MAS Journal of Applied Sciences
Uygulamalı Bilimler Dergisi
ISSN: 2757-5675
masjaps.com

OPEN ACCESS
DOI: http://dx.doi.org/10.5281/zenodo.8023251
Derleme Makalesi / Review Article

An Outlook of the Cultivation, Medicinal Properties, and Tissue Culture Techniques of Centaurea in Türkiye: A Comprehensive Review

Çiğdem Alev ÖZEL[1*], Siti MAESAROH[2]

[1]Gazi University, Faculty of Gazi Education, Department of Biology Education, Ankara

[2]Ankara University, Faculty of Agriculture, Department of Field Crops, Ankara

*Sorumlu yazar (Corresponding author): cigdemozel@gmail.com

Geliş Tarihi (Received): 10.02.2023 **Kabul Tarihi (Accepted)**: 28.03.2023

Abstract

Centaurea L., one of the largest genera within the Asteraceae family, has undergone taxonomic revisions resulting in the separation of certain sections into three distinct genera: *Psephellus* Cassini, *Rhaponticoides* Vaillant, and *Cyanus* Miller. This review presents an overview of Centaurea in Türkiye. The genus Centaurea in Türkiye involves118 taxa consisting of both endemic and non-endemic species. These taxa have been classified into various endangered categories, including Endangered (EN), Vulnerable (VU), Lower risk (LR) - conservation dependent, LR- near-threatened, LR- least concerning, Data deficient (DD), and Critically Endangered (CR). Furthermore, the review acknowledges the importance of researching Centaurea's medicinal properties, traditional agricultural practices, and tissue culture techniques. It is essential to conduct comprehensive studies on germplasm conservation, evaluate their suitability as forage and ornamental plants, and explore their medicinal and traditional agricultural applications, given their conservation status and potential uses. These studies will enhance Centaurea's potential applications and benefits in various fields.

Keywords: *Centaurea*, endangered, endemic, medicinal, tissue culture

1. Introduction

Asteraceae is a cosmopolitan family with 1600-1700 genera and 24.000 species worldwide, except in Antarctica (Funk et al., 2009). This family contains approximately 138 genera and 1336 species in Türkiye (Yılmaz, 2021). They consist of milk-containing grasses, shrubs, and rare trees or climbers based on morphological characteristics (Seçmen et al., 2011). *Centaurea* L. is one of the largest genera in the Asteraceae. Recently, some of these sections were separated to split the genus into three genera: *Psephellus* Cassini, *Rhaponticoides* Vaillant, and *Cyanus* Miller (Wagenitz and Hellwig, 2000; Greuter et al. 2005, Bancheva and Greilhuber, 2006). Centaurea, one of the most critical taxa of this family, is a perennial herbaceous plant with approximately 700 species that have a natural distribution in Asia, North Africa, America, and Europe (Brummitt, 2004). The *Centaurea* genus is derived from Greek, called Centaur, and has its basis in Greek mythology with the upper body of a human, the lower body, and the legs of a horse. The Centaurea flowers were used to heal wounds or injuries after the war by centaur Chiron and others. Hence, Chiron, who taught humanity about the healing powers of Centaureas and plants, is known as the father of pharmacy due to his knowledge and skill in medicine, herbs, and pharmacy (Aksoy and Gönüz, 2015). Many species of *Centaurea* are used for medicinal purposes today (Dülger et al., 1997; Arif et al., 2004; Ertaş et al., 2014; Özkan et al., 2016). *Centaurea* flowers can be pink, purple, blue, yellow, or whitish (Seçmen et al., 2011). Aksoy and Gönüz (2015) emphasized that flower colors can effectively paint carpet wool. The importance of the Centauera taxon for honey bees has been determined in pollen analyses (Taşkın and İnce, 2009); Bakoğlu et al., 2013; Özler, 2018). This variety of colors in the flowers of the genus *Centaurea* is also a great addition to a garden arrangement. During the colonial periods, the seeds were brought from Europe to America and started to be grown in gardens, and they have been a part of the gardens for centuries. Even if they are not large cut flowers, the variety of colors makes them desirable, and takes place in many gardens or parks (National Garden Bureau, 2004).

2. *Centaurea* in Türkiye

Türkiye has vegetation richness due to an exceptional belt at the intersection of three phytogeographic regions, and this geography changes and surface shapes throughout the geological ages. *Centaurea* species, which have an important place in this richness, have flowers with various colors also increases the potential of ornamental plants. In recent years, Global warming that caused overheating or excessive rainfall affected adversely to cities. Urban planners choose dry landscape plants suitable for the region's needs in their landscaping works due to these adverse conditions, such as drought and water scarcity. Each city varies according to its climate, topography, and demographic characteristics. It is very advantageous to use these plants in landscape architecture applications since natural plant species are the ones that can best adapt to the local conditions due to their origin or the nature of the geography. Using biological species in landscape architecture applications is essential for adapting to the local environment, providing healthy plant tissue during this adaptation, and reducing maintenance costs. Natural resource management has gained importance in Türkiye in recent years. Turkoglu et al. (2009) emphasized that *Centaurea*, with its different flower colors and appearances, is used as an alternative for cultivating plants in landscape architecture studies, and the temperature requirement of the plants is an essential factor for adaptation to the ecology of the plant species. The researchers studied the germination rates and percentages of *C. balsamita* Lam., *C. iberica* Trev. Ex Spreng., and *C. virgata* Lam. based on the temperature factor, which largely determines the seed's germination time under field conditions. These seeds taken from roadsides and fields at an altitude of 1750 had been germinated at different temperatures. The best germination percentage and rate were determined on *C. balsamita* at 15°C. Eroğlu et al. (2019) observed the endemic plant species of *C. yaltirikii* N. Aksoy, H.Duman & Efe subsp. *yaltirikii*, and it determined the possibilities of evaluating these plants in landscape designs to be made in urban areas in terms of aesthetics and functionality. Similarly, Bozkurt (2019) assessed the aesthetic and functional uses of *C. solstitialis* L. ssp. *solstitialis*, *C. iberica* Trev., *C. virgata* Lam., *C. cyanus* L. taxa in landscape architecture studies, which were grown in the geographical conditions of Sivas's Gürün district and used medicinally and aromatically by the public. Bozkurt stated that taking these plants into the culture and participating in landscape architecture studies is essential both for the recognition of plants by people and for the extinction of species. The geographical distribution of *Centaurea* species in our country is as follows.

1. Marmara region (Istranca Section, Catalca-Kocaeli Section, Ergene Sectioni, and South Marmara Section)
2. Black Sea region (Western, Central, and Eastern of Black Sea Section)
3. Aegean Region (Actual Aegean Section and Central Western Anatolia Section)
4. Central Anatolia Region (Upper Sakarya Section, Middle Kızılırmak Section, Upper Kızılırmak Section, and Konya Section)
5. Eastern Anatolia Region (Upper Euphrates Section, Erzurum-Kars Section, Upper Murat- Van Section, and Hakkari Section)
6. Mediterranean Region (Antalya Section and Adana Section)
7. Southeastern Anatolia Region (Middle Euphrates Section and Tigris Section)

Within the scope of CR, *C. tchihatcheffii* Fisch. & C.A.Mey., an endemic that started to be used in landscape architecture as an ornamental plant for festivals in upper Sakarya and known as the flower of love or iridescent, was included in the genus Cyanus / Gökbaş from the Asteraceae family in 2003 with the current name *Cyanus tchihatcheffii* (Fisch. & C.A.Mey.) Wagenitz & Greuter. Due to similar examples, *Centaurea* has declined from 280 to 238 taxa of both endemic and non-endemic, and their local names are below (Bizimbitkiler, 2013). The common Turkish names of the species belonging to the genus *Centaurea* are as cornflower (peygamber çiçeği), turmeric thistle (zerdali dikeni), shepherd lifter (çoban kaldıran), thymus thistle (timur dikeni), point button (gelin düğmesi). Although the genus Centaurea is most commonly known as cornflower, it is also referred to by many local names (Bizimbitkiler, 2013).

2.1. Conservation of *Centaurea* Genetic Diversity

The world carries out activities to protect plant diversity with organizations such as the International Union for Conservation of Nature (IUCN), the Committee on Endangered Plants, the World Wildlife Fund (WWF), the World Conservation Union, and countries take various measures to protect their plants. In our country, it has been classified according to the international IUCN hazard categories, which were first adopted in the 1980s. The Red Book of Plants of Türkiye was published, and the list of plant species under threat and threat categories were determined (Ekim et al., 2000) as follows:

1. EX- Extinct: There is no doubt that the taxon is the last individual
2. EW- Extinct in the wild: It is living for surviving in cultivation due to losing its historic habitat. It has not been found in detailed research at different times of the year and in the native habitat.
3. EN - Endangered: It is at very high risk and is in danger of extinction shortly, but it is not yet in the CR group.
4. VU- Vulnerable: It cannot be placed in the CR and EN groups but is highly threatened in the medium-term future in nature.

5. LR- Lower risk: Those populations are good and known from at least five localities. It has three subcategories according to their future status, as follows:

a. (cd) – conservation dependent: Taxa that may fall into one of the above categories within five years. Those that require a special conservation status, both in terms of species and habitat.

b. (NT) – near-threatened: Those who could not be placed in the last category but were close to being placed in the VU category.

c. (lc) – least concerning: Those who do not require any protection and are not threatened.

6. DD- Data deficient: The information about the distribution and abundance of a taxon is insufficient

7. CR- Critically endangered: It is at risk of extinction very shortly

8. NE- Not evaluated: Those who cannot be assessed by any of the above criteria

The endemic and non-endemic 118 taxa of the genus *Centaurea* are included in the endangered categories EN, VU, LR-cd, LR-nt, LR-lc, DD, and CR (Bizimbitkiler, 2013; Tubives, 2023). Germplasm protection of these taxa, mainly in danger categories, is needed to preserve this genetic diversity. The primary objective is to raise public awareness of these taxa's sustainability. They should be reproduced by traditional agricultural practices and in vitro methods, and endangered taxa should be protected in gene banks.

2.2. Studies on the medicinal properties of Centaurea

Although synthetic drugs are produced for many diseases today, it is known that many pharmaceuticals have many side effects. For this reason, people want to use natural products that they have been using since ancient times and know their side effects (Baytop, 1999). Therefore, herbal medicine raw materials increase in importance because they have fewer side effects than chemical, pharmaceutical raw materials, and most synthetic substances (Ötün, 2015). Since some species of Centaurea are used intensively among the public in this sense, they have emphasized the determination of the biological characteristics of other species belonging to this genus (Arif et al., 2004, Khammar and Djeddi, 2012). *Centaurea* species are used alone or in combination with other plants for antidiabetic, antidiarrheal, antirheumatic, anti-inflammatory, cholagogue, choleretic, digestive, stomachic, diuretic, diuretic, asthmatic, hypotensive, antipyretic, cytotoxic, antibacterial purposes. For this reason, studies have been carried out on the phytochemical and biological activities of endemic and non-endemic centaurea species in Turkiye. Salan et al. (2001) investigated the flavonoid of *C. salonitana* Vis and the endemic *C. kilaea* Boiss. The five flavones were found: salvigenin (scutellarein-6,7,4'-methyl ether), 6-hydroxy luteolin-6,7,3',4'-tetramethyl ether, luteolin-7,3',4'-trimethyl ether, jaceocidin (6-hydroxy luteolin-6,3'-dimethyl ether) and pectolinarigenin (6-hydroxy ap i genin-6,4'-d i methyl ether) in *C. kilaea* Boiss and pectolinarigenin, 6-hydroxyluteolin-4'-methyl ether, cirsiliol (6-methoxyluteolin-7-methyl ether), hispidilin (6-methoxy apigenin), and apigenin (7-O-glucoside) in *C. salonitana* Vis. Yayli et al. (2005) observed 46 compounds of essential oil of *C. armena* Boiss, with β-eudesmol as an identified main compound in ratios 19.3%. In addition, it was noted that moderate antibacterial activity against Gram-positive as *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923 and Gram-negative bacteria such as *Yersinia pseudotuberculosis* ATCC 911 with inhibition zone diameter < 5 mm. However, it had no antifungal activities for pathogenic yeast such as *Candida albicans* ATCC 60193; Ct, *Candida tropicalis* ATCC 13803. Shoeb et al. (2007) discovered phenolic nature, five flavonoids (2’’- (4’’’-hydroxy benzoyl), orientin, isoorientin, isoquercitrin, and cirsiliol), and chlorogenic acid of aerial parts of *C. gigantea* Sch. Bip.

ex Boiss. belong to antioxidant and anti-colon cancer. Karamenderes et al. (2006) studied extracts of 10 Centaurea species discovered in Turkiye using hexane, chloroform, and methanol solvent solution. The chloroform extracts of the whole plant of *C. calolepis* Boiss. and *C. hierapolitana* Boiss.; aerial plant of *C. cariensis* subsp. Maculiceps (O.Schwarz) Wagenitz, *C. cariensis* subsp. Microlepis (Boiss.) Wagenitz and *C. cadmea* IZEF5670 - IZEF5671; and rhizome of *C. cadmea* IZEF5670 - IZEF5671 with highest antileishmanial activity of *C. hierapolitana* Boiss. (IC50 1⁄4 8.7 mg=ml, IC90 1⁄4 17 mg=ml). While the hexane extracts of the whole plant of *C. hierapolitana* Boiss.; and aerial of *C. cadmea* IZEF5671 had low antileishmanial activities. All of the tested methanol extracts of plants had no antileishmanial activities. Karamenderes et al. (2007) found two elemanolide sesquiterpenes and two eudesmane-type sesquiterpene glycosides named hierapolitanins A, B, C, D with five known compounds as two flavones (hispidulin and jaceosidin), a flavon-C-glycoside (shaftoside), a flavonol glycoside (kaempferol-3-O-rutinoside), a neolignan, and dehydrodiconiferyl alcohol from the aerial parts of *C. hierapolitana* Boiss. Erel et al. (2014) extracted *C. aphrodisea* Boiss., *C. athoa* DC., *C. hyalolepis* Boiss., *C. iberica* Trev, *C. polyclda* DC using three different solvents: hexane, chloroform, and methanol. The methanol extract of these plants has antioxidant activities shown by total phenolic and flavonoid contents. The cytotoxic activity was noted in chloroform extract of these extracts, with the most substantial effect of *C. polyclada* on BT-549, KB, and SK-OV-3 cell lines (30. 33, and 47 mg ml^{-1}, respectively). The significant anti-inflammatory capacity of chloroform was noted compared to n-hexane and methanol extracts, with the most potent anti-inflammatory activity of chloroform extract of *C. athoa* on both assays (6 mg ml^{-1} for Nf-kB and 16 mg ml^{-1} for iNOS assay). Some studies of endemic Centaurea of Turkiye have been presented at International Congress on Medicinal and Aromatic Plants held in 2017 as an essential oil, fatty acid components, antimicrobial and antibacterial activities, phytochemical content, and antioxidant activity. These studies were conducted on *C. paphlagonica* (Bornm.) Wagenitz (Tüfekçi et al., 2017), C. polypodiifolia Boiss var. *polypodiifolia* (Eser et al., 2017), *C. babylonica* (L.) (Güvensen et al., 2017), *C. solstitialis* ssp. *solstitialis* L. (Tüzün et al., 2017), *C. virgata* Lam. (Aydemir et al., 2017), *C. urvillei* DC. subsp. *armata* Wagenitz (Şen et al., 2017). Yırtıcı (2019) studied on antioxidant and enzyme of the methanol extract of *C. fenzlii* Reichardt with total phenolic and flavonoid of 16.72 mg GAE g^{-1} ka and 173.16 mg KAE g^{-1} ka, respectively. Antioxidant capacity was determined to belong to reducing power as iron (FRAP) of 0.256 mmol TE g^{-1} ka, copper (CUPRAC) of 0.878 mmol TE g^{-1} ka, and radical scavenging effect as ABTS of 0.354 mmol TE g^{-1} ka, and DPPH of 0.661 mmol TE g^{-1}. The enzyme inhibitory effect was noted as α-glucosidase 0.331 mmol AKE/g ka, α-amylase 0.354 mmol AKE g^{-1} ka, AChE to 0.367 mmol GAE g^{-1} ka, BChE to 0.878 mmol GAE g^{-1} ka and tyrosinase for mmol 0.256 KE g^{-1} respectively. Keser et al. (2020) studied *C. virgata* Lam., *C. kurdica* Reichardt, and *C. saligna* (K.Koch) Wagenitz using water, ethanol, methanol, and acetone solvent extract solution. The results showed antiradical activity with high ABTS scavenging of *C. saligna* (K.Koch), Wagenitz methanol (99.94%), *C. virgata* Lam. methanol (98.23%), and water (98.10%) extracts and low of *C. kurdica* Reichardt extracts for all the antiradical assays compare to Trolox (96.79%) and. In addition, it was also reported that three Centaurea extracts showed antiproliferative properties due to high LNCaP, HCT-116, and MCF-7 cancer cell lines and had rich in total flavonoid, phenolic and proanthocyanidin, phenolic acids, phytosterols, vitamin, and unsaturated fatty acids. Aksoy (2020) studied the biological activities *of C. virgata* Lam. using ethanol, n-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions solvent extract. Its ethyl acetate fraction had the highest radical scavenging activity by DPPH value with an IC_{50} of 138.7 µg mL^{-1} and ABTS value compared to the other extracts. In comparison, n-hexane and aqueous ethanol fractions revealed poor antioxidant activity with IC_{50} values of 824.8 µg mL^{-1} and 610.3 µg mL^{-1}, respectively. The highest phenolic content was noted on ethyl acetate. All extracts showed antiproliferative activity depending on the dose excluding aqueous ethanol fractions. The study on *C. urvillei* DC. subsp. urvillei conducted by Erecevit Sönmez and Çakılcıoğlu (2021) showed that 40 mg of its plant solution could inhibit the growth of all tested microorganisms as yeast, dermatophyte isolates, and other bacteria with sensitive inhibition zone (14.66 mm-11.66 mm inhibition zone- 1 diameter). It is implied that it has antibacterial and antifungal against infectious diseases caused by *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Klepsiella pneumoniae* clinical isolate, *Candida albicans* RSKK 02007, *Candida glabrata* RSKK 014019, *Candida tropicalis* RSKK 02011, *Epidermophyton floccosum* RSKK 14024, and *Trichophyton rubrum* RSKK 03022. Alper et al. (2021) reported that phenolic and flavonoid content were noted on the ethanolic extract of *C. solstitialis* with the value of 52.31 mg GAEs g^{-1}(gallic acid) and 30.10 mg QEs g^{1}(quercetin), respectively. It was also noted that caffeic acid was the most abundant phenolic compound.

2.3. Studies on Traditional Agricultural Practices

Centaurea cyanus cornflower leaves, whose chemical classification is Benzopyrone dyes, Indigoid dyes Indole dyes, known to impart a purple-blue color, are also used in salads, cornbread cakes and to decorate foodstuffs (Chaitanya Lakshmi, 2014). Therefore, this plant, which grows naturally in our country, has value as a food color substance. It also has agricultural production potential. However, there has yet to be a study on the production of this plant for this purpose in Turkiye. *Centaurea* genus, widely distributed in our country, is an essential plant with medicinal properties, and it is needed at low production cost, is easy to harvest, and requires labor for cultivation (Gül and Mülayim, 2018). The researcher states that some species of Centaurea are used as fodder plants. According to the results obtained regarding some herbal and chemical properties, and microelement content of *C. balsamita,* it is predicted that it is suitable for use as a fodder plant by cutting it or applying different processes. Furthermore, "Regulation on the placing on the market and use of feeds" of the Feed Catalog published on 22.09.2016, Centaurea species " Centaurea cyanus L. seeds" can be used as feed in the Feed Ingredients Catalog (Anonymous, 2016).

2.4. Studies for Tissue Culture and Production

Unfortunately, many species worldwide are lost due to human-induced causes before determining what purpose they will serve. Propagation by tissue culture is a valuable method for conserving threatened species. The limited study on this subject in Türkiye is given below. Göztaş (2008) investigated the effects of gibberellic acid (GA_3), indole 3 acetic acid (IAA), 2-4 dichlorophenoxy acetic acid (2.4D) and kinetin hormones on the germination of seeds of *C. kotschyi* (Boiss. & Heldr.) Hayek var. *kotschyi* with MS medium. The highest germination rate was 32.5% at 1000 ppm of GA_3 compared to other concentrations of 1, 10, 100, and 1000 ppm of GA_3. The highest germination rate was 62.5% in 2 mg L^{-1} IAA compared to 0.5, 1, and 4 mg L^{-1} IAA. The highest germination rate was defined as 50% at a concentration of 5 mg L^{-1} 2.4D opposed with 0.5, 1, and 2.5 mg L^{-1} 2.4 D. While the highest germination rate was observed as

57.5% in 0.215 mg L^{-1} compared to 21.52 and 2.152 mg L^{-1}. *In vitro* culture is essential for the ex-situ conservation of rare, endemic, or threatened plants. This study developed an effective protocol for seed germination, seedling development, and axillary shoot regeneration of *C. zeybekii* Wagenitz. Seeds collected from a wild population were subjected to surface sterilization and germinated after six weeks under *in vitro*. The effects of photoperiod and temperature during germination on seed germination have also been investigated. A high germination rate was achieved when vitamins and 1 mg L^{-1} GA_3 were added to the nutrient mediums. The highest germination frequency was performed at 24 ± 2°C. High-frequency axillary shoot regeneration was observed in MS with 1 mg L^{-1} BA. The axillary shoots were then transferred to the MS medium with or without a plant growth regulator for rooting. Root was promoted after six weeks in a 1/2 MS setting containing only MS and 0.5 mg L^{-1} IBA. The rooting process is prolonged, and the percentage of shoot rooting is also meager (15%). The researchers emphasized that this protocol strengthens wild plant populations but also stated that many aseptic seedlings can be produced in clonal micropropagation studies (Kurt and Erdağ 2009). Yüzbaşıoğlu et al. (2012) studied *C. arifolia*, which belongs to DD (Data Insufficient) according to the IUCN category. No studies have been identified aimed at in vitro breeding of this species. This study showed seeds germinating aseptically in Petri dishes in Murashige and Skoog ½ MS-free media without growth regulators. Leaf explants of six-week-old grown seedlings were taken to MS medium containing 1 BAP mg L^{-1} + 0.1 mg L^{-1} NAA (MS1) and 2 mg L^{-1} BAP + 0.2 mg L^{-1} NAA (MS2) for indirect regeneration. After three weeks, callus growth was observed, and shoot regeneration was observed in 150% (MS1) and 120% (MS2) over calluses. The best root of the shoot regeneration was noted in the MS medium containing 1 mg L^{-1} IBA. They succeeded *in vitro* reproduction of *C. arifolia* through indirect organogenesis from leaf segments. Aydoğan and Erdağ (2015) looked at callus development of the endemic *C. zeybekii* Wagenitz by using the leaves of shoots obtained from *in vitro. They investigated* the effect of different cytokinins on adventitious shoot regeneration. The callus formation did not occur in the control medium of MS. The 100% callus has been observed in MS medium containing 0.005 mg L^{-1} and 0.01 mg L^{-1} TDZ. The best adventitious shoot regeneration per explant at the end of 1.5 months was seen in the MS medium containing 1 mg L^{-1} of KIN with 6.2 shoots. The highest average shoot was observed in the same environment at 4.17 cm. MS and 1/2 MS have been used for rooting. However, the rooting rate remained very low, and only 15% of the resulting shoots were rooted in an MS nutrient medium containing 0.5 mg L^{-1} IBA. Demirel et al. (2017) investigated in vivo and in vitro germination of endemic and threatened species *C. fenzlii* and *C. derderifolia* species. Seeds were moistened with distilled water in Petri dishes, covered with blotting paper, and left to germinate in a climate chamber at 25°C and 16 hours of light/8 or darkness between Petri dishes for *in vivo* germination. At the same time, the seeds were left in MS, B5, SH, and WH medium to germinate in a climate chamber with 6 seeds in each petite at 25°C with a 16-hour light/8-hour dark photoperiod at 25°C after sterilization for *in vitro* germination. The highest germination medium for *C. fenzlii* and *C. derderifolia* seeds was observed in the WH nutrient medium compared to others *in vivo* and *in vitro*. Turkoglu et al. (2017) studied the *in vitro* shoot regeneration of the endemic and endangered ornamental plant *C. fenzlii* Reichardt. The plant seeds were germinated at a rate of 35% in MS medium with 2.60 µM $GA^{3,}$ and young leaves of germinated seedlings were used as a source of explants. The leaves were regenerated in the MS medium, which contained 1.11, 2.22, 4.44, 8.88 µM BAP + 2.685, 5.37, 10.64 µM

NAA. The best percentage of callus regeneration was seen in the MS setting using 4.44 µM BAP + 10.64 µM NAA. Although the shoot regeneration rate ranged from 5.66% to 36.87%, the most shoot regeneration percentage was obtained in the MS medium with 8.88 µM BAP + 2.685 µM NAA. The number of micro-shoots per explant ranged from 1.66 to 5.87. The highest number of shoots per explant was recorded in the MS medium, having 4.44 µM BAP + 10.64 µM NAA. The best rooting was recorded after seven weeks of culture on 4.90 µM IBA with a low percentage. Uzun et al. 2020 studied in vitro regeneration of *C. amaena*, which is endemic and endangered in Turkiye. The cotyledon, leaf, and cotyledon node explants were cultured in Murashige and Skoog (MS) medium with or without different concentrations of 6-benzyl amino purine (1-4 mg L^{-1}, BAP), thidiazurone (0.3-1.2 mg L^{-1}, TDZ), or meta-Topolin (0.5-4 mg L^{-1}, mT), α-naphthalene acetic acid (0.5 mg L^{-1}, NAA). The maximum axillary shoot per explant was 9.975 pieces, with 70.83% of shoot regeneration noted on cotyledon nodes cultured in the MS medium containing 4 mg L^{-1} mT. Furthermore, it was pointed out that the maximum number of shoots per explant with 4.152 shoots from media containing 1 mg L^{-1} mT and the 55.00% of shoot regeneration percentage on indirect organogenesis of cotyledon explant. The leaves cultured on medium containing 4 mg L^{-1} mT brought 4.132 shoots and 50.00% of shoot regeneration percentage per explant. Only callus induction was observed in a medium with TDZ or TDZ combinations with NAA. The shoots are approximately 50.00% rooted from 1/2 MS medium containing 2.0 mg L^{-1} indole-3-butyric acid.

3. Conclusion and Recommendations

Human-induced effects mainly cause the extinction of plants. The main reasons are air, and water pollution, pollution of soils due to excessive use of herbicides, overgrazing, stubble burning, environmental wastes, and distorted urbanization. Therefore, the priority should be public awareness-raising. In particular, the primary goal should be introducing and protecting these plants for local people living in natural areas. In addition, it should aim to produce, store, and market these plants with traditional or biotechnological methods. Comprehensive studies can be carried out for germplasm conservation of threatened endemic or non-endemic *Centaurea* species. Although the morphology, anatomy, pollen morphology, and total chromosomes of many Centaurea species have been identified, molecular research is needed to determine the genetic diversity of Centaurea species and the phylogeny of the species found in Turkiye.

Considering the potential of *Centaurea* to grow as an ornamental plant, particular areas should be created in parks and gardens, considering the climate and geographical features.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the article's final version, which is ready for publication.

Declaration of Conflicts of Interest

All authors declare no conflict of interest related to this article.

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To Cite: Özel, Ç.A., Maesaroh, S., 2023. An Outlook of the Cultivation, Medicinal Properties, and Tissue Culture Techniques of Centaurea in Türkiye: A Comprehensive Review. *MAS Journal of Applied Sciences*, 8(2): 362-373.
DOI: http://dx.doi.org/10.5281/zenodo.8023251.