IJIE Int. J. Indust. Entomol. 45(1) 35-41 (2022)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2022.45.1.35

ISSN 1598-3579 (Print)
ISSN 2586-4785 (Online)
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Investigation of mulberry farm's soil properties and mulberry leaf nutritive components in local areas of Korea

Wan-Taek Ju[1†], Chan Young Jeong[1†], Seong-Wan Kim[1], Jong Woo Park[1], Nam-Suk Kim[1], Sang Kuk Kang[1], Kee-Young Kim[1], Hae-Yong Kweon[1], Seul-Bi Lee[2], Woong Kim[3], Byeong-Sam Gwak[4], Bong-Tae Han[5], Moon-Tae Choi[6], Yoo Beom Lee[7], and Young-Seek Seok[8,*]

[1]*Department of Agricultural Biology, National Institute of Agricultural Sciences, Rural Development Administration*
[2]*Agricultural research & extension services, Gyeongsangnamdo*
[3]*Agricultural Research and Extension Services, Jeollabukdo*
[4]*Silkworm & Insect Management Center, Gyeongsangbukdo*
[5]*Sericulture Division, Agricultural Production Supporting Center, Chungcheongbukdo*
[6]*Industrial Entomology Research Institue, Chungcheongnamdo*
[7]*Agricultural Research & Extension Services, Insect & Sericultural Research Institute, Jeollanamdo*
[8]*Agricultural Product Registered Seed station, Gangwondo*

Abstract

Mulberry is a hardy, perennial, deep-rooted plant capable of thriving under diverse agro-climatic conditions. The selection of suitable land and appropriate variety can help the sustainable mulberry field. However, no conclusive and comprehensive investigation has been conducted on the mulberry soil properties and nutritional composition of mulberry cultivars from Korea local areas in previous studies. In our study, soil properties and mulberry leaf components of Korea local mulberry farms were briefly investigated. In result, the soil organic matter (OM) content was significantly high in Buan (6.81%) and Jangseong (6.14%). In contrast, available phosphate (P_2O_5) was different in each local area. To investigate relationship between soil property and nutritive component of mulberry leaf, Cheongil leaf samples from 8 local areas were analyzed. Among the macrominerals (K^+, Ca^{2+}, Na^+, and Mg^{2+}), the concentration of K varied from (1884±9.36) mg/100 g to (2685±11.5) mg/100 g. The potassium (K^+) of Cheongil leaf in Sangju was the highest at 2685 mg/100 g. Besides macrominerals, flavonoids, total dietary fiber contents and moisture of Cheongil leaf samples were studied in the 8 local areas. In terms of these contents, the variation was largely depending on the local areas. This study provides a possible industrial use of mulberry, and holds promise to enhance the overall profitability of sericulture.

Int. J. Indust. Entomol. 45(1), 35-41 (2022)

Received : 12 Aug 2022
Revised : 19 Sep 2022
Accepted : 26 Sep 2022

Keywords:
Mulberry leaf,
Soil properties,
Minerals,
Nutritive components

Introduction

Mulberry (Morus spp.) trees have traditionally been cultivated as silkworm feed. In addition, the market demand for their use in health foods is increasing (Kimura *et al.*, 2007). Especially, mulberry leaf quality plays a predominant

†Authors contributed equally to this study.
***Corresponding author.**
Young-Seek Seok
Agricultural Product Registered Seed Station, Chuncheon-si, Gangwondo, Republic of Korea
Tel: +82332486183 / FAX: +82332610537
E-mail: air5738@korea.kr

role in healthy growth of silkworm and the leaf composition depends on various factors like a mulberry variety, season, irrigation, beside, temperature, length of sunshine hours, nature and type of soil profile, water table, pruning, maturity of leaf, and method of leaf harvesting, etc (Nakagawa, 2013). Production of leaves in the tropical countries is largely dependent on the application of inorganic fertilizers (Ahmed *et al.*, 2015).

Mulberry is a hardy, perennial, and deep rooted tree can grow under various climate conditions (Adeduntan and Oyerinde, 2010; Ahmed *et al.*, 2015; Rathore *et al.*, 2010). The right selection of land and mulberry variety greatly helps sustainable mulberry farming. So it is very much needed to increase the leaf yield of mulberry plant for per unite area. The native soil fertility alone cannot be relied upon for the improvement in mulberry leaf yield and quality unless the nutrients are replenished with external sources through customized fertilizers which act quickly in improving the leaf quality for feeding (Rajaram *et al.*, 2013). In general, most plants grow by absorbing nutrients from the soil. Their ability to do this depends on the nature of the soil (Dick, 1983). Depending on its location, a soil contains some combination of sand, silt, clay and organic matter (Asai *et al.*, 2009). Also attendant soil acidity, nutrient imbalance and soil physical degradation hinder sustainable use of inorganic fertilizers in the tropics (Aulakh *et al.*, 2001; Erf and Proctor, 1987)

However, no comprehensive investigation has been conducted on the nutritional composition of mulberry leaf and soil properties of Korea local areas in previous studies. In our study, soil properties and mulberry leaf component were briefly investigated to characterize mulberry farms in each other local area of Korea. And according to this study, the results would provide a relation between soil properties, nutrient of mulberry leaf, and information of mulberry farm conditions for sericulture farmer.

Materials and Methods

Preparation of soil samples and soil chemical assessment

Soil samples were collected from silkworm feed mulberry farms of sericultural institute in Rural Development Administration (RDA, Jeonju), Chuncheon, Cheongju, Gongju, Buan, Jangseong, Sangju, and Jinju, Republic of Korea. The soil samples from silkworm feed mulberry farms were obtained in August 2020. For investigating the physicochemical properties of the soil in 8 local areas, it was analyzed on soil-pH, EC (Electrical Conductivity), OM (Organic Matter), Available phosphate (Available P_2O_5), and CEC (Cation Exchange Capacity). Subsamples were pooled to make a composite sample, thus a total of nine individual samples were collected. After removal of visible root debris, field moist soil samples were sieved (< 2 mm) and stored in sterile bag and placed on ice when transported to laboratory. Subsamples for molecular analyses were stored at −20°C. Soil pH was determined in 1:5 soil water suspensions using an automatic glass pH electrode (Orion Star A215, Thermo Scientific, USA). EC (1:5) of soils was measured in saturated paste extracts using an EC meter (Orion Star A215, Thermo Scientific, USA). OM was measured by Tyurin titrimetric method (Heczko *et al.*, 2011). Available P_2O_5 was determined using the lancaster method where 5 g of sample was extracted with 20 mL 0.33 M CH_3CHOOH, 0.15 M lactic acid, 0.03 M NH_4F, 0.05 M $(NH_4)_2SO_4$, and 0.2 M NaOH at pH 4.25 and P_2O_5 content was measured as reported by Lee *et al.* (2002). The determination of CEC and exchangeable cations was performed: 50 mL of 3 M NH_4Ac solution at pH 7.0 react for 24 h with soils material (2.0 and 0.5 g using two repetitions for each mass) in batch experiments. After centrifugation, filtration, and decantation, additional 50 mL are added, the reaction time being 1 h. The procedure is repeated with 40 mL for 1 h as well as with 30 mL of deionized water for 45 min. The filtrates are combined for the determination of exchangeable cations, and the samples were analyzed by Perkin Elmer Optima 8300.

The mineral, total flavonoids contents, total dietary fiber, and moisture assessments of silkworm feed mulberry leaf

Cheongil leaf of the main silkworm feed mulberry cultivars was collected on 8 local areas (Jeonju, Buan, Jangseong, Cheongju, Gongju, Sanju, Jinju, and Chunchen) in Korea. To promote the development of the rich minerals and flavonoid content, total dietary fiber content in mulberry leaf, the mulberries were cultivated in open field. Also, they were irrigated once weekly for 2 h with 21 L water. The leaf samples were cleaned and dried in a lyophilizer. All the dried samples

Table 1. The soil properties of silkworm feed mulberry farm from 8 local areas in Korea.

Properties	Jeonju	Chuncheon	Cheongju	Gongju	Buan	Jangseong	Sangju	Jinju	Range of optimal
pH(1:5)	6.3	5.7	5.4	6.8	6.4	5.9	6.6	6.2	6.0-6.5
EC (dS/m)	0.40	0.19	0.30	0.32	0.43	0.40	0.38	0.76	-
Available P_2O_5 (mg/kg)	364	907	417	987	1924	970	420	1049	300-500
Organic Matter (%)	2.22	2.41	2.62	2.78	6.81	6.14	4.34	3.49	2.0-3.0
Exch. K ($cmol^+$/kg)	0.86	0.61	0.43	1.51	1.68	1.48	1.54	1.37	0.5-0.6
Exch. Ca ($cmol^+$/kg)	7.18	4.37	4.45	9.33	11.04	8.02	5.87	8.46	5.0-6.0
Exch. Mg ($cmol^+$/kg)	2.22	1.04	0.98	1.94	2.88	1.04	1.72	1.38	1.5-2.0
Exch. Na ($cmol^+$/kg)	0.04	0.04	0.05	0.07	0.05	0.06	0.06	0.07	-

*EC: Electrical conductivity; Exch.: Exchangeable cation.

were pulverized and stored below −18℃ prior to the proximate analysis of minerals. The minerals (nine types of inorganic elements) and total dietary were determined using the methods described by the Association of Official Analytical Chemists (AOAC, 1990), and were analyzed using an inductively coupled plasma optical emission spectrometer. All the proximate analyses were reported per 100 g of leaf. All data are expressed as mean ± standard deviation from three independent experiments. Of the test solution (standard or sample) for flavonoid content, 1 mL was mixed with 0.3 mL of $NaNO_2$ (5%, w/v) and after 5 min, 0.5 mL of $AlCl_3$ (2%, w/v) was added. Flavonoid standard solutions of 100 µM were used. A sample was mixed and 6 min later was neutralized with 0.5 mL of 1 M NaOH solution. The mixture was left for 10 min at room temperature and then subjected to spectral analysis in the range of 300–600 nm against the blank, where $AlCl_3$ solution was substituted by water. Catechin was the standard of choice for the expression of results at 510 nm.

Statistical analyses

Each experiment was repeated at least three times using soil samples of other sites. The one-way analysis of variance and Tukey's test at 95% confidence were utilized for statistical analyses.

Results and Discussion

Changes of properties and mineral compositions about 8 local mulberry farms in Korea

The chemical properties of each other areas are presented in Table 1. In results, there was no significant difference in the pH and EC, while the soil OM content was significantly high in Buan (6.81%) and Jangseong (6.14%) in Table 1. In contrast, available P_2O_5 was largely different each local area. The ranges of optimal about OM and available P_2O_5 content were 2.0-3.0% and 300-500 (mg/kg). The reason of high OM and different available P_2O_5 content in these areas was likely caused different fertilizing or compost treatments.

The nutritive value of mulberry leaf is influenced by the soil nutrient status (Bongale, 2006). Generally, the contents of the crop's functional components are influenced by cultivation conditions, such as the variety planted, period of cultivation, growth stages, environmental conditions, and fertilizers applied. Application of organic fertilizers to mulberry had a significant influence on cocoon yield, shell ratio, silk productivity and single cocoon filament length (Nasreen *et al.*, 1999; Sannappa *et al.*, 2005). Singheal *et al.* (1999) pointed that quality of mulberry leaf fed to silkworms is the most important factor that influences successful cocoon production. Also, the inorganic fertilizers have increased

Table 2. Mineral contents of Cheongil mulberry leaf from 8 local areas in Korea. (mg/100 g)

Local area	K	P	Ca	Mg	Na	Cu	Fe	Mn	Zn
Jeonju	2258.65±75.1^{b}	365.96±33.2^{b}	2285.02±82.1^{c}	302.61±15.3^{d}	18.96±3.5^{c}	0.15±0.0	8.78±0.5^{b}	5.2±0.3^{e}	2.01±0.3^{b}
Buan	2325.39±78.2^{b}	356.37±31.4^{b}	1941.93±65.3^{d}	490.67±19.2^{a}	20.12±3.7^{c}	0.06±0.0	5.71±0.5^{cd}	9.65±0.7^{c}	1.62±0.1^{c}
Jangseong	2590.08±82.7^{a}	463.66±32.5^{a}	1475.7±52.1^{g}	303.59d±11.2^{c}	40.68±3.6^{a}	0.05±0.0	6.5±0.4^{c}	6.35±0.5^{d}	3.2±0.4^{a}
Cheongju	2135.94±64.2^{c}	475.76±35.4^{a}	2267.28±64.7^{c}	342.33±14.3^{d}	21.23±2.5^{c}	0.28±0.0	12.08±1.5^{a}	9.28±0.9^{c}	2.77±0.3^{a}
Gongju	1947.74±58.1^{d}	480.09±34.2^{a}	2805.13±58.6^{a}	315.66±10.1^{b}	46.78±3.9^{a}	0.25±0.0	10.91±1.2^{a}	3.94±0.2^{f}	1.45±0.2^{c}
Sangju	2685.91±81.4^{a}	458.71±31.2^{a}	1580.16±48.5^{f}	394.74±18.2^{e}	15.16±1.5^{d}	0.03±0.0	6.67±0.4^{c}	11.78±1.1^{b}	2.25±0.2^{b}
Jinju	2208.2±75.7^{bc}	435.17±27.5^{a}	1703.38±59.1^{e}	270.51±9.1^{e}	36.32±3.9^{b}	0.08±0.0	9.62±1.2^{ab}	4.01±0.3^{f}	1.54±0.1^{c}
Chuncheon	1884.17±60.2^{d}	318.77±21.4^{b}	2582.17±54.5^{b}	284.39±9.2^{e}	21.04±2.3^{c}	0.28±0.0	5.58±0.3^{d}	24.52±2.5^{a}	3.28±0.3^{a}

*Each value calculated as means ± SD of three replicates. Scores with different letters are significantly different based on the analysis of variance ($P<0.05$)

mulberry yield leading to better silk worm productivity (Bose and Majumder, 1999). Constantinides and Fownes (1994) stated that the 1-deoxynojirimycin contents were different depending on the cultivation location. Mudau *et al.* (2006) reported that the polyphenolic content of bush tea increased after nitrogen applications, but Stewart *et al.* (2001) reported that the flavonol levels of plant tissues were higher after lower nitrogen applications. Previous study reported that the catechin content of tea plants increased with decreasing amounts of applied nitrogen (Matsunaga *et al.*, 2009). It has been realized that, in the past, this was achieved by the expense of soil health, moreover, some portions of the nutrients applied to the soil are still bound to be unused as they are not available to the plant. In the result of CEC difference, the CEC Ca^{2+} was significantly different in all area. Buan and Sangju are the areas where available phosphate and organic matter fall within the appropriate range, and in other areas, appropriate fertilizer and compost should be required. The CEC of a soil is a measure of the quantity of negatively charged sites on soil surfaces that can retain positively charged ions (cations) such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}), by electrostatic forces (Mahboubi *et al.*, 1993). If the effective phosphate and exchangeable calcium are excessive, the growth of stems and leaves is delayed, resulting in poor fruit and poor quality. Therefore, it is necessary to monitor soil composition analysis every year with an appropriate amount of fertilizer. CEC is a good indicator of soil quality and productivity. Cation exchange sites are found primarily on clay minerals and OM surfaces, and the soil OM will develop a greater CEC at near-neutral pH than under acidic conditions (pH-dependent CEC) (Karlen *et al.*, 1994). Thus, addition of an organic material will likely increase a soil's CEC overtime. On the other hand, a soil's CEC can decrease with time as well, through e.g. natural or fertilizer-induced acidification and/or OM decomposition. However, it should be carefully supervised to avoid the temptation to over-fertilizing. Over-fertilizing depletes the root zone of much-needed oxygen, thus reducing both root growth and nutrient uptake and leading to a host of potential root disease problems.

The mineral, total flavonoids contents, total dietary fiber, and moisture assessments from Cheongil mulberry leaf

The knowledge of the levels of trace elements in mulberry leaves is necessary because of their benefits for silkworm raising and productivity. Mulberry as a good source of minerals, could provide nutritionally useful amounts of most of them including K, Ca, Na, Mg, Cu, Fe, P, Mn, and Zn in Table 2. All the selected essential elements were detected from Cheongil leaf sample of the 8 local areas. As can be seen in Table 2, K and Ca was predominant element in Cheongil mulberry leaf of the 8 local areas. One of the local areas, the K of Sangju cheongil leaf was the highest on 2685 mg. Among the macrominerals (K, Ca, Na, and Mg), the concentration of K was varied from (1884±9.36) mg/100 g to (2685±11.5) mg/100 g. The mineral compositions of mulberry leaves (*M. alba*, *M. rubra*, and *M. nigra*) grown in Turkey were: K [1141 (834–1668) mg/100 g], Ca [139 (132–152) mg/100 g], Mg [109 (106–115) mg/100 g], Na [60 (59–61) mg/100 g], Fe [4.3 (4.2–4.5) mg/100 g], and Zn [3.1 (2.8–3.2) mg/100 g] (Ercisli and Orhan, 2007). Okwu (2005) even reported much lower amounts of these elements in the studied plants. The present study showed a higher mineral profile as compared with previously reported data (Ercisli and Orhan, 2007; Okwu, 2005). The mineral composition of mulberry not only depended on the species or cultivars, but also on the growing conditions such as soil, climatic, geographical conditions, the addition of fertilizers, and cultural management techniques (Ercisli, 2009). The lack of phosphate causes the leaves to change color to dark green or greenish purple, and the protein content decreases, causing the silkworm's quality to deteriorate. The Na content was low and the K/Na ratio was relatively high, which has been considered to be an advantage from the nutritional point of view, and calcium, the second most common factor in mulberry leaves, is a factor that helps silkworm to grow well by increasing consumption rate (Ruth *et al.*, 2019). The silkworm reared on mulberry leaf of high amount of calcium and potassium had significant level of fibroin protein (Laity *et al.*, 2019; Ruth *et al.*, 2019). Thus, existence of sufficient quantities of essential minerals in the studied leaves may act as better supplements of these elements by including them in daily diet, allowing to easily meet a reasonable amount of the daily requirements.

Table 3 shows total flavonoids, total dietary fiber contents, and moisture of the Cheongil leaf samples in the 8 local areas. The reason of the investigation is these contents affect to silkworm weight, functional silkworm product, and mulberry growth. Total dietary fiber can affect the weight of silkworms, and mulberry leaf moisture is related to mulberry growth. As shown in Table 4, there was no significant difference. The total flavonoids of produced mulberry leaves in Sangju was the highest on 3004.33 mg. The phenolic content of mulberry leaves also varied with cultivars and harvesting time. For example, mulberry leaves collected in May are considered to be good sources of phenolic compounds (Lee and Choi, 2012; Zou *et al.*, 2012). High total flavonoid content indirectly shows that mulberry leaves are valuable as human foods, but the silkworms fed with high quercetin which is major one of the flavonoid in plants had low growth performance and survival rates (Shi *et al.*, 2020; Zhang *et al.*, 2012). For rearing silkworm, therefore, monitoring of flavonoid level should be required. Total dietary fiber ranged from 29.91 (Jinju) to 42.11 (Gongju). In the case of moisture, the leaves of Jinju were the highest at 8.78% and those of Chuncheon were the lowest at 4.98%. Even the same variety Cheongil mulberry leaf is considered to show a difference in fiber and moisture of the leaf because it is greatly affected by growth conditions such as the amount of light and soil composition depending on the region.

Table 3. Functional components of Cheongil mulberry leaf from 8 local areas in Korea.

Local area	Total dietary fiber (%)	Total flavonoid content (mg/100 g)	Moisture (%)
Jeonju	41.00	1170.45±55.3e	6.19
Buan	37.15	1688.22±62.1d	5.40
Jangseong	38.30	2397.47±72.3b	6.04
Cheongju	33.13	2320.58±71.5b	6.70
Gongju	42.11	2168.47±65.8c	5.48
Sangju	39.83	3004.33±82.1a	5.55
Jinju	29.91	2245.94±59.1b	8.78
Chuncheon	36.55	2258.45±58.2b	4.98

*Each value calculated as means ± SD of three replicates. Scores with different letters are significantly different based on the analysis of variance ($P<0.05$)

In this study, we managed to difference the properties of the local areas' mulberry farms in Korea. These variations might be due to the growth conditions and geographical variations. The amounts of minerals, and flavonoids content in Cheongil mulberry leaf were different in 8 local areas. The amount of phosphate contained in the soil varied greatly depending on the region. Howerver, the phosphate content of the Cheongil leaves collected there did not show as much difference as the soil composition. This means that the excess phosphate component of the soil does not directly affect the nutritional component of mulberry leaves, and mulberry trees are able to absorb and use the component properly. In addition, there was a big difference in the total amount of flavonoids depending on the region. In the case of flavonoids, it has been announced that there is a possibility of inhibiting the growth of silkworm, but the standard, which negatively affect silkworm growth, for the amount of flavonoids consumed through mulberry leaves are still unclear. This will need to be identified through further research. In conclusion, a standard of soil condition and nutrient element contents should be established for sericulture farmers in Korea. This study provides a possible industrial use of mulberry, and holds promise to enhance the overall profitability of sericulture in Korea local areas.

Acknowledgment

This study was supported by the Department of Agricultural Biology, National Academy of Agricultural Science of the Rural Development Administration (project: PJ01486701).

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