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A CASE STUDY ON THE CONTENT OF 1-DEOXYNOJIRIMYCIN IN SOME MULBERRY VARIETIES (*MORUS ALBA* L.) CULTIVATED IN BULGARIA

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Abstract

In recent years, more and more attention has been given to the naturally occurring alkaloids due to their unique bioactive properties. In particular, 1-Deoxynojirimycin (DNJ), an iminosugar derivative, has been attracting growing attention as anti-diabetic, anti-obesity, anti-microbial, anti-cancer, and cardioprotective agent. This alkaloid is detected in various parts of the mulberry plant, such as leaves, latex, and root bark.

The predominant active substance in the mulberry leaf extract is 1-deoxynojirimycin (DNJ), known for its role as a blood sugar level regulator. The present research aims at analyzing the impact of vegetation stages and mulberry variety on the content of 1-deoxynojirimycin in dried mulberry leaves. It has been concluded that the vegetation stage and variety have an effect on the content of 1-deoxynojirimycin in mulberry leaves, as the values of DNJ are the highest in the end of July and the lowest in the middle of June. The highest DNJ content is detected for the following varieties: Kokuso 21, Kinriu and Italian106; and the lowest content in Bulgarian 24 and Kokuso 27 varieties.

Keywords: mulberry leaves, DNJ, vegetation, variety

INTRODUCTION

Mulberry is a perennial plant growing in many world regions, from temperate and sub-tropical to tropical. Its leaves are used mainly as the sole food source of the silkworm (*Bombyx mori L.*) (Balik et al., 2019; Kafkas et al., 2008). Although there are more than 150 mulberry varieties known, only a few have therapeutic properties - *Morus alba, Morus nigra* and *Morus rubra*. The mulberry leaves contain anthocyanins, resveratrol, quercetin, rutin (rutoside), 1-deoxynojirimycin, etc. (Kumar & Chauhan, 2008). Amongst all, 1-deoxynojirimycin (DNJ) is considered one of the most important substances found in mulberry leaves (Aramwit et al., 2013).

Mulberry is used in East Asian countries (Korea, China and Japan) as a natural remedy for hyperglycemia (Singab et al., 2005). It has also anti-allergic (Chai et al., 2005) and immuno-modulating properties (Bhrani et al., 2010).

Mulberry leaves *(Morus alba L.)* have been used in traditional medicine (folk medicine) for reducing the symptoms of diabetes. Mulberry contains phenol compounds that lower the concentration of blood glucose. The dried root rind of white mulberry *(Morus alba L.)*, the leaves and fruits have been used for medicinal purposes in China for centuries.

Mulberry leaves are extremely rich in nutrients and contain a wide range of vitamins and active compounds beneficial to human health. The predominant active substance in the mulberry leaf extract is 1-deoxynojirimycin (DNJ), which has been proven to regulate the blood sugar level. Moreover, it is assumed that the biochemical properties of 1-DNJ in mulberry leaves have many more useful effects.

Some of the key properties of 1-DNJ are as follows:

- ✓ Slowing down sugar absorption and stabilizing the blood sugar levels after consumption;
- ✓ Stabilizing the blood sugar levels on an empty stomach;
- ✓ Improving the symptoms of insulin resistance.

Clinical studies with people have shown that one-time oral dose of a food supplement enriched with DNJ suppresses the increase of blood sugar levels as well as the insulin levels. These findings have been confirmed by studies conducted with rodents. Using these models, researchers have proven that the mulberry leaf extract lowers the disfunction of glucose processing in rats with diabetes. Also, other studies have shown that the addition of the mulberry leaf extract lowers the oxidative stress in fattened mice.

There have been clinical studies with people carried out to examine the efficacy of mulberry leaf supplements. Researchers have tested the compound and have concluded that the mulberry leaf extract can be additionally beneficial to health, for instance, in cancer prevention, as immune system booster having anti-inflammatory and neuro-protective effects.

The mulberry leaf extract 1-DNJ has been proven to successfully inhibit an enzyme called α-glucosidase responsible for catalyzing the hydrolysis of sugars and other carbohydrates to glucose. By inhibiting the activity of this enzyme, the mulberry leaf extract lowers the quantity of carbohydrates that turn into glucose. Consequently, this leads to stabilizing the blood sugar level and thus avoiding a potential negative effect on brain vasculature.

1-deoxynojirimycin (DNJ) in mulberry leaf has high inhibitory, anti-oxidant, anti-microbial and anti-inflammatory properties. It is a functional nutritive component widely used in China, Japan, Korea, Thailand and other Asian countries (Xue-Qin Hu et al., 2013).

In 1976, Yagid extracted 1-deoxynojirimycin (DNJ) from a dried mulberry root. Soon after 1-DNJ was found in other varieties, including *Bacillus subtilis, Streptomyces, Hyacinthus orientalis, Commelina communis, Adenophora triphyllavar varjaponica, Angylocalyx pynaertii* and *Bombyx mori*.

1-deoxynojirimycin (DNJ) was recognized as an important source for prevention or treatment of hyperglycemia. In the meantime, it is necessary to find a more effective method for producing higher quantities of DNJ because its content in mulberry leaves is only 0,1 %. Many researchers have cstudied the impact of numerous factors influencing DNJ content in mulberry leaves, such as the season of crop harvesting, fermentation induced by microorganisms, the optimal conditions of cultivation, and the optimal conditions of extraction.

It has been established that the silkworm has the highest content of DNJ in comparison to mulberry leaves and fruits. It can be suggested that the silkworm body accumulates a high concentration of DNJ (Ryu et al., 2005).

In a clinical study with diabetics the optimum result has been obtained with the dose of 900 mg of fine flour made of silkworm taken per day during eating.

Mulberry trees have been cultivated in many countries since ancient times. Taking into account more than 400 mulberry varieties belonging to 15 different types of Morus in China (Liu et al., 2010), 173 of them contain DNJ (Song et al., 2009; Xue-Qin et al., 2013). Former studies have shown that the DNJ concentrations in mulberry mature leaves vary from 0,134 mg/g to 1,472 mg/g in dry leaves among 132 examined varieties (Xue-Qin et al., 2013). Other bioactive components have not been analyzed.

The present research aims at analyzing the presence of 1-deoxynojirimycin (DNJ) in dried mulberry leaves depending on vegetation stages and mulberry varieties. The obtained

results could be useful for future recommendations regarding the development of therapeutic applications of mulberry.

MATERIALS AND METHODS

The present study was conducted in collaboration with the Scientific Center of Sericulture – Vratsa. An analysis of DNJ content in mulberry leaves was carried out by using the following varieties: Kinriu, Kokuso 27, Kokuso 21, Bulgarian 24 and Italian 106. Two separate experiments were performed as follows:

1) **Studying impact of mulberry vegetation stage on the DNJ content in leaves.**

The experiment was carried out with Kinriu variety, which is of Japanese origin. Uterine medium-stemmed plantation, 45-year-old, planted by the scheme 4 m x 4 m, was used. Trees were trimmed only once annually in the winter period via removing the whole one-year wood. Soil surface was maintained via autumn deep ploughing and 3-4 times of milling during vegetation from April to September. Each year, in the middle of April, the plantation was fertilized with NPK. Artificial irrigation was not applied. Leaf sample was taken around every 15 days – on 15 June, 27 June, 13 July and 27 July, correspondingly. Mulberry leaves were picked up by hand from the trees. In the experiment there were included only leaves from 3rd to 7th leaf counted downwards from the top of growth. Each sample consisted of 1 kg fresh mulberry leaves. The samples were dried in a drying oven with hot air at 55º C.

2) **Studying the impact of variety on the DNJ content in mulberry leaves.**

The experiment was conducted with the following varieties: Kokuso 27, Kokuso 21, Bulgarian 24, Kinriu and Italian 106. Uterine medium-stemmed plantation, 45-year-old, planted by the scheme 4 m x 4 m, was used. Trees were trimmed once annually in the winter period via removing the whole one-year wood. Soil surface was maintained via autumn deep ploughing and 3-4 times of milling during vegetation stage from April to September. Annually, in the middle of April, the plantation was fertilized with NPK. Artificial irrigation was not applied. Leaf samples were taken in the end of September. Mulberry leaves were picked up from the trees by hand. The samples consisted of leaves only from 3rd to 7th leaf downwards from the top of growth. Each sample was made of 1 kg fresh mulberry leaves. The samples were dried in a drying-oven with hot air at 55 º C.

The taken samples were analyzed in a laboratory in China (http://www.botaniccentury.com), by high-effective liquid chromatography with ELS detector (HPLC-ELSD).

RESULTS AND DISCUSSION

The obtained results are presented in Table 1 and Table 2, as well as in Figures 1-5.

Table 1. DNJ content depending on the time of mulberry leaf collecting.

Code	Time of collect	DNJ (%)
1	15 June 2014	0.078
2	27 June 2014	0.096
3	13 July 2014	0.084
4	27 July 2014	0.106

The data in Table 1 show that the DNJ content is the highest in the end of July (0,106 %), and the lowest in the middle of June (0,078). The difference between the highest and the lowest values was 0,028 %, as the increase of 0,028 % DNJ was for a period of 42 days while the mulberry leaves were maturing.

Kukoso 27, 1.26mg/g

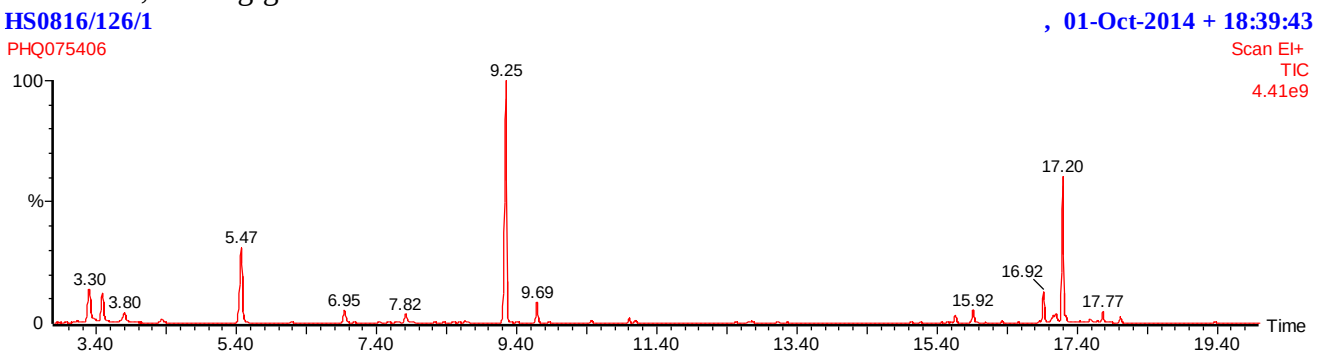

Figure 1. DNJ content in leaves of Kokuso 27 variety.

Kukoso 21, 1.6mg/g

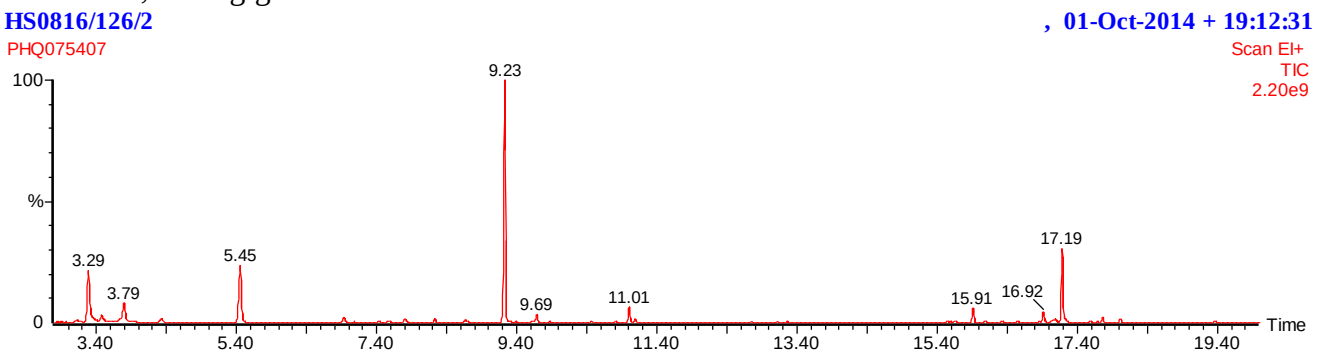

Figure 2. DNJ content in leaves of Kokuso 21 variety.

Bulgarian 24, 0.9mg/g

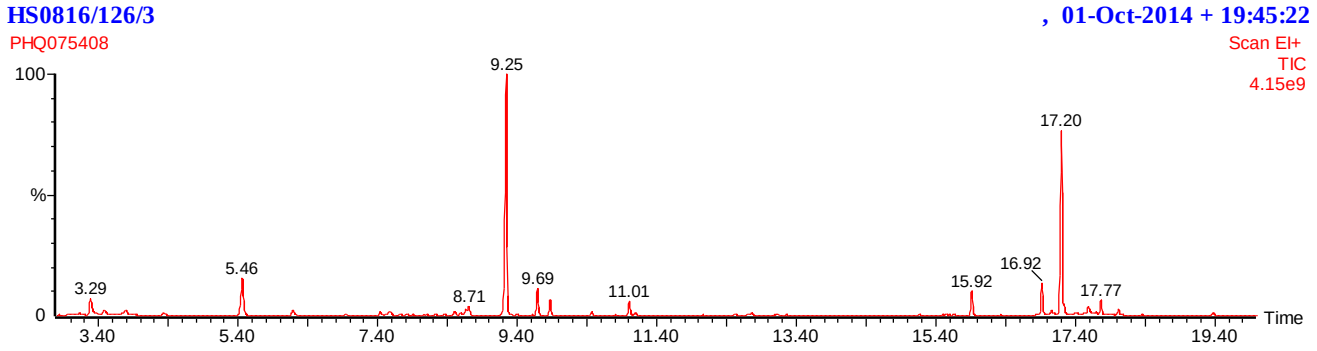

Figure 3. DNJ content in leaves of Bulgarian 24 variety.

Kinriu (Japanese), 1.6mg/g

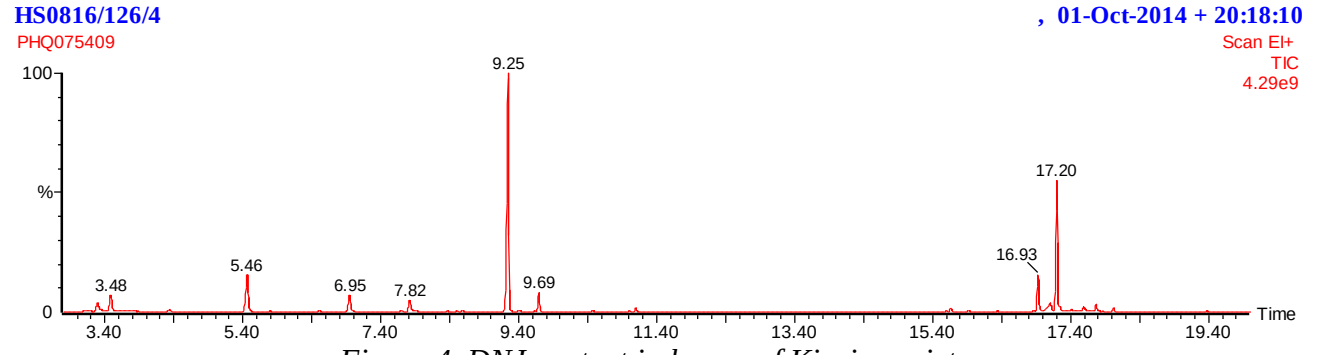

Figure 4. DNJ content in leaves of Kinriu variety.

Italian 106, 1.4mg DNJ/g

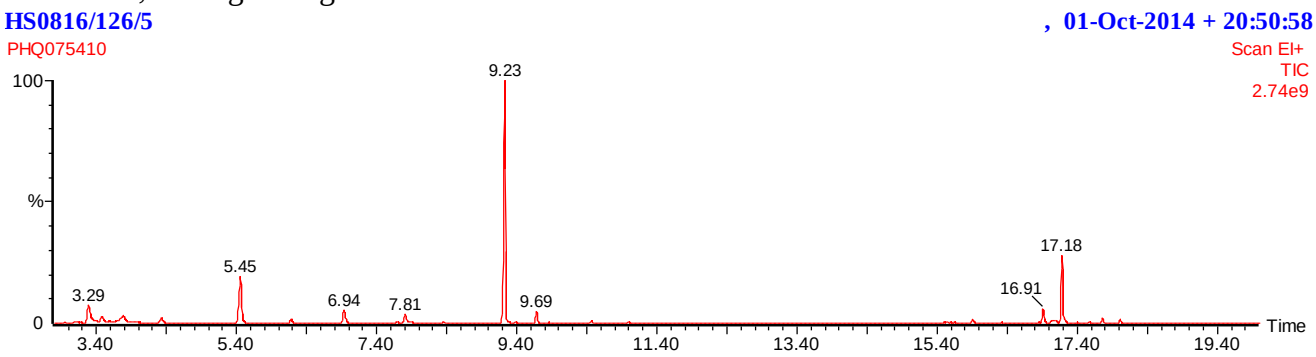

Figure 5. DNJ content in leaves of Italian 106 variety.

With regards to the 1-DNJ content in some of the most popular mulberry varieties cultivated in Bulgaria (Table 2 and Figures 1-5), it was obvious that the highest content was found in Kokuso 21 variety – 0,16%, Kinriuy – 0,16% and Italian 106 variety - 0,14%. The lowest value belonged to Bulgarian 24 variety - 0,09%, followed by Kokuso 27– 0,126%

Table 2. DNJ content in different mulberry varieties cultivated at the National Center of Sericulture – Vratsa.

Variety	Origin	mg/g	DNJ (%)
Kukoso 27	Japan	1.26	0.126
Kokuso 21	Japan	1.6	0.16
Bulgarian 24	Bulgaria	0.9	0.09
Kinriu	Japan	1.6	0.16
Italian 106	Italy	1.4	0.14

All examined varieties showed DNJ values within the range from 0,9 mg/g to 1,6 mg/g. They correspond to the studies performed by (Xue-Qin et al.,2013), who analyzed the DNJ content in 132 varieties of *Morus genus*, and the values were within the range from 0,71 mg/g to 1,019 mg/g, i.e., lower than the varieties cultivated in Bulgaria.

The highest DNJ content was found in Kokuso 21 and Kinriu varieties (1,6 mg/g).

CONCLUSION

The vegetation stage, as well as the variety of the mulberry, have an effect on the 1-deoxynojirimycin content in the mulberry leaves. DNJ content values are the highest in the end of July, and the lowest – in the middle of June. The highest DNJ percent is registered for Kokuso 21, Kinriu and Italian 106 varieties, and the lowest value is reported for Bulgarian 24 variety, followed by Kokuso 27.

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