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*Review*

# A Status Review on the Importance of Mulberry (*Morus* spp.) and Prospects towards Its Cultivation in a Controlled Environment

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**Abstract:** One of the major challenges that global society is facing nowadays is finding sustainable and safe methods for crop growth and development. Besides the traditional crops cultivated worldwide (tomatoes, potatoes, lettuce, strawberries, etc.), there is a general trend in the exploitation of polyvalent plants. Mulberry (*Morus* spp.) faced no exception; with its undeniable proprieties, it became suitable not only to be used in the sericulture industry, but in the food chain, the pharma industry, and environmental safety. Spare parts of the plants can be used in a very wide range, starting from introducing mulberry leaves in supplements to increase the protein content of a meal to extracting biologically active compounds from fruits and roots to be used in phytotherapy. However, the outstanding proprieties of this plant come with some requirements related to space availability and watering; requirements that can be easily surpassed by using vertical farming methods, such as hydroponic, aeroponic, or aquaponic systems. The present paper aims to evaluate vertical farming techniques' applicability to mulberry propagation in a controlled environment and their prospects for a more sustainable and safer agricultural practice.

**Keywords:** mulberry; agriculture; vertical farming; hydroponics; aeroponics; aquaponics



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## 1. Introduction

Mulberry is a deciduous wooden tree, belonging to the *Morus* genus and *Moraceae* family. It is a fast-growing plant, widely spread across the globe, from temperate to tropical areas, and used for sericulture and Ayurveda for centuries [1,2]. There are more than 15 species of mulberry with over 500 varieties, out of which *M. alba*, *M. indica*, *M. nigra*, *M. bombycis*, *M. australis*, *M. cathayana*, *M. rubra*, and *M. atropurpurea* are the ones with significant importance either in foliage or in fruits production [3]. The mulberry is typically a unisex dioecious plant, meaning that some of its individuals only produce male blooms, while others only produce female blossoms. Additionally, it is possible to find unisexual monoecious plants, which have both male and female flowers on the same plant. Mulberry is a seed-propagated, highly cross-pollinated species, and it can grow in countless varieties or shapes, with distinct fruit and leaf traits [4]. Mulberry leaves are used primarily in sericulture, being the sole feed for *Bombyx mori* silkworms and are intensively cultivated in countries with great silk production (China, India, Uzbekistan, Thailand, etc.). In contrast, European countries prefer to use mulberry trees for their fruit production and transform them into different food products (jams, vinegar, wine, pastries, etc.).

Besides the nutritional proprieties of mulberry, the physicochemical composition of both leaves and fruits rich in biologically active compounds gives the plant an added value. As such, a considerable quantity of polyphenols, among which the most common are rutin, quercetin, kaempferol, gallic acid, and chlorogenic acid, present in the fruits plays an important role in antioxidative processes and have cardio-protective, anti-inflammatory, antidiabetic effects [5]. In addition, mulberry fruits synthesize unique polyhydroxy alkaloid, 1-Deoxynojirimycin (DNJ), with remarkable applications in medicine and pharmacy. In vivo studies show that patients with cardiovascular diseases reported an amelioration of symptoms after the mulberry leaf powder had been administrated [6–8]. Positive results have been also reported when type 2 diabetes model rats were treated with mulberry leaves extracts [9–12], thus increasing the interest in cultivating it as a nutraceutical plant worldwide (Figure 1).

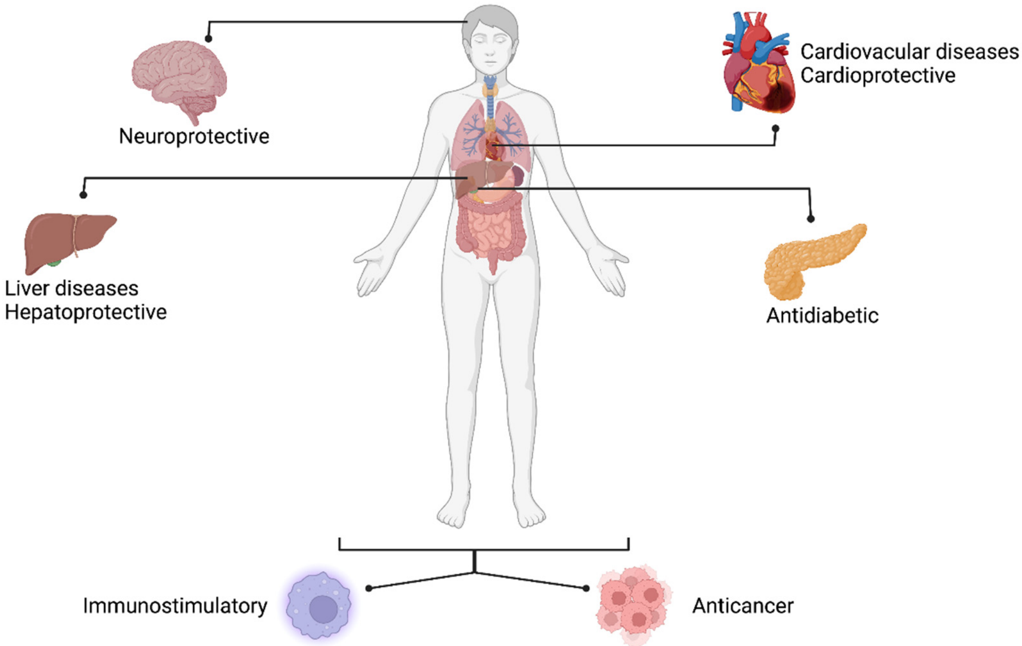


**Figure 1.** Illustration of the applicability of mulberry in biomedicine (created with BioRender.com, accessed on 10 February 2023).

Mulberries can be cultivated in a variety of soil types and topographical conditions that are unsuitable for most agricultural crops. However, it is generally known that mulberries develop normally when being exposed to an atmosphere temperature range from 13 °C to 37.7 °C, but the maximum sprout of buds happens when the environmental temperature is 24 °C to 28 °C, with a relative humidity of 65–80% and sunlight duration of 5 to 12 h per day. Mulberry can easily be developed under rainfed conditions when the downfall range is between 600 mm and 2500 mm. If the water conditions are poor due to insufficient rain, an additional irrigation system is necessary to be implemented [13]. Mulberry can grow in various types of soils; it usually thrives in sandy loam soils that range from being slightly acidic to neutral (pH 6.2–6.8) and have significant levels of organic carbon [14]. The general maintenance work required for a mulberry plantation is, in addition to irrigation, fertilizer application, and very effective management of diseases, pests and competitive plants. The application of pesticides and herbicides must be avoided and carried out only in extreme cases, when other treatments are not applicable, due to the compromise of leaves and fruits, that cannot be further used in feeding silkworms, obtaining pharmaceutical extracts, or used in the food industry. This makes mass production susceptible to losses. Moreover, the application of a relatively large amount of water and manure are less environmentally friendly solutions.

Although mulberry is a versatile plant, which can easily adapt to different altitudes, soils, or climates, it represents a challenge for third-millennium agriculture to exploit its potential in unfavorable environments and obtain all-year-round production. Biotechnology played a major role in overcoming biotic and abiotic stress that occurs in mulberry, using genetic engineering techniques. Some recent examples of lowering the biotic stress in Mulberry include transgenic plants that developed resistance to the fungal pathogen

*Botrytis cinerea,* which causes gray mold [15], or to *Lasiodiplodia theobromae*, which causes black root rot [16]. The use of some antimicrobial peptides for disease control has also been reported [17]. In terms of abiotic factors, caused mainly by cold temperature and soil salinity, stress resistance mediated by MuNPR1, MuNPR4 [18], or miR171 [19] genes have been proven to be efficient in some tested varieties. Furthermore, obtaining mulberry seedlings, regardless of the environmental conditions, has been successfully achieved through in vitro propagation. Murashige and Skoog basal medium, supplemented plant growth regulators, showed performances in cell division, elongation, shoot formation, or callus initiation (auxins), regulate differentiation, stimulate protein and enzymes' activity (cytokinins), promote flowering, stem elongation, and awaking the seeds or buds when applied to mulberry explants [20–23]. However, although the micropropagation technique offers the possibility of having seedlings that can develop in the winter months, this solves only part of the problem. Obtaining the mass production of leaves and/or fruits still represents a real challenge for countries with a temperate climate, areas with acidic soils or with a high level of salinity, but also with poor light and rain conditions. Thus, the prospects of using vertical farming technology have been brought into consideration. In comparison to conventional agriculture, vertical farming may produce yields while using less land and water and may not need any pesticides. In order to build resilient crop-producing systems, especially in and around highly populated places, vertical farming technology (VFT) can help producers to meet their expected yield all year round. Fruits, vegetables, and herbs are among the products already produced by VFT, using multi-layer indoor crop cultivation systems such as hydroponic, aquaponic, and aeroponic systems [24].

Based on the latest literature in the field, this paper aims to review the physicochemical composition of mulberry, correlated with their applicability in the medicine and food industry and overall economic importance, as well as the prospects of growing mulberry in more sustainable alternative methods, such as vertical farming technology.

## 2. Significance of Mulberry (*Morus* spp.)

### *2.1. Medicinal Value of Mulberry*

Since the beginning of time, humans have had a major need to treat various diseases, and their first instinct was to use plants for this purpose. Nowadays, the pharmaceutical industry and medicine are making tremendous progress. Despite that, therapeutic plants are still being highly used to prevent or treat a wide range of medical conditions. Therefore, numerous scientific laboratories are focused on herbal medicine, and new plant-based drugs are being developed [25]. It is well known that botanical medicine was first used in traditional Chinese and Indian medicine [26], although, as reported by the WHO, nowadays, no less than 80% of the globe's population uses therapeutic plants for health-related situations [27].

Nowadays, cancer is one of the leading causes of death worldwide. Aiming to decrease morbidity and mortally cancer related rates, the scientific community is constantly developing new treatment approaches [28]. Considering the great potential of plants as a rich reservoir of various bioactive elements, tremendous efforts are being made to develop anticancer plant-based therapeutics. Moreover, a wide range of anticancer agents that are frequently used in clinical practice originated from plants, such as irinotecan or paclitaxel [29,30]. In this direction, due to its complex composition, numerous studies explored the anticancer effects of mulberry [31–36].

In 2021, it was explored, in a novel study, the cytotoxic potential of M. nigra fruit extract by using two human cancer lines, specifically, MDA-MB-231 (breast cancer) and PC3 (prostate cancer) [37]. The authors applied the mulberry extract on the cell lines for three days by using five different concentrations, 1%, 1.33%, 2%, 4%, and 10%, respectively. For measuring the cell viability, the MTT assay was used, and the results revealed that mulberry exhibits cytotoxic effect in a concentration-dependent manner. When it comes to the prostate cancer cell line, the cell viability was affected only by applying a mulberry extract of 10% concentration. On the other hand, when applying mulberry extracts of lower concentrations, no cytotoxic effect was registered. As for the breast cancer cell line, similar results were registered. The highest concentration led to the most powerful cytotoxic effect; however, lower anticancer activity was reported at 1%, 1.33%, 2%, and 4%, respectively. Moreover, when comparing the anticancer effect of the same concentrations of the doxorubicin and mulberry extract, the cytotoxic effect of the fruit extract was higher than the outcome of using the chemotherapy drug.

In another study [38], a research team compared the cytotoxic impact of the mulberry flavonoid extract (*M. alba*) with the effect of doxorubicin on a human colon cancer cell line, specifically HT-29. Furthermore, the authors investigated the cytotoxic action of the two products combined. Aiming at that, they evaluated the adenomatous polyposis coli (APC) expression level induced by the two products, individually and combined. This APC gene expression was assessed due to the fact that it plays a key role in tumor suppression. Furthermore, the poly (ADP-ribose) polymerase (PARP) concentration in the cancer cell line was evaluated after applying the treatments. PARP represents a key protein that plays various roles in certain biological processes, and several studies showed that it is found in higher concentrations in tumor cells. Accordingly, there is a great interest in developing new therapeutics based on PARP inhibitors. Firstly, cancer cell viability was determined by MTT assay after the cell line was exposed to different concentrations of target products for 24 h. The results showed cell viability was affected by administering mulberry flavonoids extract, depending on the applied concentration. Moreover, the effect of the natural extract was similar to the cytotoxic action of doxorubicin. Subsequently, in order to evaluate if cell viability was run-down due to the increased APC gene expression, the authors used the quantitative real-time PCR technique. Their data revealed that all three tested products led to a significant increase in the APC gene expression level in colon cancer cell lines. Pursuing to analyze if the PARP level is correlated with the loss of cell viability, the ELISA assay was performed. Interestingly, in the presence of mulberry leaf extract, the level of PARP not only was reduced, but it was even lower than in the other two groups.

Another major threat, and one of the most widespread chronic diseases, is diabetes. When it comes to this specific disorder, it is crucial to manage blood sugar levels in order to prevent regrettable outcomes [39]. Considering the undesirable side effects of the drugs that are available today, it is imperative to develop new plant-based therapeutics in order to investigate the hypoglycemic potential [40]. It is well documented that mulberry is used especially in Asian countries to treat diabetes.

Yang et al. [41] carried out in vivo research that aimed to evaluate the hypoglycemic action of mulberry leaf extract. For this purpose, the pivotal alkaloid was extracted from mulberry leaves, namely DNJ. For 15 days, the extract was administered to diabetic mice, and the authors observed that DNJ significantly inhibits glucose absorption by suppressing the activity of the enzyme that is responsible for decomposing sugar. Considerably, in the animal model, the blood sugar level was reduced by up to 50%.

In another research, Wistar rats were used to evaluate the potential hypoglycemic effect of mulberry leaf extract (*M. alba*). For 35 days, the induced-diabetic animals were treated with the target extract and several parameters were measured, for instance, blood glucose, triglyceride level, LDL, HDL, blood urea, the amount of β cells, and so on. Compared with the control group, in the diabetic-induced animals, all studied parameters, except HDL, were highly elevated. However, when applying 600 mg/kg/day, all investigated parameters were restored to the initial values that were determined in the control group. On the other hand, when a smaller amount of mulberry leaf extract was administered, specifically 400 mg/kg/day, hyperglycemia was highly reduced, but the studied levels of certain parameters were not restored [42].

Eo et al. (2014) evaluated the anticancer effect of *M. alba* root bark. The authors used the mouse macrophage cell line (RAW264.7) to analyze the anti-inflammatory activity, keeping in mind the role of macrophages in producing inflammatory mediators, including NO or TNF-α. NO represents a pivotal factor in controlling the inflammatory response, and in this study, it was shown that mulberry root bark suppresses the over-production of NO.

A colorectal cancer cell line (SW480) was used to assess cell viability and apoptosis; two techniques were employed, specifically the MTT assay and Western blot, to evaluate both parameters. Moreover, they investigated if the mulberry root bark treatment included the expression initiation of the activating transcription factor 3 (ATF3), and the regulation of the cyclin D1 level, which are related to the cell growth suppression process and apoptosis. Their study was the first report that confirmed the anti-inflammatory and anticancer effects of mulberry root bark [43].

Yin et al. (2017) [44] used diabetic-induced mice to evaluate the mulberry branch bark potential for regulating insulin secretion. Their data revealed that it is effective in regulating insulin release, but also in controlling glucose levels. In a novel study, Qiu and Zhang (2019) [45] explored the impact of mulberry (*M. multicaulis*) branch bark on diabetic model mice. The mice were injected with streptozotocin and were fed with a high-fat diet for five weeks. After this time, diabetes symptoms were perceived, including food and fluid intake or high glucose levels, also great insulin resistance was observed. Their results confirmed that mulberry branch bark exhibits a re-balancing impact in diabetic mice by ameliorating the disturbed metabolic processes involved in this endocrine disease.

In addition to mulberry's antioxidative, anticancer, and hypoglycemic therapeutic effects, a wide range of studies investigated and confirmed other great curative actions of this outstanding plant, highlighting its hypolipidemic, hepatoprotective, antibacterial, and anti-obesity activities. These therapeutic effects are correlated with the main bioactive compounds from leaves, fruits, and root bark, as shown in Table 1.

**Table 1.** Main biological compounds from different parts of mulberry and their health benefits.

| Plant Part | Health Benefits | Biological Compounds | References |
|---|---|---|---|
| Leaves | Anti-diabetic activity | 1-Deoxynojirimycin, Moranolin, 2-aryl-benzofuran | [46,47] |
| | Anticancer activity | Morin, Kuwanon S, 8-granilapigenin, ciclomulberrin, morusin, ciclomorusin, atalantoflavones, Kaempherol-3-O-glucoside (Astragalin) and derivates, Quercetin and derivates, Epicatechin, moracins, phenolic acids (gallic, protocatechuic, vanilinc, p-coumaric, ferulic), apigenin and derivates | [46–52] |
| | Antioxidant activity | Quercitrin, rutin, Eriodictyol, gallic acid, chlorogenic acid, sinapic acid | [47–50,53,54] |
| | Hypolipidemic effect | 1-Deoxynojirimycin, Caffeic acid, Cyanidin-3-O-rutinoside | [46,47,49] |
| | Neuroprotective activity | $\gamma$-aminobutyric acid, gallic acid, chlorogenic acid | [46,48,49] |
| | Anti-inflammatory effects | Chlorogenic acid, Caffeic acid | [47,49,50] |
| | Anti-viral activity | Cyanidin-3-O-rutinoside, 1-Deoxynojirimycin | [46,47] |
| | Cardio-protective | Mulberrofurans, Luteolins | [48,55] |
| Fruits | Anti-diabetic activity | Polysaccharides (FMAP, MFP, MFP-1, MFP-2, MP, PMF1, PMF2, PMF3, MFP3P, JS-MP-1) | [47] |
| | Anticancer activity | Vanillic acid, caffeic acid, ferulic acid protocatechuic acid, gallic acid | [49,51,56] |

**Table 1.** *Cont.*

| Plant Part | Health Benefits | Biological Compounds | References |
|---|---|---|---|
| | Antioxidant activity | Isoquercitrin, rutin, phenolic acids (gallic, chlorogenic, phloridzin, citric, malic, tartaric, lactic, succinic, fumaric, acetic), anthocians (Cyanidin-3-O-β-D-glucopiranoside, Cyanidin 3-β-D-glucopyranoside, Cyanidin-7-O- β-D-glucopyranoside, Cyanidin-3-O-(6′′-O-α-rhamnopyranosyl-β-D-glucopyranoside), Cyanidin-3-O-(6′′-O-α-rhamnopyranosyl-β-D-galactopyranoside), catechins | [47–51,56–58] |
| | Neuroprotective | Quercetin and derivates, gallic acid, chlorogenic acid, Cyanidin-3-O-β-D-glucopiranoside | [46,49,56,57] |
| | Anti-inflammatory effects | Chlorogenic acid, p-coumaric acid, Caffeic acid, Protocatechuic acid, o-coumaric acid | [47,49,56,57] |
| Root bark | Anti-diabetic activity | 1-Deoxynojirimycin | [56] |
| | Anticancer activity | Morusin, oxyhidromorusin, moracins, Glycoside,5,2′Dihidroxyflavone-7, 4′-di-O-D-glucoside | [46,47,56] |
| | Antioxidant activity | Mulberroside A, Mulberoside C | [47,59] |
| | Neuroprotective and hepatoprotective activities | Sanggenones (B, D, E, G, M, O, T) | [56] |
| | Anti-inflammatory effects | Mulberrofurans, Morusin, oxyhidromorusin, sanggenones | [47,56] |
| | Anti-viral activity | Leachianone G, 1-Deoxynojirimycin, Eudraflavone B hydroperoxide, α-acetyl-amyrin | [46,47,60] |
| | Anti-bacterial effect | Kuwanones (G, H, M, L, O) | [47] |
| | Anti-hypertensive effect | Moracenin A, Moracenin C, Moracenin D | [56] |

### *2.2. Economic Impact of Mulberry*

Besides the fact that mulberry exhibits great interest in the scientific community due to its distinctive medicinal values, it plays key roles in numerous industries; therefore, it is a significant player in the global economy (Figure 2).

Mulberry has an indispensable role in sericulture, being the exclusive source of food for silkworms. There is an increasing demand when it comes to the silk produced by *B. mori*; therefore, the role played by mulberry is even higher for the economic sector [61]. Furthermore, among the most important insects, such as *Apis mellifera* or *Drosophila melanogaster* [62], *B. mori* plays a pivotal role in the biomedicine and pharmaceutical industry. It has been reported that the mulberry leaf content influences silk production. In a particular study, a research team investigated if the moisture content determines certain *B. mori* parameters, such as larval weight, and filament length, just to mention a few. In terms of mulberry leaves' moisture level, they demonstrated that by feeding the silkworms with tender leaves that involve higher moisture, all the studied parameters were positively impacted. On the other hand, when the silkworms received coarse leaves that have a lower moisture content, there was observed a negative correlation [63]. In another study, Susanti et al. (2021) [64] evaluated the impact of mulberry leaf quality on silkworms' nutrition index (growth rate, consumption rate, efficiency of conversion of digested and ingested food, and approximated digestibility). There were defined two groups of newly hatched larvae, one group was fed with unfertilized mulberry leaves and the other group received leaves of fertilized mulberry. Their results showed that by feeding the silkworms with leaves of mulberry plants that were treated with fertilizer, their nutrition index was positively impacted.

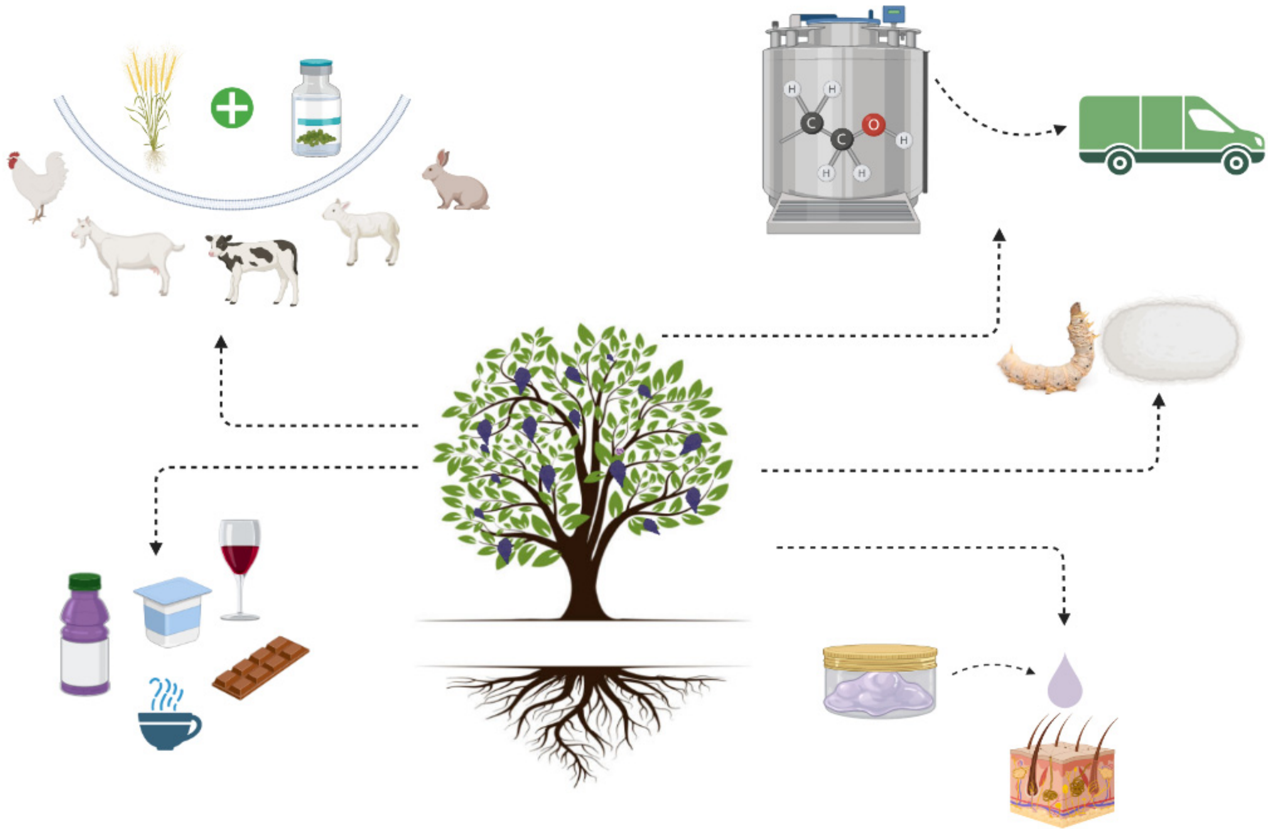

**Figure 2.** Main applications of mulberry in various industries (created with BioRender.com, accessed on 10 February 2023).

Even if the main reason for cultivating mulberry is the silkworms' rearing, it has been demonstrated that it is a great alternative to replace fodder crops in order to fulfill the high demand of feeding domestic animals. In China, India, but also in Korea, the leftover branches and leaves are used to feed domestic animals. Furthermore, mulberry leaves are currently utilized as supplements to fulfill a balanced diet. Several studies showed that by adding mulberry leaf as a supplement in dairy animals' nourishment, milk production was increased. Moreover, due to the addition of mulberry leaves, the lipid and fat contents were increased in cow and goat milk. Other researchers successfully used mulberry leaf in order to substitute the commercial concentrate used for pigs and rabbits. In other studies, mulberry leaf was used to supplement hens' nourishment, and the formulation not only led to bigger eggs, but the yolk color was also improved [65]. Keeping in mind the great potential of the mulberry plant as a promising food supplement, Cai et al. (2019) [66] investigated the toxicity potential of mulberry leaves by using Sprague–Dawley rats. The experimental animals were divided into three groups, as follows: a control group that had not received mulberry leaves, the second group that received 1 g/kg body weight, and the third group that received the highest mulberry quantity, 2 g/kg body weight, respectively. For 28 days, the treatment was administrated once a day, via intragastric gavage. In this study, they analyzed several traits, including rats' behavior, body weight, feed intake, and numerous blood parameters. By comparing with the control group, neither one of the experimental groups that received mulberry leaves as a food supplement registered any adverse effects. In terms of the livestock feed solution, from an economic point of view, mulberry represents one of the most feasible options for increasing the forage production for livestock due to its extended geological distribution. It is displaying a wide range of ecotypes. On the other hand, by being in the scientific community's attention for a long period of time, there have been described numerous techniques for mulberry reproduction, cultivation, or pest and disease management. However, there are several studies that certified that mulberry represents a cheaper alternative for supplementing livestock feed [59,60]. For instance, in a study conducted by Islam et al. (2014), a broiler chicken diet was developed, more specifically, a low-cost recipe of a grower diet, which led to the production of low-cholesterol meat [61].

It is well documented that mulberry composition includes various bioactive elements that make mulberry a great functional food. Nowadays, there is a wide range of applications for mulberry in food industries; moreover, it has been processed in different types of products, such as jams, wines, syrups, just to mention a few. For instance, by using *M. alba*, a group of researchers obtained wine that contained a high level of phenolic compounds. However, in another research, vinegar was obtained from *M. alba*, and the authors confirmed its antioxidative and antibacterial activities. On another note, by using *M. nigra*, a research group obtained enriched pasta that displayed hypoglycemic action on human organisms [2]. In a recent study, On-Nom et al. (2020) [67] formulated a mulberry fruit jelly and evaluated its therapeutic effects on 60 dyslipidemia subjects. For seven days, the subjects received one serving size of anthocyanin-rich mulberry fruit jelly (191 mg). After receiving the treatment, various parameters were analyzed, including blood glucose, lipid, insulin, but also oxidative stress. The results revealed that acute consumption of anthocyanins-rich mulberry fruit jelly could lead to a lower risk of cardiometabolic disorders, by significantly decreasing the levels of blood total cholesterol, low-density lipoprotein, and inflammatory markers. Furthermore, by consuming this product, the subjects' insulin sensitivity was improved [67].

When talking about mulberry's impact on the global economy, it plays a great role in the cosmetic industry. Mulberry is a component of several creams or bath gels, but, also, it is a constituent of other products that are currently on the market. It is well documented that mulberry has a great contribution in diminishing skin conditions associated with aging [2]. Moreover, it was revealed that mulberry has a great role as a skin whitening agent [2]. It is an inhibitor of tyrosinase which is involved in melanin biosynthesis. There have been described several mulberry-based cosmetic products that aim to counterattack hyperpigmented disorders by inhibiting tyrosinase activity [65].

Another significant role played by mulberry is in the environmental safety area, as shown in Figure 3.

Plants have, in general, a high positive impact on reducing global warming by uptaking an important amount of carbon dioxide from the atmosphere and releasing oxygen in return. This process leads to purified air to be used by living organisms. In addition, plants have a so-called "evapotranspiration mechanism of water cycle", which helps to prevent overheating urban areas and contributes to an improvement of soil health [68]. In this regard, and according to Jiang et al. (2017), mulberry is an excellent green species that can be used to purify the air, as one ha of mulberry trees can absorb around 1000 kg of $CO_2$ a day and release around 730 kg oxygen, the equivalent of 1000 peoples' breathing demand [69].

Another notable role played by the mulberry tree in the environmental safety is represented by its ability to absorb heavy metals and some toxic organic compounds. Having a well-developed root system (up to 4 m deep into the ground) and increased resistance to environmental conditions, mulberry can grow and develop without any visible changes in soil contaminated with 734 mg/kg of Pb, 1194 mg/kg of Zn, or 53 mg/kg of As. Regarding organic compounds, the phytoremediation power of Morus in soils contaminated with polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and trichloroethylene (TCE) [68] has been studied.

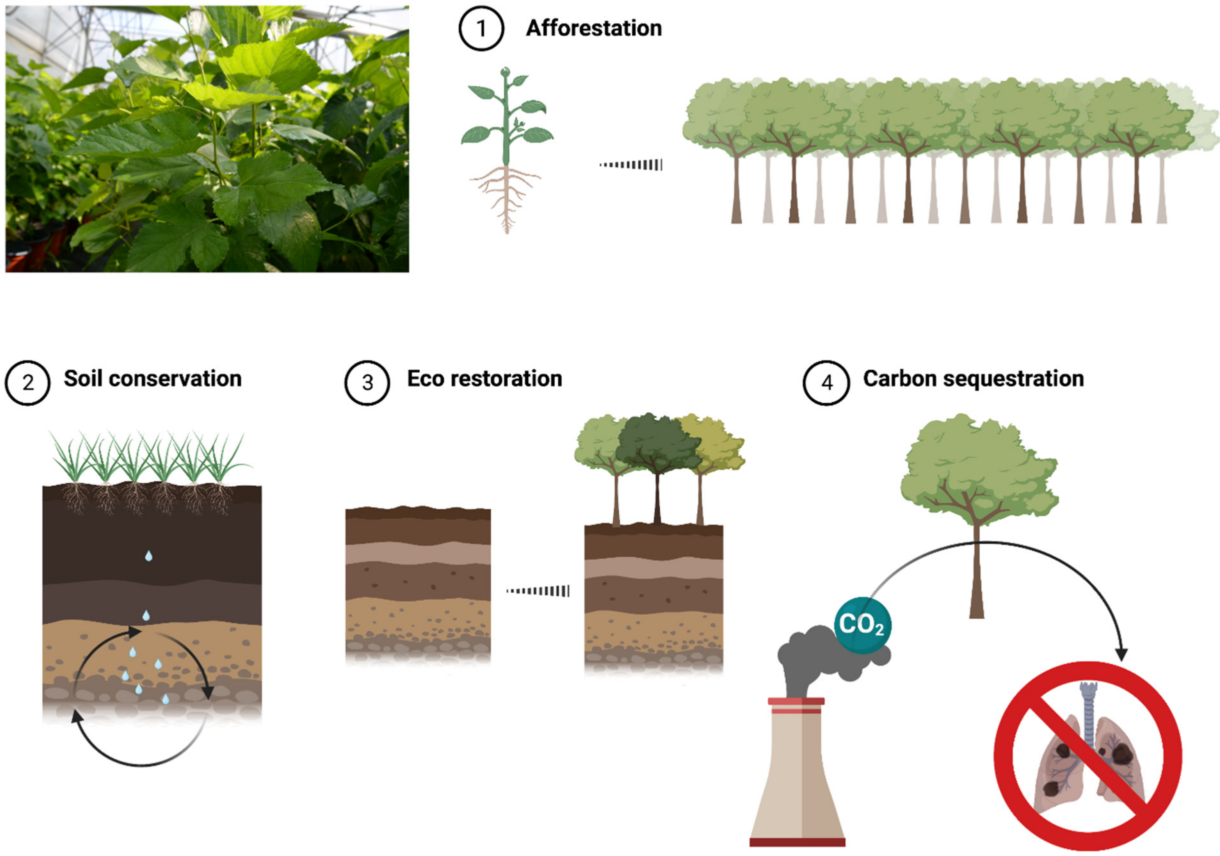


**Figure 3.** Importance of mulberry in environmental safety (created with BioRender.com, accessed on 10 February 2023).

Side roles of mulberry in this area are linked to its ability to grow and develop in various environmental conditions, meaning a diverse climate (from very dry areas to temperate countries), altitudes (from below 100 m sea level to over 4000 m above sea level) and soils. Thus, mulberry trees have been planted in desert areas (minimum irrigation required) and salinized lands, aiming for a natural restoration. In both cases, the eco restoration has been successful. Moreover, it has also been demonstrated that mulberry trees can be effectively used in landscaping, as they help prevent floods, drought, and wind currents. Besides the afforestation advantages given by this plant, mulberry plantation allows the cultivation of grass and other crops on the same field [68].

## 3. Alternative Farming Methods

There is a great interest in food quality and safety among consumers, keeping in mind that conventionally grown foods are known to have negative health effects due to their higher levels of pesticide residue, nitrate, heavy metals, hormones, or antibiotic residues, but these foods also lack nutrients and antioxidants. As a result, the demand for organically grown foods has increased in recent decades due to concerns over food safety and potential health benefits. Organic food production involves cultivating crops without using synthetic fertilizers, weedicides, pesticides, or other chemicals. The popularity of organically grown foods is on the rise because of their positive impact on nutrition and health. Additionally, organic farming practices help to protect soil health and promote biodiversity, leading to more sustainable agricultural systems [70–72]. However, it can also be more labor-intensive and may require more land to achieve the same level of productivity as conventional methods. Mulberry organic farming implies the process of growing mulberries by using natural methods such as crop rotation, mulching, composting, vermicomposting, and so on. However, many farmers prefer chemical-based inputs due to their short-term results and cost-effectiveness. On the other hand, the use of synthetic agents can potentially pollute the environment and affect the quality of mulberry by-products; therefore, it negatively impacts the consumer's health, the silkworms' welfare, and the beneficial micro-organisms, just to mention a few. Although chemical farming initially results in higher yields, farmers eventually experience a reduction in leaf yield and quality, and in terms of sericulture, it impacts cocoon productivity [73]. Therefore, promoting organic farming is crucial in sericulture to prevent the use of chemicals in mulberry cultivation. Moreover, it has been revealed that by using organic agents, due to soil improvement, the quality of mulberry leaves was enhanced [74]. As such, it is worth considering growing mulberry in alternative systems to ensure proper organic crop management for this species.

Alternative farming methods usually refer to agriculture types other than the conventional ones, such as in vitro micropropagation, vertical farming (including soil-less techniques such as hydroponics, aquaponics, aeroponics, etc.), permaculture, polyculture, and biodynamic farming (Figure 4).

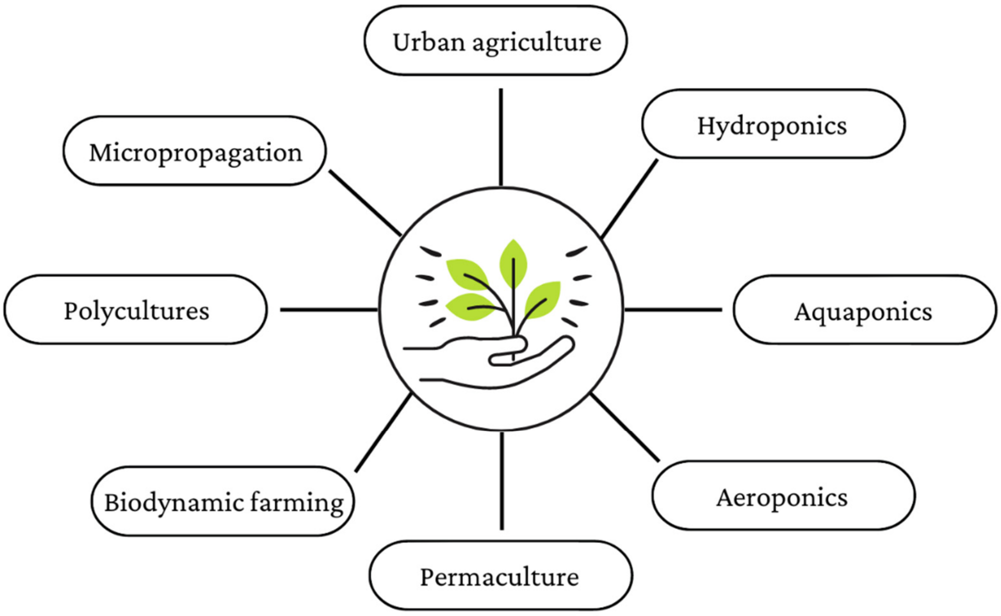


**Figure 4.** Schematic representation of the main alternative crop cultivation methods (created with Canva.com, accessed on 10 February 2023).

The subject of the current review is represented by the vertical farming systems, as possible change makers of food chains in urban areas or in areas with infertile ground or excessive dryness. The United Nations estimated a population growth of 2 billion people by 2050 [75]; as such, actions towards achieving the Sustainable Development Goal 2 ("End hunger, achieve food security and improved nutrition and promote sustainable agriculture") should be taken as soon as possible, worldwide. In terms of food, its production has a direct impact on the environment and consumers; thus, conventional agriculture systems require major updates in making them more sustainable, diverse, environmentally friendly, and accessible.

Vertical farming is an alternative crop cultivation method that aims to ensure global food security by increasing food production per unit of space and resource [76]. Empty industries, contemporary structures built on environmentally damaged sites, and even repurposed ocean freight shipping containers could all be used as alternatives to greater agriculture spaces in our community. Vertical farming is a technique that involves raising crops in controlled indoor conditions with specified lighting, nutrients, and temperatures, Plants are stacked in layers and can reach a height of several floors. Despite the fact that inhabited vertical farming has been around for more than a decade, business-range vertical farming has only recently come under examination. Curiosity about this cutting-edge agricultural technology is rapidly growing, and businesspersons in many places are paying close attention to it [77], not only for its sustainable character, but because even though the initial investment is comparable to a greenhouse set-up, the general profit was higher [76]. Out of the most commonly used vertical farming system, the general hydroponic, aquaponic, and aeroponic systems are further described.

### *3.1. Hydroponic System*

A hydroponic system is a soilless type of crop cultivation, very promising when it comes to geographic areas with limited spaces that can be dedicated to conventional agriculture [78]. The principle of the hydroponic method is described by Lei and Engeseth in 2021, as follows: The plants grow on a liquid substrate (water enriched with essential nutrients) that replaces the soil. The roots are soaked in the mineral-rich solution and absorbed with significantly less effort than soil-based growth methods, which eventually leads to an increased growth rate and yield [79]. There are two main types of hydroponic systems: circulating (closed) and non-circulating. Circulating hydroponic systems, which also can be divided into deep-water culture (DWC) and nutrient film technique (NFT), are proven to give the best results in terms of environmental impact and cost-effectiveness [78]. A basic one-level circulating hydroponic system has, as its main components, a tank full of nutrient solution, a growth tray, an air pump, and a water pump. The water pump is used to generate bubbles that deliver oxygen into the tank and the air pump to force air into the nutritional solution [78–81], as shown in Figure 5a. For more advanced growing technology, the system can be equipped with UV LEDs linked to a timer, so that plants can have the necessary amount of light during their developing stages when cultivating indoors, as well as an AI device that helps with the controlling and monitoring of the parameters and processes that occur when the hydroponic system is on. In this regard, several pieces of research have been conducted in order to develop the smart farming area, including the applications of Raspberry Pi in modern agriculture [82] or the provision of the IoT framework for an automatic robotic system, designed by Shrivastava et al. in 2021, to route the protocols [83].

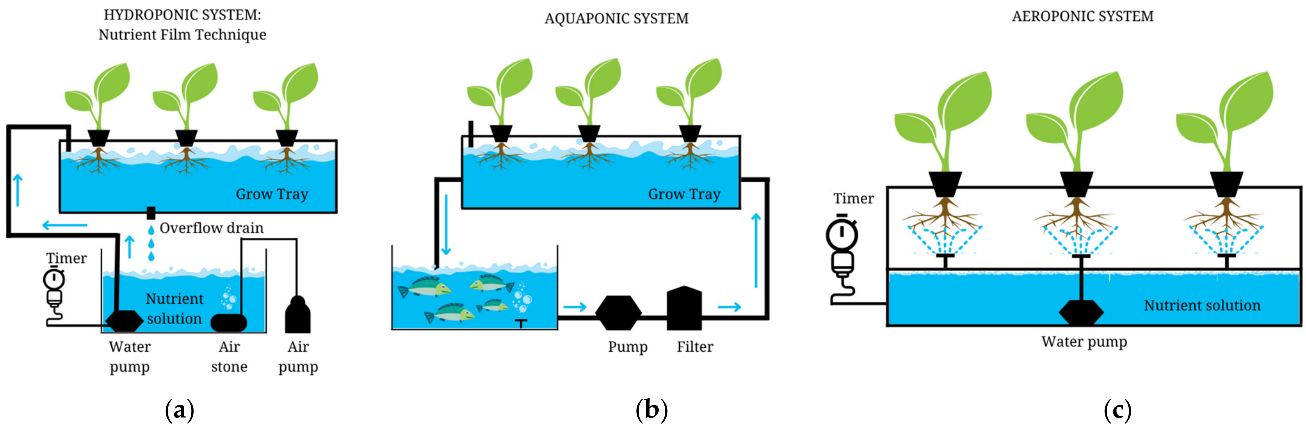


**Figure 5.** Hydroponic system (**a**), aquaponic system (**b**), and aeroponic system (**c**). A basic representation of one-layer cultivation techniques (created with Canva.com, accessed on 10 February 2023).

Over the past 50 years, experiments on cultivating plants (Table 1) in the hydroponic system have been conducted with remarkable results in terms of texture, nutritional values, taste, and overall quality of the final product [84]. One of the main influencing components of any hydroponic system is represented by the nutrient solution. It is well known that, in plant cultivation, there are some essential chemical elements that are used for the proper growth and development of any plant. As such, the tank with the nutrient solution should contain basic organic compounds (carbon, hydrogen, and oxygen), macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur), and micronutrients (manganese, iron, boron, zinc, copper, molybdenum, nickel, and chlorine) [85]. As organic elements are widely spread in the environment (in the air and water in the case of hydroponic systems), the mineral elements should be given greater attention when optimizing the nutrient solution. Absorption in the hydroponic crop is normally proportional to the concentration of nutrients in the solution near the roots, and it is highly influenced by environmental parameters, such as pH, salinity, oxygen levels, temperature, and electric conductivity. In addition, the intensity of light, photoperiod, and air humidity have a big impact. Each physicochemical element performs a specific role in the plant, and their excess or deficiency causes symptoms of insufficiency or toxicity [86].

Recently, white mulberry (*Morus alba* L.) has been successfully reported as grown in hydroponic conditions by Sakurai et al. (2022). The key findings of their study are related to the possibility of growing mulberry in hydroponic conditions and are also related to quality of leaves and roots from obtained biological material. The tasted leaves were noticeably better in terms of the organoleptic measurement, making them edible. When the levels were tested for 1-deoxynojirimycin (DNJ) and polyphenols, the DNJ level increased in the hydroponically grown plants compared to the conventional ones. Contrarily, polyphenol levels dropped in comparison to the field variety. Indicating increased relative levels of lipophilic polyphenols, HPLC profiling found a clear variation in leaf components between hydroponic and field cultivars. When compared to So-Haku-Hi, the root's polyphenol content—particularly its lipophilic polyphenol content—was exceptionally high (Sang Bai Pi in Chinese). Moreover, anti-obesity efficacy of the hydroponically grown mulberry was further investigated in rats. As a result, the hydroponic cultivar's leaf and root both demonstrated potential anti-obesity and anti-hyperlipidemic properties via reducing insulin resistance. Differential effects of leaf and root powders suggested that, in addition to DNJ, the lipophilic polyphenols of hydroponically cultivated white mulberry may play a significant part in its anti-diabetic action. In order to cultivate a novel source of mulberry for creating functional foods and medications, hydroponics can become a suitable alternative method [87].

### *3.2. Aquaponic System*

Aquaponics is a technique that falls under the IAAS, meaning the Integrated Agri-Aquaculture Systems. The use of these systems aims to create sustainable farming and food systems through the integration of crop and livestock production principles [88]. This technique integrates the practices of recirculated aquaculture with the manufacture of crops in a hydroponic system [89]. This kind of symbiotic environment was firstly discovered in South China, in the mid-fourteenth century, in a form of a "dike-pond system". Later on, in sixteenth century Mexico, the chinampas (floating gardens) created by the Aztecs became famous for its unique operating system that consisted of the base of nowadays' aquaponics [90].

The workflow of a basic aquaponic system is as represented in Figure 5b and has the following components: a growth tray for plants (the hydroponic part), a fish tank, a pump, and a filter [91]. The hydroponic subsystem receives water from the aquaculture subsystem. A bacterial community converts the metabolic waste from fish and unconsumed feed into easily digested nutrients (such as nitrates and phosphates) that plants use to develop. The water might be put back into the fish tanks after being extracted for plant nutrients, when the systems are linked [92]. Some treatment applied to the circulating water system enables the purification and recycling of aquaculture wastewater through the use of machinery for oxygen enrichment, sterilization, and physical and biological filtration. In most cases, sterilization and disinfection are performed by using ozone and UV light [93].

The fish species that can be used in aquaponic systems have a broad spectrum, not being restrictive as long as they provide the necessary nutrients for the development of the plants [94]. Commonly, the Nila tilapia (*Oreochromis niloticus*) species is extensively used in aquaponics practices, due to its rapid development rate, tolerance for crowded environments, and resistance to temperature variations and poor water quality [95]. Besides the Nila tilapia, several other species have been tested and proved to become well-adapted to the aquaponic system. Love et al. provide some very good examples, based on their international survey that was conducted in 2019, on the topic of aquaponic farming: ornamental fish, shark catfishes (*Pangasius* spp.), brook trout (*Salvelinus fontinalis*), black bass (*Micropterus* spp.), yellow perch (*P. flavescens*), shrimps, and prawns were successfully used in different countries [96]. In order to maintain an adequate environment for fish development, it is important to constantly maintain good-quality water, to ensure proper nutrient management, and to provide the necessary amount of feed. For fish with a body mass of more than 100 g, the current feeding recommendation for aquaponics is to provide fish feed at 1% of body weight every day [97].

Nutrient management is essential in running aquaponic systems, according to the dynamic that occurs in the solution. Although the wastewater coming from the fish tank contains important amounts of nitrogen, phosphorus, potassium, calcium, magnesium, sodium, and sulfur which serves as a nutrient solution for the plants, the required ratio of micro and macronutrients are not always in normal parameters. A notable example of dysregulation is given by Teng Yang and Hye-Ji Kim in 2020, when cultivating basil, tomato, and lettuce in an aquaponic system. They observed a significant reduction of nitrogen, magnesium, and calcium during the development of the plants, while phosphorus, sodium, and chlorine remained in optimal amounts [98]. Although these processes were conducted manually for several decades, the rapid advances of technology allowed the integration of artificial intelligence in managing the systems. Remarkable examples are shown by Haryanto et al. (2019) [99], Dhal et al. (2022) [100], and Lauguico et al. (2020) [101]. More than that, prospects in using computational tools in assessing the quality of the products obtained have also been approached by Reyes-Yanes et al. (2021) [102].

In terms of economical significance, pilot studies show an increased income when several vegetables were grown in aquaponic system, compared to conventional ones [103–105].

### 3.3. Aeroponic System

The general idea of aeroponics dates back to the 15th century, when random observations about plants that were growing with roots suspended in the air, near waterfalls, were made by scientists [106]. Along the way, they replicated conditions in laboratories for studying the roots' physiology, but the starting point of modern aeroponics was in 1942, when Carter published his research about "a method of growing plants in water vapor" for a better evaluation of roots [107], cited in [108].

The basic principle of the modern aeroponic system is having a nutrient solution tank from which nozzles linked to a programmable timer sprinkle fine fog onto the plant roots in a given time-frame (Figure 5c) [109]. Although this system represents a variation of hydroponics, the clear advantage is that the roots are constantly aerated, so no additional equipment is required for this purpose. More than that, it allows a lot of flexibility in terms of space required and helps in increasing crop density [110].

The quality of the products obtain in an aeroponic system is nowadays widely studied. Johnson M et al. (2015) evaluated the algal biomass production in aeroponic systems and, interestingly, the lipidic content and fatty acids' profile were not affected when compared to the conventional growth. Thus, prospects of using such substrate can be explored for increasing the yield that can be further used in biofuel production, feed, or nutraceuticals [111]. Another study reveals that tomatoes maintain higher quality parameters (growth rate, yield and antioxidant capacity, nutrients, etc.) when cultivated in an aeroponic system [112]. Similar results were obtained also by Patil N.L. et al. (2021) when cultivating wheatgrass [113].

The economical aspect of using aeroponics in growing edible plants is also significant. In a study conducted by Pasch J. et al. (2021), the wet and dry leaves weight of basil was up to 40% higher when cultivated in an aeroponic system, compared to a dynamic root-floating system and decoupled aquaponic system. Therefore, the income of selling the final product was also higher [114]. Same results were reported by Tunio and Gao (2020). They obtained not only a higher yield when cultivating lettuce in an aeroponic system, but also the highest shoot and root length, using the less amount of water compared to traditional and hydroponic system [115].

### *3.4. Plants Adapted to Hydroponic, Aquaponic and Aeroponic Conditions*

Several common plants, especially vegetables and spices, have been successfully grown in soil-less vertical farming systems, as shown in the Table 2.

**Table 2.** Examples of crops successfully grown in vertical farming systems.

| Type of the Crop | Examples | Hydroponic Culture | Aquaponic Culture | Aeroponic Culture | References |
|---|---|---|---|---|---|
| Fruits | strawberries, melons | ✓ | ✓ | ✓ | [116–120] |
| Vegetables | tomatoes, spinach, lettuce, green beans, bell peppers, cucumbers, eggplants, celery, potatoes | ✓ | ✓ | ✓ | [118,120,121] |
| Spices | basil, parsley, mint, coriander, ginger, echinacea, licorice | ✓ | ✓ | ✓ | [84,121,122] |
| Cereals | rice, maize, sorghum, oats, wheat | ✓ | ✓ | ✓ | [84,123–125] |
| Woody plants | Coffee tree, bamboo, mangrove tree | ✓ | ✓ | ✓ | [126] |
| | Mulberry | ✓ | | | [87] |
| Othe plants | Indian aloe, roses, philodendron, Cineraria | ✓ | ✓ | ✓ | [84,123,126–128] |

Compared to the other plants mentioned above, mulberry has a biological particularity which makes it suitable for intensive growth: the excitability of dormant buds. Annually, a large number of buds form, but just a part of them start to grow, while others (from the bottom of the tree crown) stay in a dormant state. They are the ones that, stimulated by the production cuttings, start in the vegetation. This process leads to the development of new vigorous shoots, ensuring leaf production for the following year [129]. Further exploitation of mulberry could be possible if its availability would not be dependent on the climate, type of soils, or land size. As such, it is safe to consider mulberry as one the most potent plants while using alternative farming methods in completion of the conventional ones to ensure year-round production and maximize income [68].

However, there are several disadvantages and challenges that are associated with vertical farming systems, the most important being energy dependency. These kinds of systems are dependent on LED lighting, and not on sunlight, but also are dependent on $CO_2$ management. These throwbacks include constant monitoring, which implies certain technical knowledge. One of the biggest challenges is the cost involved in the use of vertical farming systems, which are higher than the costs implied in traditional agriculture [130].

## 4. Conclusions

Among a wide range of medicinal plants, mulberry is one of the most important major players when it comes to bioactive composition. Recently, there is a high demand for this specific plant, as numerous researchers obtained a wide range of mulberry-based products that exhibit constructive biological effects. It is well documented that oxidative stress plays a crucial role in multiple diseases, which represent death-leading causes worldwide, such as cardiovascular diseases or cancer. As stated by previous research, mulberry exhibits strong antioxidant activity, and it could play a key role in treating the aforementioned target diseases. Furthermore, mounting evidence revealed that mulberry could be used in the treatment of hyperlipidemia-based diseases by contributing to the prevention of lipid accumulation. This event occurs via the stimulation of lipolysis and the repression of lipid synthesis. These results revealed that the mulberry plant represents a powerful prophylactic agent for fatty liver disease. In addition to mulberry's role in alternative medicine, it constitutes the unique nourishment source for *B. mori*. However, fresh leaves are not the only way of feeding silkworms. There are described several recipes for artificial diets that facilitate the silkworms' rearing when the climate does not permit its rearing during the whole year. These recipes mainly consist of mulberry leaf powder and represent a great advantage for sericulture. Besides the pivotal role of mulberry in various industries, it also represents a key player in the environmental safety area.

In terms of global food security, achieving sustainable development goals requires new technologies and strategies to make agriculture and food production, in general, more resilient. One of the most recommended solutions is the transition from conventional agriculture to alternative systems, such as indoor hydroponics, aquaponics, and aeroponics. Several plants have been successfully cultivated in such environments independent of the outside climate, free from pesticides and insecticides, and with a minimum amount of water. Therefore, the applicability of these techniques may be extrapolated to a larger collection of functional plants, especially to mulberry, when cultivating bush-type for a maximum yield per surface unit. The aforementioned use of mulberry, not only in sericulture but also in medicine, pharmacy, and the food industry, makes it a very suitable candidate for year-round production in countries that do not benefit from it due to cold weather, inappropriate soil, or limited available land.

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