miyakawa, T., Hou, F., Qin, H. M., Fukui, K., ... & Asami, T. (2013). Molecular mechanism of strigolactone perception by DWARF14. *Nature Communications*, *4*(1), 2613.

Nelson, D. C., Riseborough, J. A., Flematti, G. R., Stevens, J., Ghisalberti, E. L., Dixon, K. W., & Smith, S. M. (2009). Karrikins discovered in smoke trigger Arabidopsis seed germination by a mechanism requiring gibberellic acid synthesis and light. *Plant Physiology*, *149*(2), 863-873.

Nelson, D. C., Flematti, G. R., Riseborough, J. A., Ghisalberti, E. L., Dixon, K. W., & Smith, S. M. (2010). Karrikins enhance light responses during germination and seedling development in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences*, *107*(15), 7095-7100.

Nelson, D. C., Scaffidi, A., Dun, E. A., Waters, M. T., Flematti, G. R., Dixon, K. W., ... & Smith, S. M. (2011). F-box protein MAX2 has dual roles in karrikin and strigolactone signaling in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences*, *108*(21), 8897-8902.

Nelson, D. C., Flematti, G. R., Ghisalberti, E. L., Dixon, K. W., & Smith, S. M. (2012). Regulation of seed germination and seedling growth by chemical signals from burning vegetation. *Annual Review of Plant Biology*, *63*(1), 107-130.

Park, Y. J., Kim, J. Y., & Park, C. M. (2022). SMAX1 potentiates phytochrome B-mediated hypocotyl thermomorphogenesis. *The Plant Cell*, *34*(7), 2671-2687.

Rolando, S. S., Norgrove, L., Sutter, L., Dienes-Nagy, Á., Bergoënd, A., & Simonnet, X. (2026). Smoke water and smoke-derived karrikinolide, KAR1, improved germination in some medicinal and aromatic plants. *Scientific Reports*, *16*, 865.

Ruduś, I., Cembrowska-Lech, D., Jaworska, A., & Kępczyński, J. (2019). Involvement of ethylene biosynthesis and perception during germination of dormant *Avena fatua* L. caryopses induced by KAR1 or GA3. *Planta*, *249*(3), 719-738.

Saleem, M. H., Mfarrej, M. F. B., Khan, K. A., Ercisli, S., Elsharkawy, M. M., & Fahad, S. (2024). Advances in physiochemical and molecular mechanisms of abiotic stress tolerance in plants. Journal of Crop Health, *76*(4), 753-767.

Samanta, S., & Roychoudhury, A. (2025). Molecular crosstalk of jasmonate with major phytohormones and plant growth regulators during diverse stress responses. *Journal of Plant Growth Regulation*, *44*(1), 62-88.

Sami, A., Rehman, S., Tanvir, M. A., Zhou, X. Y., Zhu, Z. H., & Zhou, K. (2021). Assessment of the germination potential of *Brassica oleracea* seeds treated with karrikin 1 and cyanide, which modify the ethylene biosynthetic pathway. *Journal of Plant Growth Regulation*, *40*(3), 1257-1269.

Shah, F. A., Wei, X., Wang, Q., Liu, W., Wang, D., Yao, Y., ... & Wu, L. (2020). Karrikin improves osmotic and salt stress tolerance via the regulation of the redox homeostasis in the oil plant *Sapium sebiferum*. *Frontiers in Plant Science*, *11*, 216.

Shah, F. A., Ni, J., Tang, C., Chen, X., Kan, W., & Wu, L. (2021a). Karrikinolide alleviates salt stress in wheat by regulating the redox and K^+/Na^+ homeostasis. *Plant Physiology and Biochemistry*, *167*, 921-933.

Shah, F. A., Ni, J., Yao, Y., Hu, H., Wei, R., & Wu, L. (2021b). Overexpression of karrikins receptor gene *Sapium sebiferum* KAI2 promotes the cold stress tolerance via regulating the redox homeostasis in *Arabidopsis thaliana*. *Frontiers in Plant Science*, *12*, 657960.

Sharifi, P., & Bidabadi, S. S. (2020). Strigolactone could enhances gas-exchange through augmented antioxidant defense system in *Salvia nemorosa* L. plants subjected to saline conditions stress. *Industrial Crops and Products*, *151*, 112460.

Smith, S. M., & Li, J. (2014). Signalling and responses to strigolactones and karrikins. *Current Opinion in Plant Biology*, *21*, 23-29.

Soós, V., Sebestyén, E., Posta, M., Kohout, L., Light, M. E., Van Staden, J., & Balázs, E. (2012). Molecular aspects of the antagonistic interaction of smoke-derived butenolides on the germination process of G rand R apids lettuce (*Lactuca sativa*) achenes. *New Phytologist*, *196*(4), 1060-1073.

Stirling, S. A., Guercio, A. M., Patrick, R. M., Huang, X. Q., Bergman, M. E., Dwivedi, V., ... & Dudareva, N. (2024). Volatile communication in plants relies on a KAI2-mediated signaling pathway. *Science*, *383*(6689), 1318-1325.

Stirnberg, P., van De Sande, K., & Leyser, H. O. (2002). MAX1 and MAX2 control shoot lateral branching in Arabidopsis. *Development*, *129*(5), 1131–1141.

Sunmonu, T. O., Kulkarni, M. G., & Van Staden, J. (2016). Smoke-water, karrikinolide and gibberellic acid stimulate growth in bean and maize seedlings by efficient starch mobilization and suppression of oxidative stress. *South African Journal of Botany*, *102*, 4-11.

Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., & Mittler, R. (2014). Abiotic and biotic stress combinations. *New Phytologist*, *203*(1), 32-43.

Takei, S., Uchiyama, Y., Bürger, M., Suzuki, T., Okabe, S., Chory, J., & Seto, Y. (2023). A divergent clade KAI2 protein in the root parasitic plant *Orobanche minor* is a highly sensitive strigolactone receptor and is involved in the perception of sesquiterpene lactones. *Plant and Cell Physiology*, *64*(9), 996-1007.

Toh, S., Kamiya, Y., Kawakami, N., Nambara, E., McCourt, P., & Tsuchiya, Y. (2012). Thermoinhibition uncovers a role for strigolactones in Arabidopsis seed germination. *Plant and Cell Physiology*, *53*(1), 107-117.

Toh, S., Holbrook-Smith, D., Stogios, P. J., Onopriyenko, O., Lumba, S., Tsuchiya, Y., ... & McCourt, P. (2015). Structure-function analysis identifies highly sensitive strigolactone receptors in *Striga*. *Science*, *350*(6257), 203-207.

Ullah, G., Ibrahim, M., Nawaz, G., Khatoon, A., Jamil, M., Rehman, S. U., ... & Tariq, A. (2023). Plant-derived smoke mitigates the inhibitory effects of the auxin inhibitor 2, 3, 5-Triiodo Benzoic Acid (TIBA) by enhancing root architecture and biochemical parameters in maize. *Plants*, *12*(14), 2604.

Van Staden, J., Jäger, A. K., Light, M. E., Burger, B. V., Brown, N. A. C., & Thomas, T. H. (2004). Isolation of the major germination cue from plant-derived smoke. *South African Journal of Botany*, *70*(4), 654-659.

Varshney, K., & Gutjahr, C. (2023). KAI2 can do: karrikin receptor function in plant development and response to abiotic and biotic factors. *Plant and Cell Physiology*, *64*(9), 984-995.

Waheed, M. A., Jamil, M., Khan, M. D., Shakir, S. K., & Rehman, S. U. (2016). Effect of plant-derived smoke solutions on physiological and biochemical attributes of maize (*Zea mays* L.) under salt stress. *Pakistan Journal of Botany*, *48*(5), 1763-1774.

Wang, L., Waters, M. T., & Smith, S. M. (2018). Karrikin-KAI2 signalling provides Arabidopsis seeds with tolerance to abiotic stress and inhibits germination under conditions unfavourable to seedling establishment. *New Phytologist*, *219*(2), 605-618.

Wang, L., Xu, Q., Yu, H., Ma, H., Li, X., Yang, J., ... & Wang, B. (2020a). Strigolactone and karrikin signaling pathways elicit ubiquitination and proteolysis of SMXL2 to regulate hypocotyl elongation in Arabidopsis. *The Plant Cell*, *32*(7), 2251-2270.

Wang, L., Wang, B., Yu, H., Guo, H., Lin, T., Kou, L., ... & Li, J. (2020b). Transcriptional regulation of strigolactone signalling in Arabidopsis. *Nature*, *583*(7815), 277-281.

Wang, Q., Smith, S. M., & Huang, J. (2022). Origins of strigolactone and karrikin signaling in plants. *Trends in Plant Science*, *27*(5), 450-459.

Wang, Y., Han, Y., Zhu, M., Li, X., Liu, Y., Tan, Z., ... & Zhang, X. (2025a). Karrikin1 mitigates salt stress in creeping bentgrass (*Agrostis stolonifera*) via physiological regulation and metabolite reprogramming. *Plant Physiology and Biochemistry*, 110800.

Wang, Y., Tan, Z., Han, Y., Zhu, M., Li, X., Yang, Z., & Zhang, X. (2025b). Foliar application of Karrikin 1 enhanced heat tolerance of creeping bentgrass by activating antioxidant defense, signaling transduction, and stress responsive pathways. *Grass Research*, *5*(1), e016.

Wang, H., Chen, F., Wang, D., Leng, J., Deng, Q., Xu, X., ... & Zhang, H. (2026). KAR1 Priming Breaks Seed Dormancy by Regulating Antioxidant System and Plant Hormones Under High Temperature in Lettuce Seeds. *Journal of Plant Growth Regulation*, 1-12.

Wani, S. H., Kumar, V., Shriram, V., & Sah, S. K. (2016). Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *The Crop Journal*, *4*(3), 162-176.

Waters, M. T., Nelson, D. C., Scaffidi, A., Flematti, G. R., Sun, Y. K., Dixon, K. W., & Smith, S. M. (2012). Specialisation within the DWARF14 protein family confers distinct responses to karrikins and strigolactones in Arabidopsis. *Development*, *139*(7), 1285-1295.

Waters, M. T., Scaffidi, A., Sun, Y. K., Flematti, G. R., & Smith, S. M. (2014). The karrikin response system of Arabidopsis. *The Plant Journal*, *79*(4), 623-631.

Waters, M. T., & Nelson, D. C. (2023). Karrikin perception and signalling. *New Phytologist*, *237*(5), 1525-1541.

Wicklow, D. T. (1977). Germination response in *Emmenanthe penduliflora* (Hydrophyllaceae). *Ecology*, *58*(1), 201-205.

Xiong, L., Schumaker, K. S., & Zhu, J. K. (2002). Cell signaling during cold, drought, and salt stress. *The Plant Cell*, *14*(1), 165-183.

Xu, Y., Miyakawa, T., Nakamura, H., Nakamura, A., Imamura, Y., Asami, T., & Tanokura, M. (2016). Structural basis of unique ligand specificity of KAI2-like protein from parasitic weed *Striga hermonthica*. *Scientific Reports*, *6*(1), 31386.

Xu, P., Hu, J., Chen, H., & Cai, W. (2023). SMAX1 interacts with DELLA protein to inhibit seed germination under weak light conditions via gibberellin biosynthesis in Arabidopsis. *Cell Reports*, *42*(7), 112740.

Yao, J., & Waters, M. T. (2020). Perception of karrikins by plants: a continuing enigma. *Journal of Experimental Botany*, *71*(6), 1774-1781.

Zhang, H., Zhu, J., Gong, Z., & Zhu, J. K. (2022). Abiotic stress responses in plants. *Nature Reviews Genetics*, *23*(2), 104-119.

Zheng, J., Hong, K., Zeng, L., Wang, L., Kang, S., Qu, M., ... & Xiong, G. (2020). Karrikin signaling acts parallel to and additively with strigolactone signaling to regulate rice mesocotyl elongation in darkness. *Plant Cell*, *32*(9), 2780-2805.

Zheng, Y., Wang, X., Cui, X., Wang, K., Wang, Y., & He, Y. (2023). Phytohormones regulate the abiotic stress: An overview of physiological, biochemical, and molecular responses in horticultural crops. *Frontiers in Plant Science*, *13*, 1095363.

Zhao, Z., Tan, L., Dang, C., Zhang, H., Wu, Q., & An, L. (2012). Deep-sequencing transcriptome analysis of chilling tolerance mechanisms of a subnival alpine plant, *Chorispora bungeana*. *BMC Plant Biology*, *12*(1), 222.

Zhao, M., Zhang, N., Gao, T., Jin, J., Jing, T., Wang, J., ... & Song, C. (2020). Sesquiterpene glucosylation mediated by glucosyltransferase UGT91Q2 is involved in the modulation of cold stress tolerance in tea plants. *New Phytologist*, *226*(2), 362-372.

Zhou, T., Xiao, Y. S., Fu, Y. L., Li, H., Cui, R., Feng, Y. N., ... & Hua, Y. P. (2025). Unveiling the multifunctional roles of karrikin as a novel phytohormone in cadmium detoxification of bread wheat (*Triticum aestivum* L.). *Journal of Hazardous Materials*, 139568.

BİDGE