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REVIEW PAPER

Assessing *Indigofera tinctoria* L. as a forgotten medicinal industrial plant and the importance of its revitalization for the sustainability of Iran's agricultural ecosystems

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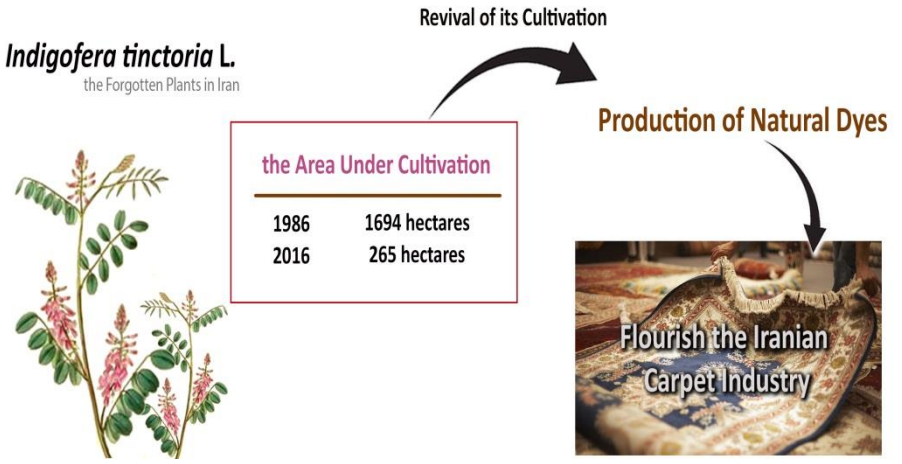
Highlights

- Indigo plant is one of the forgotten functional plants in Iran.
- This plant can be considered in order to increase the sustainability in agriculture and the appropriate planting pattern in the region.
- In addition to being used as a medicine in traditional medicine, indigo is also used to produce natural dyes.
- This plant has the ability to be introduced and used as a forage edible plant.

Graphical Abstract



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Abstract

Indigo (*Indigofera tinctoria* L.) belongs to the legume family and is originally native to India. The dye extracted from its leaves was known in ancient civilizations such as Mesopotamia, Egypt, Greece, Rome, England, Central America, Peru, Iran and Africa. In 1986, the area under cultivation of this plant was about 1694 hectares. In 2016, the cultivation of this plant has reduced to 265 hectares. This study is a kind of development research and review, its method is a library survey, and the data was collected by taking notes. The results show that indigo can be considered as one of the forgotten crops in the country due to the decrease in cultivated area. This plant is currently cultivated only in some areas of Kerman province such as Fahraj, Jiroft and Regan, while in most of these areas it grows wild in pastures and in the mountains at an altitude of 700 meters above sea level. Therefore, to conserve and protect this useful and important species, it should be used in crop rotations and stable ecosystems. It is also necessary to identify and generalize all aspects of this medicinal-industrial plant, and proper planning should be done to prevent it from being forgotten.

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1. Introduction

Over thousands of years, our ancestors have collected many plants and learned a great deal about them. Medicinal herbs are still very valuable resources to meet health and hygiene needs so the demand is increasing for medicinal herbs, aromatic and natural products in the world. According to the World Health Organization, 80% of the world's population traditionally uses herbs and natural products for primary health care (Modanlo et al., 2021; Mulholland, 2005). Iran, as a country with a long history in the production and consumption of medicinal herbs, has only a very small share of these plants in the global trade market. According to the official statistics, in 2017, in Iran, the area under cultivation for medicinal herbs was about 605,876 hectares and 262,428 tons were produced, of which 13,694 tons belonged to Khorasan Razavi province with 19,348 hectares of area under cultivation of medicinal plants. The main crops were cumin, henna, coriander, indigo, fennel and Damask rose. Important export items of Iran are licorice, garlic, tragacanth, cumin, saffron, henna, rhubarb, and thyme, which are mainly exported to European countries and some Arab countries (Jain et al., 2010).

In recent years, the tendency to use herbal medicines in most human societies is expanding due to the side effects of some industrial drugs. Therefore, the cultivation of many forgotten medicinal plants that grow wild in natural areas has become common as new plants in many parts of the world. Following the need, the destruction of natural areas has also increased. According to available statistics, five percent of 7,000 plant species are endangered in the country, which is more than the amount predicted by the International Union for Conservation of Nature and Natural Resources (Lee, 2010). Therefore, in order to preserve the genetic resources of medicinal species in natural areas, we should be careful about the proper use of natural areas instead of an improper collection. Also, these plants should be planted in the agricultural ecosystems and their characteristics (including distribution, associated species, cover density, biological characteristics, yield, etc.) should be studied in order to preserve genetic diversity (Hamrouni et al., 2001). Since the global approach to the production of medicinal plants is mainly aimed at improving the quality, quantity and health of active, therefore, it is necessary to pay attention to the principles of ecological agriculture, which guarantees the quality of these plants and reduces the possibility of their negative effects. Indigo (*Indigofera tinctoria* L.) is a forgotten medicinal-industrial plant belonging to the legume family. Indigo is one of the plants that Heravi has mentioned in his agricultural guidance book “Ershad-O-Zeraa”. This plant is cultivated in the Bam region in two ways; after fallow and after harvesting wheat and barley. Indigo had about 1,694 hectares of cultivated area in Iran in 1986, which decreased to 520 hectares in 2006.

At present, with about 300 hectares of indigo cultivation area in Kerman province, this plant is one of the most important summer crops in these areas. However the area under cultivation for this plant is very small in Iran, but the increasing tendency to use medicinal herbs over the past decades due to their fewer side effects has caused the indigo to be given more attention. Reviving the cultivation of this product can be useful in creating employment and diversity in cultivation patterns in different regions of the country. The medicinal properties of indigo include laxative, expectorant, anti-intestinal parasite, treatment of diabetes, epilepsy, liver strengthening, chronic bronchitis and asthma (Tyagi et al., 2010). A substance called indigo is extracted from indigo leaves. Indigo is used in the dyeing industry. The blue color is extracted which has very high stability and is widely used in dyeing fabrics and objects. Replacing artificial colors with natural colors is one of the reasons for forgetting these plants. At present, the production of chemical dyes has led to marginalization in Iran. This research is about studying indigo, as a forgotten medicinal-industrial plant in Iran, which on the one hand it can increase and improve the livelihood of its producers and it can reduce the outflow of currency to buy chemical dyes, and on the other hand, revive this plant in crop rotation will play an important role in stabilizing sustainable crop ecosystems.

2. Materials and methods

In this study, data were collected from the available information and documentary sources in the provinces, information bases, and the Internet. Also, theoretical researches, library studies, studying and reviewing available reports and documents were done. In some areas, field studies were also conducted.

2.1. Botanical characteristics of Indigo

Legumes are one of the oldest plants cultivated by humans. They date back to the Neolithic period as crops that human went from the stage of hunting and gathering food to the stage of food production and they entered a stage of life based on agricultural and rural communities (Duponnois et al., 2001). Indigo or woad is an industrial and forgotten plant belonging to the legume family (Hossain et al., 2012). In Iran, the genus Indigofera includes six species of shrub plants that mainly grow in the southern regions. These species are *I. intricate*, *I. oblongifolia*, *I. argentea*, *I. articulatae* and *I. tinctoria* (Mozaffari et al., 2000). This plant is an annual, biennial or perennial with a height of one to two meters. The leaves are 2.5 to 7.5 cm long and have small stipules.

The leaflets are 9 to 13 and the color of the leaves is greenish-gray and covered with hairs and their margins are serrated (Warrier et al., 1993). The flowers are pink with a length of 4 mm and are located on a peduncle 5 to 11 cm long. The flowering of this plant usually lasts from June to August, depending on the climatic conditions of the region. The fruit of this plant has short and black pods and its root is vertical and somewhat thick. Its leaves are harvested 4 to 5 times a year when it is thick enough, shiny and oily. The leaves are arranged alternately, the leaflets are opposite to the triangular earrings with a length of 1.5 to 3 mm. The stipule is more than 2 cm long. The inflorescence axis is up to 7 cm long and has one mm petiole. Every 100 grams of dried leaves has 1.5 grams of nitrogen, 1.35 grams of phosphorus, 1.4 grams of potassium and 3.9 grams of calcium (Felicia and Muthulingam, 2012).

Its propagation is usually done by sowing seeds in the spring on lines at a distance of 25-30 cm. Its growth is very slow until July, but from then on it is fast. Indigo seeds vary in appearance but are generally oval to cylindrical and flat on one side. The seeds of this plant were small; So its length is between 2.3 to 2.5 mm and width is 1.8 to 2 mm (Jahan et al., 2013). The seeds of this plant contain 12 to 38% protein and also they contain six types of rotenoids including degatin, dehydrodguatin, rotenol, rotenone, tefrosin and somatrol (Felicia and Muthulingam, 2012). Rotenoids have insecticidal properties (Felicia and Muthulingam, 2012; Asuntha et al., 2010). The active ingredients are alkaloids, glycosides, flavonoids, tannins and phenolic compounds, amino acids, carbohydrates, minerals, other compounds such as ash, acid-soluble ash, water-soluble ash, etc (Asuntha et al., 2010).

2.2. Distribution of Indigo in Iran and the world

Indigo is native to East Asia, but it is also found in southeastern and parts of central Europe. This plant has been cultivated in Europe since the Stone Age, and the presence of its main long root enables it to withstand drought. The plant consists of approximately 750 species that are distributed throughout the tropics and subtropics of Africa, Asia and America. Large areas of the Netherland are covered by indigo. In some areas, including the United States, it grows wild. This plant also grows in Pakistan, Sri Lanka, Indonesia, Saudi Arabia, Egypt, India, hot and humid regions of Africa and Syria (Mozaffari et al., 2000).

Indigo has a long history of cultivation in Jiroft, Kahnooj, Iranshahr, and Bam (Jain et al., 2010), some people believe that indigo was imported to Iran from India during the Sassanid period (Anushirvan), and some reports showed that it was entered in Iran in the sixth century AD Some sources also mention that indigo was cultivated in Kerman province (during the Sassanid period) and Khuzestan province (during the Qajar period) (Mozaffari et al., 2000) and currently, indigo cultivation in Kerman province is one of the most important summer crops in this region (Tyagi et al., 2010).

Also, this plant grows wild in the mountainous areas of Khash, Nehbandan, Iranshahr, Zabol, Mirjaveh, Fahraj, Sakhor and Regan. In the crop year of 2006, the area under indigo cultivation was 520 hectares with an average yield of 2500 kg per hectare in Jiroft, Kahnooj and Regan regions and in 2016, it was reduced to 265 hectares. Therefore, this plant is considered a forgotten plant. Indigo is cultivated in small plots and less than 0.5 hectares, along with other crops, and the number of farmers reaches is about 450 to 500 people. The

cultivation of this plant is mainly done by small owners. Replacing artificial colors with natural colors is one of the main reasons for forgetting these crops (Zargaran Khouzani, 2021).

2.3. Climatic conditions and crop management

Indigo is mainly grown after summer crops or wheat. it can be said that indigo is alternated with summer crops or wheat. According to Mangan et al., 2004, the base temperature for germination of indigo seeds is 10°C and the optimum and maximum temperatures for this plant are 28and 39°C, respectively (Mangan et al., 2004). Therefore, according to the natural habitat of this plant and its base temperature, it is specific to tropical regions and its growth of it increases with increasing temperature. Also, in areas where this plant falls asleep due to winter cold, its growth begins when the temperature rises to 11°C. This plant likes light-permeable soils. Nitrogen is considered as an essential element for it, which research has shown that adding nitrogen fertilizer in the form of urea at a rate of 100 kg per hectare causes the highest yield and color content. Excessive density will increase the longitudinal growth of the plant and competition between plants within the vegetation will eventually lead to reduced yields. Also, reducing the density to less than the desired level will certainly lead to a reduction in yield.

Also noted that the biomass yield of this plant is significantly affected by plant density; with increasing density from 5 to 21 plants per square meter, biomass yield increased, but the effect of plant density on indigo was not significant. They also stated that the growth of this plant at high levels of nitrogen consumption (200 kg per hectare) showed more negative reactions to low levels of this element consumption. However, increasing nitrogen consumption improved indigo yield by improving soil fertility (Mangan et al., 2004).

2.4. Chemical compounds

In terms of chemical composition, the indigo plant contains indical, endoxyl, isatan and labenzyme (Fig. 1). The active ingredients of Indigo are alkaloids, glycosides, flavonoids, tannins and phenolic compounds, amino acids, carbohydrates, minerals. Other compounds are ash, acid-soluble ash, and water-soluble ash. As a natural product, indigo is a secondary metabolite. This plant is derived from colorless glucoside in the form of enol, which is composed of indoxyls such as indican, which is oxidized as blue indigo by aerobic oxidation. This plant contains colorless glucoside in the form of enol that is formed indoxyls such as indican, and it is oxidized as blue indigo by aerobic oxidation.

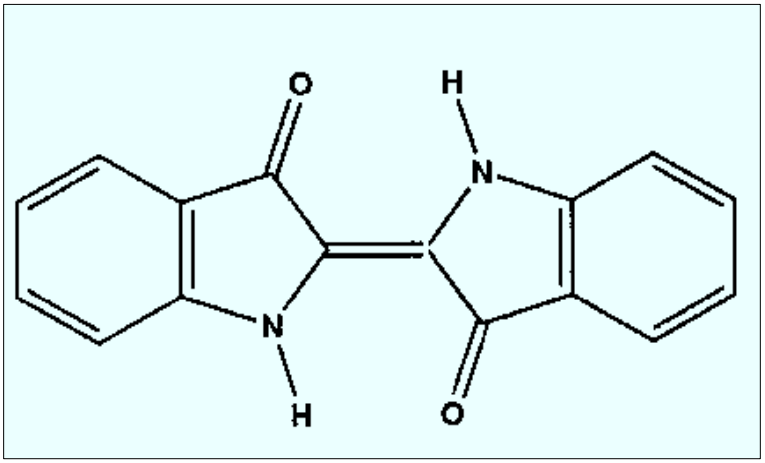

Fig. 1. The chemical composition structure of Indigo (Strobel and Gröger, 1989).

Since the biosynthetic pathway of indigo production and its quality has not been fully identified, the purity and the quality of this natural product are uncontrollable, which is one of the main reasons for replacing indigo with chemical dyes (Gilbert and Cooke, 2001). Lu (1986) concluded that the content of indigo depends on the type of texture, the plant age, and the climatic conditions of the region (Lu, 1986) (Fig. 2).

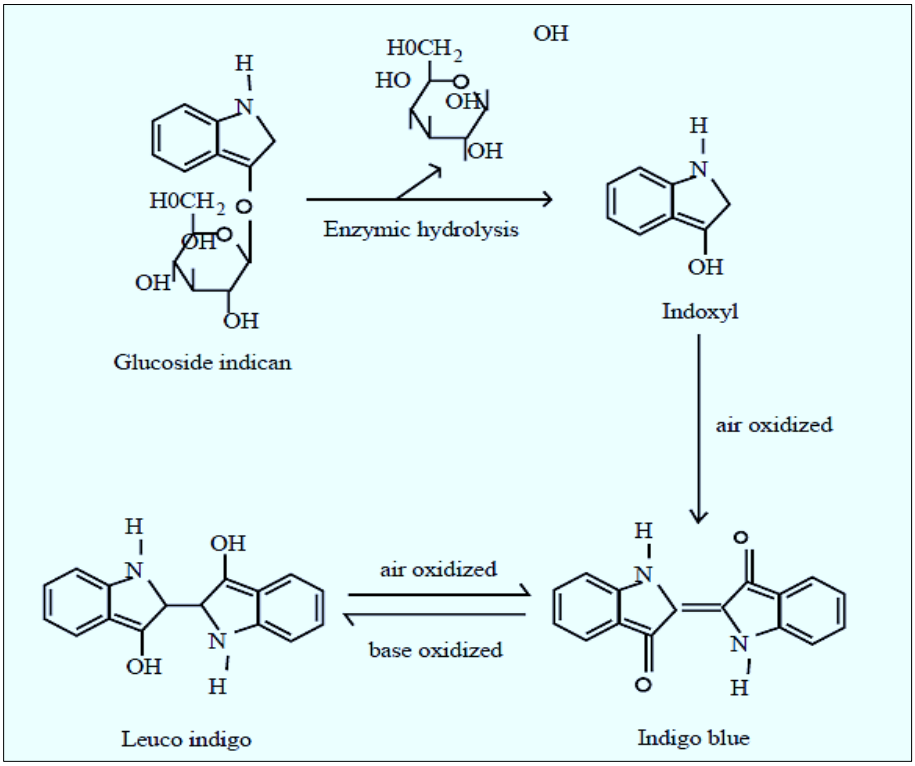

Fig. 2. The changes of Indigo pigment from chemical compounds (Kavimani et al., 2000).

The main components of indigo crude extract include two pigments, blue indigo and red indirobin (Kavimani et al., 2000). Indirobin is a pink-red pigment which is produced in the structure of *Polygonum tinctorium* (Fig. 3) (Lee, 2010).

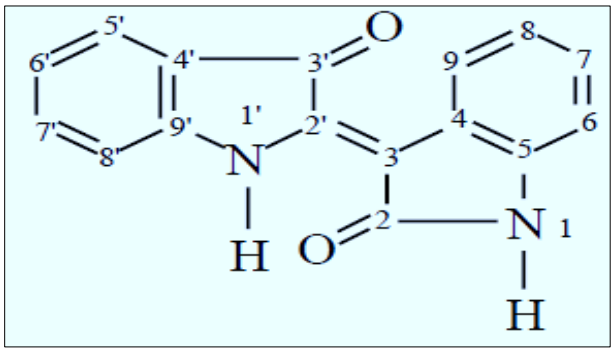

Fig. 3. Chemical structure of indirobin (Kavimani et al., 2000).

2.5. Indigo usage in traditional medicine

Indigo is widely used in traditional medicine. In Iran, in the southeastern regions, the leaves of this plant are used to treat foot pain and headaches. Also in these areas, crushed indigo leaves are used to treat burns and foot cracks. In Cameroon, indigo branches are used as toothbrushes. Indigo roots and stems have a bitter taste and they have laxative, expectorant, and anti-intestinal parasitic effects and they can be used to strengthen hair. All parts of the plant have an anti-inflammatory effect and are used to treat chronic bronchitis, asthma (especially in children), hemorrhoids, poisonous insect and reptile bites, wound healing and skin disorders (Kavimani et al., 2000). Its sap is also used to treat epilepsy and neurological disorders (Tyagi et al., 2010). It is also used as an ointment for the treatment of skin diseases and hemorrhoids. Its leaf extract is also used to treat burns and wounds of domestic animals such as cattle and horses.

In India, its root paste is used to heal wounds and also used in as an antidote against snake bites and to treat scorpion and insect bites. The indigo seeds can be useful to prevent dehydration as well as to absorb eye water at the beginning of cataracts. The leaves and roots are recommended for the treatment of epilepsy and diabetes

(Kavimani et al., 2000). Its dry powder is used in the treatment of asthma. Its leaves are recommended for liver toxicity and as an anti-inflammatory (Kavimani et al., 2000). The extract of this plant is known to be effective in treating shortness of breath, pertussis and heart palpitations (Bechtold et al., 2002). Methanolic extract of its different parts prevents the growth of the AIDS virus (Kavimani et al., 2000).

2.6. Blue indigo extraction steps

Today in the world, the tendency to use natural dyes has increased, which is effective in implementing ecological standards (Bechtold et al., 2002). The blue color of this plant is one of the oldest dyes known to human. It is called the "King of Colors" because the indigo has an attractive blue color, very high color stability and high strength to combine with a wide range of other natural colors. No other plant has had such a high status as Indigofera species in many past civilizations. A substance called indigo is extracted from indigo leaves. The most important use of indigo in the dyeing industry is that its color has very high stability and is widely used in dyeing fabrics and objects. The annual leaves of the indigo plant produce a blue dye that is used to dye cellulosic and protein fibers (Samanta and Konar, 2011), no blue dye has been identified in the structure of the indigo, but some substrates have the ability to convert to indigo for producing a natural blue dye. These substrates are in the form of needle-shaped crystals with a melting point of 180 °C. They are hydrolyzed by enzymes and converted to free indoxyl, and finally, in the presence of an oxidizer, they are converted to indigo, which is used for the natural dyeing of woolen yarns.

In middle-age Europe, most blue indigo was produced from the indigo plant, but by the 17th century, it was replaced by other plants and its consumption was reduced. By the 19th century, indigo and other natural plant sources producing indigo pigments had given way to synthetic dyes. To use indigo blue, the leaves are harvested in the first year and under a process, their blue color is extracted. The leaves are pulped in a mortar and pestle then they are turned into pellets and dried under the sun for four weeks. The dried pellets are fermented and extracted. Finally, the extracted blue dye is used for dyeing fibers and are used in the textile industry, art and painting (Tyagi et al., 2010; Zeidali et al., 2021).

2.7. The importance of indigo revitalization for Iran's agricultural ecosystems

In recent decades, the tendency to use medicinal herbs has increased in the countries of the world and due to the good quality of Iranian medicinal plants; a good opportunity has been established for their export. Therefore, the restoration of ecological capacities will improve the quality of medicinal plants, achieve a good share of the global trade of medicinal plants and, most importantly, reduce water use in the country. If indigo is planted and cultivated in appropriate ecological conditions to its habitat, it preserves a large amount of its composition and properties. This plant has a limited water requirement and with a multi-year cultivation capacity, it can be a suitable alternative for the optimization programs of the erosion in cultivation pattern. The conversion and processing of various primary and industrial products will lead to the prosperity of the natural dyes industry and the extraction of pharmaceutical products. This will be followed by an increase in employment in the agricultural, industrial, pharmaceutical, commercial, and community health sectors. In recent years, with the reduction of the area under cultivation, planning and support will be very important for its healthy products in the pharmaceutical and cosmetic industries in the country and outside the country. This program will involve training and promoting research, using the experiences of leading Asian and European countries in the production, processing and exporting of indigo plants, as well as making some necessary arrangements. Examining the views and hypotheses of production and export of valuable medicinal plants and importing agricultural products with high water consumption will be a plan in the direction of using natural capacities and preserving the country's water reserves (Zargaran Khouzani and Gharineh, 2021).

3. Conclusions

Although the area under indigo cultivation in Iran is very small, due to its diverse usages, it seems that the revival of its cultivation can be effective for creating employment and diversity in cultivation patterns in

different parts of the country. Therefore, recognizing the agronomic needs of indigo as a forgotten medicinal and industrial plant and the role of management factors in the production of natural dyes can improve the livelihood of its producers, and also it can reduce the currency export for providing chemical dyes. On the other hand, the global market of some industries will flourish again including the Iranian carpet industry. Also, due to shortage in cultivation, and the absence of its historical background, there is no significant information about its cultivation and production. With increasing natural dyes demand, increasing indigo cultivation can be considered as an alternative to chemical and synthetic dyes. Restoration and reviving of ecological capacities will improve the quantity and the quality of this plant if it is cultivated in appropriate ecological conditions. Also, with suitable placement in crop rotations, it has caused better stability for the agricultural ecosystem to protect the country's water reserves by its eco-physiological characteristics.

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