REVIEW ARTICLE

Exploring the Wonders of Duckweed: Unveiling the Intriguing World of the Smallest Free-Floating Aquatic Plant

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ABSTRACT

Duckweed is a member of the family Lemnaceae, which also includes the fastest-growing angiosperms, *Wolffia*. There are 36 species of duckweed, which are divided into five genera. The duckweed plant is widely distributed in ponds and ditches and is known for its high nutritional content, which includes a high protein as well as fat content, making it an excellent source of food for humans, poultry, and fish. Due to its ability to accumulate various chemicals and heavy metals, it plays an important role in phytoremediation. There is a high potential for biomass and starch accumulation in this plant. Biofuels can be produced from duckweed as the biomass accumulates rapidly. This review aims to present the latest research on duckweed species in an updated manner, providing information on the latest nutritional profile, molecular taxonomy, biomass accumulation, duckweed as a model plant, and duckweed's impact on the environment and sustainability.

Keywords: Duckweed, Molecular taxonomy, Nutritional profile, Aquatic flora, Sustainable environmental benefits.

Highlights

- Duckweed species are the smallest free floating aquatic plants.
- Duckweed contains good nutritional profile and potential source for livestock and aquaculture.
- It has ability to remove pollutants from water bodies.
- It is used as model plant for many molecular and genomics studies.
- Promising bioactive compounds are present in this plant

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INTRODUCTION

Duckweeds are members of the Lemnaceae family and are divided into 36 species within five genera: *Lemna, Wolffia, Wolffiella, Spirodela and Landoltia* (Xu *et al.,* 2022). Duckweeds are generally called water meal or water lentils and are illustrated in (Fig. 1). These have frond, a leaf-like structure, with some genera having roots as in case of *Lemna, Spirodela* and *Landoltia*. The size of the plant ranges from less than 1-cm in *Wolffia angusta* to 1.5 cm in *Spirodela polyrhiza* (Acosta *et al.,* 2021). Duckweed is a type of aquatic monocotyledon with extremely small organs and remarkable size reduction, probably due to their adaptive lifestyle of rapid development and dispersion. Although duckweed is a flowering plant, vegetative propagation is the primary method for reproduction in which from the pockets of the parent plant, frond primordia will first begin to emerge and develop. In Duckweeds, doubling durations of two days or less are not uncommon, resulting in high biomass production (Imron *et al.,* 2019).

Duckweeds are plants that spread and flourish quickly. This fast growth is crucial to duckweed' ability to colonize new areas of water bodies and to flourish in the natural habitat. These are well-established aquatic plants for accumulating biomass for human consumption, industrial applications and energy production (Zeigler *et al.,* 2015). Duckweed, an aquatic plant, grows significantly more rapidly than the majority of higher plants because it's biomass can be doubled in 16 hours to 48 hours (Liu *et al.,* 2019). Duckweed cultivated mixotrophically had the highest growth rate and biomass production of any of the growth conditions examined, and it also significantly improved the uptake of organic carbon and nutrients from the environment. Compared to photoautotrophic and heterotrophic conditions, the mixotrophic growth rate was 6.22 & 4.98 times greater, respectively. Furthermore, mixotrophy generated higher biomass than the basic accumulation of biomass produced by photo autotrophy & heterotrophy (Sun *et al.,* 2020).

In several South Asian nations, such as Bangladesh, Pakistan, and India, the meal is primarily composed of carbs with little protein. Duckweed which is rich in proteins, is a good alternative to a carbohydrate-rich diet. Duckweed may also increase the protein level of a vegetarian or vegan diet, which is becoming increasingly popular in industrialized nations (Appenroth *et al.,* 2016). Protein accounts for 10 to 15 of the energy in the diet of wealthy cultures. Protein is necessary for humans to increase their BCM during growth, adaptation or recovery and to sustain normal growth conditions. In general, 9 of the 21 amino acids needed to make proteins are classified as essential since the body is unable to produce them, necessitating dietary intake which includes (isoleucine, histidine, lysine, leucine, threonine, tryptophan, phenylalanine, valine and methionine) (Hoffer, 2016).

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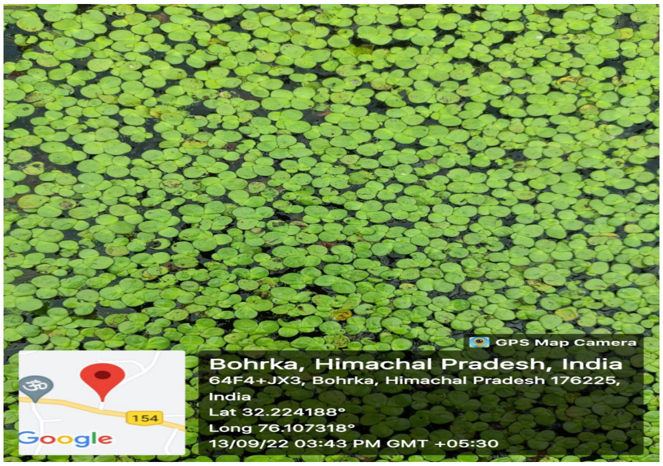

Fig. 1: Geo tagged picture of Duckweed plants growing in the stagnant pond water

Humans have traditionally consumed duckweed as food. In the study containing all 5 genera, per dry weight, the protein ranged from 20 to 35, starch from 4 to 10, and fat from 4 to 7. The distributions of amino acids are remarkably close to the WHO standards, with 4.8 Lys, 7.7 Phe + Tyr and 2.7 Met + Cys. A favorable n6/n3 ratio of 0.5 or less was achieved and there was 48 to 71 polyunsaturated fatty acids (Appenroth *et al.,* 2016). With an average value of 8.0 among the 30 species of duckweed, the Fatty acid content varies from 4.6 in the case of *Wolffiela welwitschii* to 14.2 in *W. borealis*. Among the species, 50 species fatty acid content ranges from 6.9 to 10.1. Fatty acids (Palmitic acid, ALA and Linoleic acid) constituted 80 of the total fatty acids found in all strains (Yan *et al.,* 2013). Variable carbohydrates found in duckweed include cellulose, starch, trace amounts of pectin, hemicellulose and others. The species and growing circumstances affect the precise carbohydrate content. For instance, depending on the species, the dry weight basis starch content of duckweed can range substantially from 3 in (*L. aequinoctialis*) to 75 in (*Landoltia punctata*) (Xu *et al.,* 2022).

Starch is a key photosynthate compound in duckweed. In the daytime, higher light levels and photoperiod caused more starch to accumulate in duckweed, which in turn provided more substrates for growth and metabolism at night. The generation of duckweed starch is the primary factor to be taken into account when using duckweed to produce biofuel. Duckweed's ability to produce starch largely depended on the amount of starch present and biomass growth (Yin *et al.,* 2015). Duckweed can be used to create promising alternative fuel sources, ethanol, biogas and butanol, which can help reduce reliance on scarce natural gas and crude oil. High rates of nutrient (phosphorus & nitrogen) uptake, a high biomass output, and tremendous potential as a substitute feedstock utilized in the generation of fuel source ethanol, biogas and butanol are all benefits of this plant (Cui *et al.,* 2015). Duckweed biomass's high concentrations of important fatty acids (linoleic acid and palmitic acid) and starch (3–75) point to its potential use in biorefineries (Verma *et al.,* 2015). When compared to the first and second generations, third-generation bioethanol offers additional advantages. The use of marine organisms like algae is the main goal of the third generation of bioethanol (Jambo *et al.,* 2016).

A promising method for removing or recovering extra nutrients from contaminated water is phytoremediation. Because aquatic plants have a remarkable potential to absorb and digest contaminants (phosphates, nitrates, heavy metals, etc.) from wastewater, their use in phytoremediation for wastewater is advantageous. The retrieval of nutrients like phosphates and nitrates from wastewater for use in manufacturing fertilizers and other food additives is another use for phytoremediation techniques (Mustafa *et al.,* 2021). *L. minor* is among the few aquatic plants that have been successfully used in large-scale chemical cleanup projects in aquatic environments and wastewater effluents. The efficacy of the common duckweeds in the remediation of heavy metals, chemical-free pollutants, pharmaceuticals and agrochemicals have been established through research, while its performance in the case of radioactive waste, petrochemicals, nanomaterials, and some other contaminants is average (Ekperusi *et al.,* 2019).

Duckweed is used as a model system for molecular genetics and genomics. The fundamental research efforts have already prepared the ground for a significant advancement in the re-establishment of duckweed as a desirable plant model. These efforts proceed from identifying the very first genome of duckweed to developing new chemicals that stimulate plant development (Lam *et al.,* 2014).

By means of passive dispersal, *L. minor* is an extremely mobile species. Streaming water and infrequently strong winds can aid in the local dispersal of Lemnaceae, but dissemination over greater distances (>10 km) and between distinct, isolated waterbodies is frequently aided via dispersing organisms. Particularly, birds were long identified as epizoochorous dispersers of a variety of aquatic plants, as in case of Lemnaceae (Coughlan *et al.,* 2017). Out of 31 studies species of duckweed studied, 8 were potentially high-risk invasive species which included *W. brasiliensis, Wolffiella welwitschii, W. lingulata, L. minor, W. arrhiza, L. perpusilla, L. aequinoctialis, Spirodela oligorrhiza* (Moodley *et al.,* 2016). *W. columbiana* can spread very quickly which has the capability of Invasion. The research region's vegetative reproduction is fast and productive (Ardenghi *et al.,* 2017).

Molecular Taxonomy

The family Lemnaceae (duckweed species) and Araceae are monophyletic. Despite the preference of several other researchers, the monophyly doesn't really demand the inclusion of duckweeds into the family Araceae (Sree *et al.,* 2016). Lemnaceae has always poorly fit into Araceae because of their great morphological differences. The Araceae family is characterized by having a specific inflorescence as well as vegetative characteristics, whereas duckweeds have reduced morphological characteristics which prevent the unification of these two families (Tippery *et al.,* 2021). The duckweed family was classified into two subfamilies: Wolffioideae (having absence of roots) and Lemnoideae (having varying numbers of roots). Wolffioideae, one of these subfamilies, is monophyletic, whereas Lemnoideae is definitely paraphyletic (Tippery *et al.,* 2015).

Lemnaceae family Structure related to genera (Tippery *et al.,* 2015) Genome size evolution and morphological development have been found to have a strong relationship in the phylogeny of the Lemnaceae family. The genus *Spirodela*, which is the most primitive, has the smallest genome size in comparison to *Wolffia*,

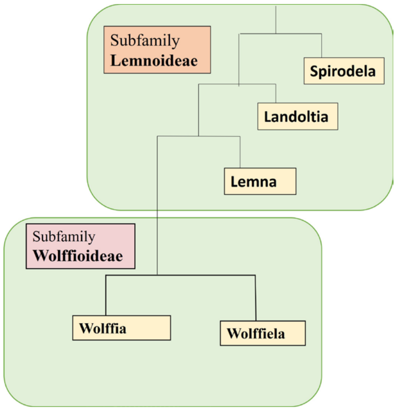

Fig. 2: Taxonomy of duckweed plants

which is the most developed has the largest genome size. This indicates that the genome size is correlated to morphological reduction, preferably than organism intricacy (Wang *et al.,* 2011). The correlation of the evolution of frond size and morphological reduction with genome size makes duckweed a fascinating subject for studies on karyotype and genome evolution (Hoang *et al.,* 2019). The phylogenetic study using chloroplast genome showed that the evolution order in different genera of Lemnaceae is in order: *Spirodela; Landoltia; Lemna; Wolffiella; Wolffia*. The study focused on the potential of using DNA from the entire chloroplast genomic sequence as a valuable tool for phylogenetic analysis of Lemnoideae (Ding *et al.,* 2017).

The species of *Lemna*, *Spirodela* and *Landoltia* have laterally placed vegetative envelopes from where daughter fronds may bud from the parent frond, a few to many roots, vegetative pouch, unique vascular system and the floral cavity in which floral tissues develop are identical are the various characteristics. On the other hand, the *Wolffia* and *Wolffiella* species only have single vegetative pouch, without roots, a unique floral cavity that opens towards the dorsal surface of the frond and lack of a vascular system (Bog *et al*., 2020). The taxonomical classification is given in Table 1 and demonstrated in Fig. 2.

Genus Spirodela and Landoltia

The classification of the three species (*Spirodela polyrhiza, L. punctata* and *Spirodela intermedia*) into two genera was supported by the extremely high genome size homology of the recently discovered clones of *Spirodela polyrhiza* to *Spirodela intermedia* and twice greater genome size in *L. punctata*. Significant variations in between these two genera were revealed by genome size analysis (Bog *et al.,* 2015). The new genus *Landoltia* was recognized, backed by molecular evidence. According to studies, *Spirodela punctata* was a transitional species that wasn't a part of either *Spirodela* or *Lemna*, which resulted in the discovery of a new genus.

This taxonomic change was made and *Spirodela punctata* was renamed *L. punctata,* a single species under the genus *Landoltia* (Les *et al.,* 1999). Only two species, *Spirodela intermedia* and *S. polyrhiza* constitute the original genus *Spirodela* and both have relatively small genomes. At the phylogenetic foundation of Lemnaceae, it is unclear which of the two has a more ancient karyotype or if both current karyotypes are equally far from the ancestral one (Hoang *et al.,* 2017). By using sequential multicolored cross-FISH, the two *Spirodela* species i.e., *S. polyrhiza* (chromosome number 40) and *S. intermedia* (chromosome number 36) were investigated. Despite having a comparable genome size and sharing the majority of morphological characteristics, investigations conclusively indicated that *S. intermedia* and *S. polyrhiza* are two distinct species (Hoang *et al.,* 2022).

S. intermedia has 6 – 21 roots, barely 2- 5 of them penetrate the prophyllum. In *S. polyrhiza*, 7 - 21 roots can be found, in which one root (sometimes two) puncturing the prophyllum. Normally, *L. punctata* has 2 - 7 roots per frond; although, 1 to many as 12 roots have infrequently been seen (Bog *et al.,* 2015).

Genus Lemna

About 84 *Lemna* clones were examined, representing each one of the 13 recognized *Lemna* species, using the AFLP marker approach. Ten of these thirteen species: *L. turionifera, L. minor, L. trisulca, L. aequinoctialis, L. obscura, L. tenera, L. japonica, L.*

Table 1: Duckweed family Lemnaceae with Genus and Species

Genus	*Lemna*	*Wolffia*	*Wolffiella*	*Spirodela*	*Landoltia*
Species	*gibba*	*arrhiza*	*Caudata*	*polyrhiza*	*punctata*
	minor	globose	oblonga	intermedia	
	minuta	neglecta	hyalina		
	tenera	borealis	angusta		
	disperma	elongata	repanda		
	obscura	columbiana	lingulata		
	trisulca	brasiliensis	gladiata		
	japonica	australiana	neotropica		
	valvidiana	cylindricea	denticulata		
	perpusilla	microscopica	welwitschii		
	aequinoctialis				
	yungensis				
	turionifera				

minuta, L. disperma and L. perpusilla were identified using NJ analysis. However, neither the structure analysis nor even the NJ cluster analysis was able to distinguish between *L. yungensis* and *L. valdiviana* (Bog *et al.,* 2010).

DNA barcoding could be utilized to separate various species of genus *Lemna*. According to barcoding data, the highly associated species L. valdiviana and L. minuta could not be distinguished from one another. Knowing that *L. minor* and *L. japonica* share similar alleles, this notion suggesting *L. japonica* originated from a hybrid between *L. minor* and *L. turionifera* is substantiated (Wang *et al.,* 2010). While *L. minuta* could be easily differentiated from *L. yungensis* and *L. valdiviana* but *L. valdiviana* could not be distinguished from *L. yungensis* using techniques of AFLP and MALDI-TOF-MS. These findings suggest that *L. valdiviana* and *L. yungensis* are similar species (Bog *et al.*, 2020).

L. yungensis is strongly related to *L. valdiviana* , but *L. perpusilla* was mostly closely linked to *L. aequinoctialis*. Barcode analysis of and *L. valdiviana* and *L. minuta* made it abundantly obvious that neither barcode can distinguish between interspecies and intraspecies changes. With varied levels of accuracy, 30 out of 37 known duckweed species were identified using method of sequencing barcodes (Borisjuk *et al.,* 2015). According to current investigations, *L. yungensis* and *L. valvidiana* should be merged into one species but *L. valdiviana* should continue to be used as a legitimate nomenclature for clones belonging to any of these two species (*L. yungensis* used as a synonym). As a result, there are now just 12 recognized species of genus *Lemna,* reduced from 13 originally (Bog *et al.,* 2019).

Genus Wolffia and Wolffiella

The smallest angiosperms pertain to the *Wolffia* genus of the Lemnaceae family. Consequently, morphology was the primary foundation for recognizing and classifying 11 species. The species were recognized as *W. cylindracea, W. angusta, W. brasiliensis, W. australiana, W. elongata, W. neglecta and W. microscopica* (Bog *et al.,* 2013). In cpDNA analysis and combined data studies, *W. brasiliensis* was identified as *Wolffiella*'s sister lineage and was not monophyletic with some other *Wolffia* species. The status of three other *Wolffia* species (*W. australis, W. borealis and W. microscopica*) in relation to *Wolffia* or *Wolffiella* remains unclear (Tippery *et al.,* 2015).

By using AFLP and barcoding techniques, two groupings of *W. globosa* have been identified. Here, the first group comprising clones was described as "conventional *W. globosa*." Another group comprising *W. globosa*, technically known as "*W. neglecta*, exhibits strong similarities to *W. borealis*. All of the techniques failed to identify *W. borealis* as a separate species (Bog *et al.,* 2013). There are 10 species in the genus *Wolffiella*, which has received the least research attention. With the highest level of morphological primitivism in this genus, *W. neotropica* seems to be the most primitive species (Bog *et al.,* 2019).

In total, 67 clones from all 10 species were chosen for this study. In conclusion, the two groupings, *W. gladiata/ W. oblonga* and *W. lingulata/ W. oblonga*, make distinguishing between these 3 species using plastidic markers difficult. Although hybridization cannot fully explain for the ambiguity in individual barcoding, it is hypothesized that the existence of interspecies genomic hybrids within genus *Wolffiella* poses a significant barrier to species identification (Bog *et al.,* 2018). The barcoding results and phylogenetic analysis indicated that perhaps the strongly related species *W. oblonga* and *W. gladiata* could not be distinguished. These related species share matching barcoding marker sequence, hence finding new barcoding markers with higher sequence polymorphism will be helpful (Wang *et al.,* 2010).

The species *W. neotropica, W. caudata, W. rotunda, W. welwitschii* and *W. gladiata* could be differentiated clearly using the fingerprinting technology AFLP. The plastidic markers potentially distinguish two extra species, *W. denticulata* and *W. hyalina*. The species *W. repanda, W. lingulata* and *W. oblonga* could only be specifically defined morphologically and could not be classified through molecular techniques (Bog *et al.,* 2018). With the upcoming developments in molecular tools, we anticipate that the molecular taxonomy of duckweeds will soon contribute to resolving the current difficulties in characterizing and distinguishing duckweed species and perhaps even clones. Nevertheless, the morphological approach would continue to be crucial for practical purposes even if molecular taxonomy is getting progressively more reliable and precise (Bog *et al.,* 2020).

Nutritional Content

Protein and Amino Acid Content

L. minor has non- essential amino acid (53.64), non-proteinogenic (7.13), and (39.20) essential amino acid. All essential amino acids i.e., isoleucine, histidine, phenylalanine, threonine, tryptophan, leucine, lysine, methionine and valine are present sufficient amount. 48.67 of the essential amino acids were made up of valine, isoleucine, and leucine. Glutamic acid which is a non-essential amino acid constituted 25.87. The non-proteinogenic amino acids, namely hydroxyproline, citrulline, taurine, was part of duckweed (Chakrabarti *et al.,* 2018). In Genus *Wolffia,* protein level varied significantly between the plant samples, ranging from 20 to 30. Nearly all ones met the requirements for human nutrition, according to the average of various amino acids. A number of species exhibited ratios greater than 1 in relation to protein content taken as reference. This ratio for cysteine plus methionine, isoleucine, leucine, threonine and valine was greater than 1. The limiting AA in duckweeds were lysine, aromatic AA histidine, and phenylalanine (Appenroth *et al.,* 2018).

The nutritional quality was taken into consideration in the experiment to check the metabolites content in *W. globosa* and *L. minor.* The two kinds of duckweed had protein levels that ranged from about 26.38 to 29.91 (Yahaya *et al.,* 2022). At 25°C, *S. polyrhiza, L. p*unctata and *L. aequinoctialis* all had the greatest levels of protein, with respective protein contents of 36.20, 31.91, and 32.63. When *S. polyrhiza* and *L. aequinoctialis* were grown in polyculture at 20°C, their protein content was 31.29, which was substantially greater than that of the monoculture (29.61, 27.97) ($p < 0.05$). Similar to this, the polyculture of *L. punctata, L. aequinoctialis* & *S. polyrhiza* at 20°C had 30.12 content of protein, which was considerably greater than the monoculture's (26.99, 27.97, and 29.61) ($p < 0.05$) (Li *et al.,* 2016).

Duckweeds' amino acid profile can be effectively compared to that of legume flours like lupine, chickpea or pea which have been promoted for vegan or vegetarian diets (Zahreis *et al.,* 2016). Duckweed will certainly be classified under the heading "fish, shellfish, fish products, mollusks as well as some freshwater and marine food products." Duckweed is a promising source of plant protein for livestock and aquaculture, whether fresh or dried. The inability to obtain fresh duckweed in particular climate zones and the expensive expense of making processed feeds restrict its utilization (Sonta *et al.,* 2019).

Fat Content

S. polyrhiza LC15 and *L. punctata* LC06 and *W. globosa* LC30 have TFA in their biomass ranging from 1.05 (of DW) to 1.62 (of DW), respectively. The composition of fatty acids of the four strains were comparable and straightforward, mostly consisting of three PUFA and four SFA. The predominant components in these profiles were linoleic acid, palmitic acid and linolenic acid, which together accounted for around 80 of the TFA detected. The concentration of four fatty acids— pentadecylic acid, myristic acid, stearic acid and oleic acid—existed in much smaller concentrations (8 of TFA), whereas linolenic acid was more than 40 of the TFA (Tang *et al.,* 2015).

Among the 30 duckweed species surveyed, with three Fatty acids alpha-linoleic acid, palmitic acid and linoleic acid, makeup to 80 of the total duckweed fat. The research study of 30 known species of duckweeds revealed that the total Fatty acid content varied around threefold between a percentage of 4.6 to 14.2 (Yan *et al.,* 2013). In six species under consideration, in terms of total fatty acid content, saturated fatty acids made between 25 to 46. The most prevalent SFA was palmitic acid, while the concentration of monounsaturated fatty acid, primarily oleic acid was quite low, reaching a maximum of 5.65 in case of *L. punctata*. Contrarily, the amount of PUFA was quite high, ranging from 48 to 71 in various duckweed species. As a result, the amount of PUFA was typically close or higher than the amount of total fatty acids. Surprisingly, all of the species under investigation had a higher concentration of n3 fatty acids than n6 fatty acids (Appenroth *et al.,* 2017).

In *L. punctata,* three saturated and three unsaturated fatty acids were mainly present. The predominant components were linoleic acid, palmitic acid and linolenic acid, which accounted for around 80 of the TFA in the various Selenite concentrations. Three fatty acids oleic, stearic and pentadecylic acid were present in much smaller concentrations (5 of TFA), whereas linolenic acid constituted more than 55 of TFA (Zhong *et al.,* 2016).

Starch Content

An important source of carbohydrates is starch. It consists of both amylopectin and amylose (Waterschoot *et al.,* 2015). A unique bioenergy crop called duckweed has a lot of potential for starch accumulation. With only pond water and no additional sediment, duckweed greatest starch content of any duckweed described in the literature, reaching 52.9 (Xiao *et al.,* 2013). Starch is among the main energy-storing components in Lemnaceae. The content of starch in four different varieties of duckweed was examined. The findings demonstrate that the starch content of the four species was similar. The starch content in *L. punctata* LC06, *L. aequinoctialis* LC33, *S. polyrhiza* LC15 and *W. globosa* LC30 was 11.20, 11.61, 11.14 and 11.05 respectively (Tang *et al.,* 2015).

S. polyrhiza had (17.18), maximum starch concentration when the temperature was 20°C. Three polycultures, *S. polyrhiza + L. punctata* (14.38 right at 25 °C), *L. aequinoctialis + S. polyrhiza + L. punctata* (14.01 right at 25°C), and *S. polyrhiza + L.. aequinoctialis* (13.04 right at 30°C), showed greater starch levels than their respective monocultures (Li *et al.,* 2016). When *S. polyrhiza* was cultivated for 25 days, the content of starch accounted for 27.75 percent of the total dry mass under heterotrophic settings, 13.50 percent under mixotrophic conditions, and just 1 percent under photoautotrophic conditions. As a result, when grown under heterotrophic settings, duckweed accumulated 25.69- and 2.06 times higher starch than it was when grown under photoautotrophic or mixotrophic conditions, respectively (Sun *et al.,* 2020). Duckweed had a lower starch content (dry basis) of

Table 2: Nutritional profile of duckweed species

S. No.	*Duckweed genus and species*	*Nutritional content*	*Percentage (%)*	*Reference*
1.	*Wolffia globosa*	Protein	26.38	(Yahaya *et al.,* 2022)
2.	*L. minor*	Protein	29.91	
3.	*S. polyrhiza*	Protein	36.20	(Li *et al.,* 2016)
4.	*L.*	Protein	31.91	
5.	*L. aequinoctialis*	Protein	32.63	
6.	*Wolffia species*	Protein	20-30	(Appenroth *et al.,* 2018)
7.	*L. minor*	Non – Essential AA	53.64	(Chakrabarti *et al.,* 2018)
8.	*L. minor*	Essential AA	39.20	
9.	*S. polyrhiza* *L. gibba* *L.* *L. gibba* *W. hyalina* *Wolffia microscopica*	Protein content	20-35	(Appenroth *et al.,* 2016)
10.	*Wolffia welwitschii*	Fatty acid	4.6	(Yan *et al.,* 2013)
11.	*Wolffia borealis*	Fatty acid	14.2	
12.	*L. punctata*	MUFA	5.65	(Appenroth *et al.,* 2016)
13.	*S. polyrhiza* *L. gibba* *L. punctata* *L. gibba* *W. hyalina* *Wolffia microscopica*	PUFA	48-71	
14.	*S. polyrhiza* *L. gibba* *L. punctata* *L. gibba* *W. hyalina* *Wolffia microscopica*	SFA	25-46	
15.	*L. punctata*	Starch	52.9	(Xiao *et al.,* 2013)
16.	*L. aequinoctialis*	Starch	11.61	(Tang *et al.,* 2015)
17.	*L. punctata*	Starch	11.20	
18.	*S. polyrhiza*	Starch	11.14	
19.	*Wolffia globosa*	Starch	11.05	
20.	*L. aequinoctialis*	Starch	3	(Xu *et al.,* 2022)
21.	*L. punctata*	Starch	75	
22.	*S. polyrhiza*	Starch	17.18	(Li *et al.,* 2016)

23.3 compared to B73 corn kernels with a starch content 66.5. Compared to the B73 maize starch (31.0), the duckweed starch had a higher amylose content (35.7). Granules in duckweed starch had the morphology of discs or domes, diameter ranging from 4 to 9 mm. (Lee *et al.,* 2016).

The highest figure recorded in the field to far for *L. punctata* is 58.2 glucose content and 52.9 starch content, however, Reid and Bieleski, who cultivated *L. punctata* in Phosphorus-deficient medium having glucose supplied, found 75 starch accumulation in the plant (Xiao *et al.,* 2013). *L. punctata* nutrient starvation redirects metabolic flux of fixed CO_2 in starch synthesis, resulting in starch deposition in *L. punctata*, indicating that nutrient deprivation down-regulated its overall metabolic status (Tao *et al.,* 2013). The nutritional profile of duckweed species has been given in tabulated form in Table 2.

Biomass Accumulation

Duckweed biomass has two most significant uses, including feedstock for biofuels and high protein generation for animal feed. Duckweed's biomass production and composition determine how much protein and starch it can produce. The amount of carbohydrates and protein produced in the study was determined by the amount of biomass produced (Li *et al.,* 2016). When wastewater was used as the nitrogen supply, duckweed produced yields substantially greater than those of most other prospective energy crops, ranging from 39.1 to 105.9 tons per hectare per year. After being starved of nutrients for time period of 5 to 10 days, duckweed developed starch levels of 31 to 45.8 (DW) and approximately 94.7 starch can be processed to ethanol by use of the currently available technology for maize starch conversion (Xu *et al.,* 2012).

S. polyrrhiza biomass accumulation efficiency in a small-scale study, in pond utilizing diluted form of pig effluent reached 12.4 g (DW) m^{-2} per day. The content of starch was increased to 64.9 after a 10 day immersion in well water, yielding a relatively high starch output of 9.42×10^3 kg/ ha (Xu *et al.,* 2011). Cytokinins have a considerably greater influence on boosting duckweed biomass accumulation as compared to all other plant hormones. Furthermore, the capacity of 6-benzyl amino purine) to enhance chlorophyll content might contribute to its capability to promote biomass accumulation. Notably, high ABA concentrations can significantly increase starch content, 20.15 in comparison to the control which was 3.52 in seven days (Liu *et al.,* 2019).

Research has shown that duckweed can effectively produce high-quality starch biomass under Nitrogen restriction. The method can significantly reduce the amount of nitrogen fertilizer needed in duckweed cultivation and the manufacturing of starch, which could lessen environmental degradation and lower input costs and energy utilization. The complete duckweed plant can be utilized for fermentation because of its structure, leaving hardly any agricultural wastes, which is a distinct advantage over all other crops (Guo *et al.,* 2020).

Role of Duckweed Species in Phytoremediation

Lemna species

L. minor has a metal removal effectiveness of between 70 and 90 from landfill leachate. *L. minor* was shown to have the highest efficiency (91) for removing copper off leachate. Pb, Ni, Zn and Fe had removal efficiencies of 78, 76, 83 and 77, respectively. All of the metals had removal efficiencies that were more than 70. Copper was the metal that accumulated the largest amount in *L. minor* out of the five metals that were being studied (Daud *et al.,* 2018). Physical examination revealed after 24 hours of dye exposure a definite sign of bio-decolorization via *L. minor*. Analysis of the UV/Visible spectra also demonstrated the decline in Methylene blue absorbance over the course of the test's 24 hours and revealed up to 80.56 + 0.44 of decolorization. With longer exposure times, *L. minor* was able to decolorize more Methylene Blue (Imron *et al.*, 2019).

Duckweed's capability in phytoremediation as for the removal of sulfate and chloride in biological oxygen treatment wastewater was suggested by the fact that it successfully removed 16 sulfate, 30 chloride and 14 total dissolved solids from BOT polluted water (Saha *et al.,* 2015). The duckweed had a 94-eradication rate for Cr with removal efficiency of 36 for lead, 33 for cadmium and 27 for copper in the duckweed pond (Sekomo *et al.,* 2012). After 28 days of being exposed to the 10 mg/l, Ni and Pb polluted water at pH 6 and pH 5 to 6, respectively, *L minor* had 99.30 and 99.99 of its Ni and Pb removed from it (Kaur *et al.,* 2012).

Spirodela species

Depending on the original cadmium concentrations, *S. polyrhiza* was able to remove 42 to 78 and 52 to 75 of the cadmium from the media. At the conclusion of the treatment period, the maximum cadmium accumulated was 7.711 g/kg at the initial concentration of 3 mg/L (Chaudhuri *et al.,* 2014). The concentration of lead in tissues increased from 0.02 to 17.30 ppm upon 10 days while the level of cadmium escalated from 0.03 to 16.9 ppm after the same amount of time. All absorbed harmful metals from growth medias were accumulated in the tissues of *S. polyrhiza*. Depending on the initial amounts of lead and cadmium, *S. polyrhiza* could remove 40 to 53 and 42 to nd of these metals from the media, respectively (Al –Balawna *et al.,* 2020). On average, inorganic nitrogen was removed from media by 83.9 and 73.2 for diluted slurry and sewage, respectively by *S. polyrhiza* (Stadtlander *et al.,* 2019).

Wolffia species

Wolffia, an aquatic plant, with a rapid growth rate, has the capacity to accumulate Cadmium. In fresh aquatic settings that are co-contaminated by lower concentrations of Cd and As, *Wolffia* can be exploited as a powerful Cd accumulator with excellent phytoremediation potential. 10 grams of fresh *W. globosa* in a 200 mL solution could almost completely reduce the Cd (2 µ M) content (Xie *et al.,* 2013). A powerful Arsenic accumulator is *W. globosa*. *W. globosa* gathered 2 to 10 times more Arsenic than other four comparable duckweed and Azolla species examined when cultivated with 1 µm arsenate, *W. globosa* was able to accumulate more than 1000 mg of arsenic per kg of frond (DW) and can withstand up to 400 mg (Zhang *et al.,* 2009).

Landoltia species

In waterways that are co-polluted with Naphthalene and Microcystin LR, *L. punctata* may be employed as an Microcystin-LR phytoremediation agent (Yang *et al.,* 2021). Conditions of starting fluoride content of 5 mg/L with pH varying from 5 to 9 was taken which demonstrated the phytoremediation capacity of *L.*

punctata. *L. punctata's* fluoride uptake was related to its removal from the solution. Maximum fluoride percentage removal for the species was 21.0, while average percentage removal was 12.1 (Braga *et al.,* 2021). For Cadmium phytoremediation, duckweeds have already been regarded as potential solutions. The high Cadmium tolerance in *L. punctata* clone 6001 was discovered through extensive screening of more than 200 duckweed clones (Xu *et al.,* 2018).

Biofuel Production-Duckweed as a Promising Substitute

In order to combat the world's energy crisis, renewable sources of energy are essential. Biofuels are a great example of sustainable energy that can be generated using living things and help minimize our dependence on fossil fuels (Rodionova *et al.,* 2016). Raw material used is plant biomass for the production of biodiesel, bioalcohal, and fermentation-derived biohydrogen. The main biofuels are biodiesel, alcohols, ethanol triglycerides, lipids, carbohydrates, cellulose, fatty acids, alcohols, ethanol or the biomass of living organisms (Poudyal *et al.,* 2016). Duckweed can be converted into biofuel using either fermentation or anaerobic digestion. *L. punctata*, one of the three species of duckweed the others being *Spirodela* and *Lemna*, shows the greatest potential for the production of biofuel. Bio-hydrogen and Ethanol conversion from duckweed is strongly affected by pretreatment (Arefin *et al.,* 2021).

Corn starch is a key component in fermentation of various biofuels such as biobutanol and bioethanol. A promising substitute source for the synthesis of these alcohols is high-starch duckweed. Duckweed has the potential to produce bio-gas which is another type of biofuel, by anaerobic digestion (Cui *et al.,* 2015). By fermentation, higher alcohols can be produced from duckweed. Duckweed *(L. punctata)* yields of 680.36 mg/L isopentanol and 24.06 g/L ethanol produced using acid hydrolysate were 15 times more than those produced using fermentation with mutant yeast. Additionally, using bioengineered strains, that of, *E. coli* to ferment the acid hydrolysate of duckweed, yields of 24.68 mg/L isopentanol, 16.27 mg/L butanol and 195.85 mg/L pentanol were obtained (Su *et al.,* 2014). Before employing duckweed as a feedstock for the creation of biofuel, it is important to enhance the starch biomass inside the duckweed plants.

Currently, nutrient starving is the most practical and cost-effective approach to enrich duckweed starch on a wide scale (Xu *et al.,* 2012). By employing duckweed in the feedstock in synthesis of biohydrogen via dark fermentation and simultaneously utilizing the fermentative waste to make microalgal lipids is a cost-effective and very environmentally friendly technique for biofuel production. The maximum hydrogen generation was reported to be 169.30 mL g^{-1} (D.W) at an initial pH of 7.0 and 35°C temperature (Mu *et al.,* 2020).

Turion: Starch Accumulating Organs

Turion is a dormant duckweed structure that accumulates many starch granules. Turions are formed under nitrate, phosphate or sulphate limitation, which may contribute to increased starch content in *S. polyrhiza* culture (Xu *et al.,* 2012). Turion derived from the species *S. polyrhiza* is a novel feedstock utilized for biofuel production. Various analysis showed that turion has a high amount of starch (65.63) and may be easily digested due to its low lignocellulose percentage (12.82).

In comparison to traditional starch production based on duckweed, turion production attributed to strain 0196 produced equivalent starch productiveness of 2.90 g/m2/d. Furthermore, the sustainable production method and streamlined harvest procedure facilitates turion production at a low cost (Xu *et al.,* 2018). Lower phosphate availability appears to be the most important element in promoting turion production.

- Growing fronds absorb phosphate, and turion formation occurs.
- Lower daytime temperatures (18 *vs* 25°C) and, specifically, lower nighttime temperatures (18 *vs* 15°C) clearly boost the effect of turion-inducing component phosphate by boosting yield.
- When manmade phosphate concentrations are substantially greater, minimum temperature cause turion production (Appenroth *et al.,* 2010).

Duckweed: A Model Plant

The fundamental advantages of model system are

- These allow for the data collection relevant to a wide range of research issues (based on intrinsic species qualities like as small height and rapid generation time)
- Have value-added properties resulting from their history of usage in research, such as completely developed molecular tools and experimental protocols, deep & broad literacy, and a global community of researchers (Laird *et al.,* 2018).

Arabidopsis thaliana is the most intensively researched flowering plant species known to science. Other plants, such as soybean, maize, petunia, pea, tomato and snapdragon, which were previously thought to be potential prospects to direct plant research in future, now fall far behind (Koornneef *et al.,* 2011). Research on Arabidopsis is quick, inexpensive, and convenient. *Arabidopsis* renders itself well to genetic study with the genome size of 132 Mbp (Woodward *et al.,* 2018).

The genomics initiative in case of duckweed began in 2009 starting from sequencing of duckweed (Fig. 3) Genus, *S. polyrhiza* clone 7498, as one of the collaborative project by Joint Genome Institute. *Spirodela* was selected because of its anticipated basal position in family Lemnaceae and because it has the smallest predicted genome size (158 Mbp) which is comparable to *Arabidiopsis* (Lam *et al.,* 2014). There is significant association

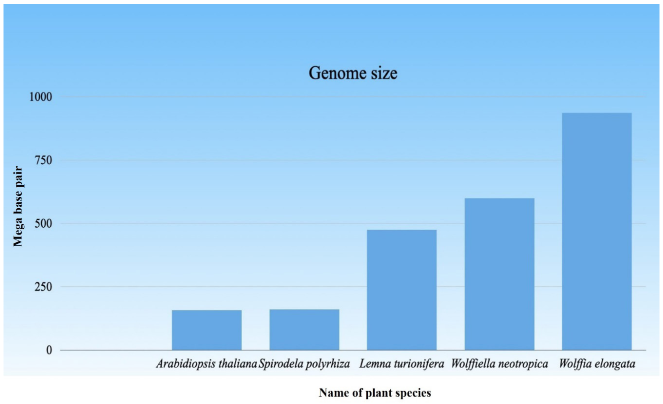

Fig. 3: Genome size of duckweed species

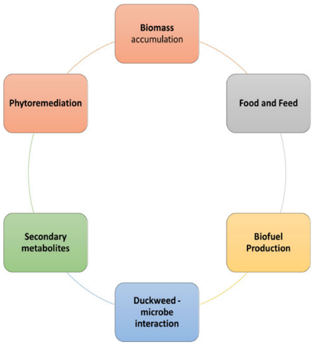

Fig. 4: Various uses of duckweed species

identified between morphological advancement and genome size progression in the Lemnaceae lineage. The primitive genus *Spirodela* now has smallest genome size, whereas *Wolffia,* the most advanced genus has the largest. The estimated genome size ranged from 150Mbp for *S. polyrhiza* up to 1,881 Mbp as in case of *W. arrhiza* (Wang *et al.,* 2011).

In recent study the least Genome size in every genus of duckweed was found to be (475 Mbp) *in L. turionifera* 8693, (936 Mbp) in *W. elongata* 9188, (599 Mbp) in *W. neotropica* 8848, (160Mbp) in case of *Spirodela* species were obtained by flow cytometry (Hoang *et al.,* 2022). The genome sizes ranged from 160 Mbp in *S. polyrhiza* to 2203 Mbp in *W. arrhiza*, representing a 14-fold variation in duckweed species. The genus *Wolffia* had the greatest variance in genome size (between 432 - 2203 Mbp**)** (Hoang *et al.,* 2019). Traits like which are small structure, anatomy, fast reproduction, short life plan, cosmopolitan in distribution, local abundance, genetic and industrial applications, transcriptome and genome availability make duckweed a model in evolution and ecology (Laird *et al.,* 2018).

Recent Advancement in Duckweed Studies

The various roles of Duckweed species were illustrated in Fig. 4.

Duckweed-Microbe Interaction

Investigation of the multifaceted interaction between diverse duckweed genus and various bacterial classes which share the same habitat as aquatic plants has rapidly progressed. The interaction of duckweed and microbe not only facilitate bioremediation in polluted waters but now it has become obvious that the association frequently promotes duckweed development (Appenroth *et al.,* 2015). Plant growth-promoting bacteria (PGPB) can promote the growth of their hosts. The growth-promoting mechanisms and phylogeny of PGPB linked to aquatic plants are little understood, whereas those of terrestrial PGPB are thoroughly investigated. MRB1-4 (NITE P-01645-P-01648), four novel aquatic strains of PGPB for duckweed *L. minor* were obtained from *Lythrum anceps*, featuring previously ignored *Pelomonas* strains.

These strains boosted the duckweed's growth, chlorophyll and biomass content (Makino *et al.,* 2022). Co-cultivation of bacterial populations and aseptic *L. minor* of various aquatic habitats results in variations in duckweed growth rates ranging from -24 to positive growth of +14 when compared to the aseptic control. The study concluded that:

- The bacterial community has a substantial influence here on the production level of duckweed total biomass;
- duckweed includes bacteria with an inhibitory, promotive and neutral effect on their growth;
- the constructive effects of PGPB strains may or may not be sustained in the proximity of all other bacteria,
- Numerous isolates from duckweed-associated bacterial communities share several common traits in their biology (Ishizawa *et al.,* 2017).

The potential of bacterial strains related to the phylum *Acidobacteria*, members of which are known to inhabit terrestrial and soils plants also promoted duckweed growth, although their microbe-plant interactions and ecological roles remain largely unknown. F-183 and TBR-22, newly isolated *Acidobacteria* strains, had shown a plant growth-promoting effect upon many Lemnoideae species *(S. polyrhiza,L. aequinoctialis, W. arrhiza, L. minor, L. punctata,* and *W. globosa)* (Yoneda *et al.,* 2021). RWX31 strain is the first known rhizosphere aerobic denitrifying bacterial strain obtained from the duckweed *L. minor*, has a high potential for practical utilization of the SND (Zhou *et al.,* 2013).

It was reported that DAB (Duckweed associated bacteria) strains that generated a shorter root phenotype in plant *Arabidopsis* were numerous on the root surface. This shows duckweed microbiome might create distinct indole-containing chemicals at varying concentrations, with various strains colonizing in different ways and perhaps occupying different niches (Gilbert *et al.,* 2022). Recent findings show that, in the absence and presence of rhizosphere microorganisms, duckweed can release fatty acid amide and fatty acid methyl ester, which have a considerable stimulating effect on denitrification. Furthermore, findings show that N-removal accelerators secreted by duckweed may act as biochemical signals instead of carbon skeletons. Given the necessity of discovering cost-effective & ecologically friendly methods of removing excess nitrogen from bodies of water (Lu *et al.,* 2014).

Secondary Metabolites in Duckweed

These are the substances which frequently play a role in plants' defense against biotic or abiotic stressors. Terpenes are represented by monoterpenes, diterpene, sesquiterpenes, poly-terpenes, phenolics represented by Coumarin, flavonoids, nitrogen-containing represented by cyanogenic glucosides, alkaloids and Sulphur containing represented by Phytoalexins, allinin, thionine, defensins) chemicals are the three chemically separate classes of secondary metabolites found in plants (Pagare *et al.,* 2015). Terpenoids and flavonoids are mostly present in medicinal plants as common secondary metabolites. Flavonoids extracted from duckweed showed immunosuppressive activity against specific antigens (Sharma *et al.,* 2017).

After processing, *L. minor* commonly had more flavonoids, alkaloids, and saponins. The amount of saponin was lowest in fresh samples and was highest in sun-dried-blanched samples (Ifie *et al.,* 2014). Due to their higher overall flavonoid content and greater percentage of flavonoids, *W. globosa* strains and *S. polyrhiza* strains are advantageous. The flavonoid content was highest in *W. globosa* at 5.85 preceded by *S. polyrhiza* at 4.22 (Zhao *et al.,* 2015). Three of the identified chemicals were derivatives of chlorogenic acid that were exclusively found in *S. polyrhiza*, while eleven others were derivatives of luteolin and apigenin that were present in different species. The flavonoids were generally identified as C-glycosides.

L. punctata had about 81 apigenin content which is cancer adjuvant was highest among all duckweed species (Pagliuso *et al.,* 2020). Significantly more anthocyanins were present in the *L. gibba* that were exposed to arsenic concentration. With an increase in arsenic concentration, anthocyanin content was continuously increased (Leao *et al.,* 2014). In the *L. paucicostata* cultures, higher concentrations of caffeic acid, campesterol, isoferulic acid, coumaric acid, sinapic acid and beta-sitosterol were found which have immunostimulatory and antioxidant properties (Kim *et al.,* 2017). Phytosterols were present in the maximum portion of the duckweed wax fraction, represented by stigmasterol, campesterol and sitosterol (Borisjuk *et al.*, 2018).

In the future, a combination of genome size, genome sequencing, chromosome number, FISH and GISH data will help to clarify the phylogenetic relationships and taxonomic position of intrageneric accessions of duckweed, which are challenging to designate as separate species based exclusively only on morphological characteristics (Hoang *et al.,* 2022). Tools, technology, and resources that enable researchers to thoroughly explore their topics are essential for successful model systems. A genotyping platform, genetic techniques and a transformation system are important technologies for future utility of duckweed. Complete exploration of resources like genomics databases (Clone collection in case of duckweed) will make it possible to swiftly adopt a new model and examine its possibilities (Acosta *et al.,* 2021).

CONCLUSION

The use of duckweed in research has a wide range of applications. These plants are well known for their fast growth rates, the ability to store biomass, efficient and cheap food sources, industrial applications and energy production. Many advances have been made in the field of molecular taxonomy and species identification. Duckweed species contain a significant amount of lipids, amino acids, and proteins. They are capable of accumulating chemicals and heavy metals, which aids in the bioremediation of polluted water bodies. There are a number of promising alternative fuels that can be made from duckweed, including ethanol, biogas, and butanol, which are promising alternatives to non-renewable energy sources. Biomass yields are high, nutrient uptake rates are high, and the utilization of biomass as ethanol, biogas and butanol has a wide range of applications. The exploration of new applications for duckweed species requires further research.

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AUTHOR CONTRIBUTION

Nikita[1] and Munish Sharma[1] collected the data, involved in original draft preparation, and designed the study. The final editing was done by Munish Sharma[2*] and also supervised the work. All authors read and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The author declare that they have no competing interests.

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