academicresearchJournals

Vol. 8(5), pp. 453-460, June 2020
DOI: 10.14662/ARJASR2020.325

ISSN: 2360-7874
http://www.academicresearchjournals.org/ARJASR/Index.htm

Academic Research Journal of Agricultural Science and Research

Review paper

Effect of micro nutrient composition of leaves of mulberry (*Morus indica*) varieties on growth and cocoon yield of bivoltine mulberry silkworms (*Bombyx mori* L.)

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Accepted 15 June 2020

The growth, yield and reproduction potential of silkworms can be affected by their feed sources to a great extent. Micronutrients are among important biochemical components of leaves and they may have very big influence in productivity of silkworms. In the present study, six different mulberry varieties (K-2, S-13, M-4, Nekemte coll, Jimma Coll and Local) were evaluated for their leaf composition and effect on mulberry silkworms (*Bombyx mori L.)* in Melkassa Agricultural Research Center (MARC), East-Shewa zone of Oromia Regional State, Ethiopia. Completely Randomized Design (CRD) with three replications was employed for carrying out feeding trial in a laboratory. The leaf micro-nutrient composition of mulberry varieties for sodium, copper, iron, zinc, manganese and molybdenum was determined by using appropriate methodologies. The relationship of micronutrient components of leaves with silkworm parameters was also determined by using correlation coefficient analysis. Overall data analysis was carried out by using SAS software at 5% probability level. Finally, the leaves of these varieties showed significant differences among themselves in their micronutrient compositions. Their effect on rearing performance of mulberry silkworms was also statistically different when they were fed. Generally, relationship of micronutrient constituents of mulberry varieties with rearing performance of mulberry silkworms showed positive correlation with manganese, molybdenum and zinc contents with larval, cocoon and grainage parameters of mulberry silkworms. However, iron content was found to affect these silkworm parameters negatively. Therefore, it can be concluded that among micro-minerals in mulberry leaves, the presence of higher managanese, molybdenum and zinc contents and lesser iron content will be helpful to the gain better mulberry silkworm productivity. As a result, these micronutrients will be considered as basic parameters for evaluation of mulberry varieties for mulberry silkworms rearing in the future.

Key words: Mulberry silkworms, Bombyx mori, Micro nutrient, Correlation

Cite this article as: Kedir S., Metasebia T., Ahmed I., Abiy T., Yohannis N (2020). Effect of micro nutrient composition of leaves of mulberry (Morus indica) varieties on growth and cocoon yield of bivoltine mulberry silkworms (*Bombyx mori* L.). Acad. Res. J. Agri. Sci. Res. 8(5): 453-460

INTRODUCTION

Silk is a functional term used to describe protein fibers that are produced by arthropods. It is a natural protein fiber and is very soft, lustrous, smooth, strong and durable than any natural or artificial fibre (Lamelu, 1998; Hiwar, 2001 and Craig and Riekel, 2002). The industrial use of silk and its economic importance of production finely contributed to the silkworm promotion all over the world (Ramesh-Babu et al., 2010).

Tzenov (2007) indicated that there is a good potential for sericulture development, not only in the East Asia, but also in Eastern Europe, Central Asia, Latin America and the Africa Region based up on overall socio-economic and agro-climatic conditions (rainfall, soil, temperature, humidity, light and air). Hence, the developing countries are directing their development strategies on the productivity levels in rural areas (Hajare *et al*., 2007). In Ethiopia, silk has played an important part in the social and religious life of Ethiopia from the earliest days of its history. This silk was imported in large quantities from India, Arabia and China and stored in vast caverns in the central highlands of Ethiopia and Ethiopian Emperors would make prodigious gifts of silk to recognized organizations and special guests. Ceremonial umbrellas, binding of sacred books, covers for wooden altars and spectacular hangings have all been produced from silk over the centuries (Spring and Hudson, 2002). Recently, silk production from mulberry silkworm (*S. c. ricini*) is practiced in Ethiopia (Metaferia *et al*., 2007).

The mulberry-silkworm*, Bombyx mori* L., is the most exploited and commercialized silkworm. It is a sericigenous insect exploited for its valuable mulberry silk. It could be classified as multivoltine, bivoltine or univoltine based on generations per year. However, it is a monophagus species feeding only on mulberry plant and it can be reared throughout the year depending on the availability this feed plant. The agro-ecology and feed availability are the major requirements which have significant effect on rearing of larvae of this insect and finally cocoon crop yield and quality (Debaraj et al., 2003 and Thangavelu and Phukon, 1983).

Mulberry (*Morus spp*.) can ensure production of good quality cocoons. However, mulberry shows a wide range of variability in nature. It has been well recognized that morphological features and nutritive values of the leaves differ significantly from variety to variety and over locations. The morphological traits and nutritional factors could be used in selection of varieties (Solanki and Joshi, 2001). In addition, the quality of feeds given to insects generally affects the economic products of insects. So, silkworm nutrition is one and the major factor which affect development and productivity of silkworms (Milner, 2004 and Baruah and Baruah, 2007). Silkworm nutrition deals about the substances required by silkworm for its growth and metabolic functions and obtained from ingested food and some other nutritional components synthesized through various biochemical pathways including proteinous silk fiber of commercial interest (Takano and Arai, 1978 and Hamano *et al*., 1986).

Silkworms have adapted to feeding to derive and store adequate energy, nutrients and water from the food they consume. Feeding silkworms provides energy for growth, development, reproduction and many of its other needs. Such nutritional requirements in food consumption have direct impact on the overall genetic traits such as larval and cocoon weight, amount of silk production, pupation and reproductive traits. Thus, successful sericulture depends on increased production of leaves with high nutritive values. Therefore, the better the quality of leaves, the greater the chances of getting good cocoon harvest (Krishnaswami, 1978).

Moreover, a significant correlation has been reported between chemical compositions of mulberry leaf and larval growth and cocoon characters. Nutritional value of food plants either alone or in combination plays an important role in the larval growth and silk productivity. Improvement in larval and cocoon characters has also been witnessed with increase in chemical composition of leaves (Sannappa and Jayaramaiah, 1999).

Therefore, feed stuffs can be analyzed in to different fractions including the ash (minerals). The ash, residue left behind after all organic matter has been burnt off, is a measure of the total content of mineral salts in a food. Minerals including micronutrients constitute very good fraction of silkworm feeds. The role of inorganic salts (minerals) is variable. It has been reported that the silkworm larval structure at different ages utilizes these absorbed minerals (Ito, 1978 and Friend, 1958). Their role in the egg stage is also recognized to regulate the hydrogen ion concentration osmotic pressure. Furthermore, sodium and potassium ions act on protein synthesis. However, the importance of nitrogen has been significantly considered in different stages together with the protein content (Legay, 1958). Among micro-minerals, sodium, iron, silicon, mangnesium and copper found orderly on the quantity basis from different feeds of silkworms (Fukuda *et al*. 1960; Maynard and Loosli, 1962 and Sannappa and Jayaramaiah, 1999). In another way, Jayaramaiah and Sannappa (1998) find out a positive significant relationship between foliar constituents and important silkworm components. They indicated that larval duration, weight, survivability and effective rate of rearing (ERR) as well as cocoon yield were found to have significant positive relationship with nitrogen, phosphorus, potassium, calcium, magnesium and sulphur.

Therefore, selection of mulberry varieties inconsideration with foliar micronutrients will be an important criterion for better growth and development of mulberry-silkworm (Joshi and Misra, 1982). However, very little information is available in Ethiopia. This work, therefore, attempts to assess the extent of micro nutrient

composition of some mulberry varieties and find out the relationship with growth and yield of mulberry silkworm, *Bombyx mori*.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Melkassa Agricultural Research Center (MARC). It is found 117 kms away from Addis Ababa and 17 km to southeast of Adama in the East-Shewa zone of Oromia region, Ethiopia. It is located 8°24'N latitude and 39°12'E longitude having an elevation of 1500 meters above sea level and a mean annual rainfall of 770 mm.

Foliar nutritional evaluation of mulberry varieties

Six selected varieties of mulberry viz. K-2, S-13, M-4, Nekemte coll, Jimma Coll and Local (check) were used in the study. These materials were selected from preliminary research observations. The methods of Sannappa and Jayaramaiah (1999) were adopted for foliar nutritional analysis on the above mentioned mulberry varieties. The leaf samples were collected after fifteen months of planting at three different heights of the plant viz. top, middle and bottom leaves in paper bags. They were shade dried and then transferred to hot air oven maintained at $70^{O}C$ until constant weight was obtained. Then, the dried-up leaf samples were grinded in to fine powder and preserved for chemical analysis.

By adopting the techniques and procedures, analysis of mineral compositions for sodium, copper, iron, zinc, manganese and molybdenum contents were carried out for each mulberry variety. To get the estimates, dry ashing (Total Minerals) and Sample preparation were done at Melkassa Food science Laboratory, Melkassa, Ethiopia. Minerals quantification reading using ICP-OES were done at Horticoop Ethiopia (Horticultural) Laboratory, Bishoftu, Ethiopia. During analysis of aqueous solutions by ICP-OES with radial plasma observation, all measurements were performed with spectro arcos optical emission spectrometer optimized with small volume and 32 linear CCD detectors the wave length range between 130-770 nm simultaneously analyze, the sample is nebulizer in to the argon filed plasma and the energy excitement is processed in the single spectra and the energy is expressed in intensity which is directly proportion to concentration, the concentration is calculated on the linear graph of the standard concentration and the corresponding intensities (Haynes, 1980).

Evaluation of mulberry varieties on rearing performance of mulberry silkworms

The above mentioned mulberry varieties were used as feed source of mulberry silkworms in a laboratory. As per the rearing recommendations of silkworms by Dayashankar (1982), a bivoltine mulberry-silkworm breed was used for this experiment and it was reared on the six mulberry varieties. The silkworm rearing room and equipments were cleaned, washed and disinfected with 2% formalin solution at the rate of 800ml per $10m^2$ before the commencement of the experiment (rearing). This breed was reared following cellular rearing techniques starting from brushing till cocoon spinning on the six varieties. Tender leaves of mulberry were fed four times a day until the larvae ends II instar stage, and semi tender leaves to III instar while more matured leaves were fed to IV and V instar larvae. The experiment was arranged in Completely Randomized Design (CRD) in three replications. In each replication, 100 worms were used and allowed to complete the larval period. As used by Neupane *et al*. (1990), Singh and Benchamin (2002) and Ramesha *et al.* (2010) matured worms were picked and mounted on the mountages for spinning. On the six day of spinning, the cocoons were harvested, counted and weighed.

Rearing parameters like larval duration (in hours), body weight of mature larvae (g), percentage of larval mortality and hatchability, cocoon traits (cocoon weight, shell weight and silk ratio), effective rate of rearing (%) and fecundity (number of eggs per female) were recorded to find out rearing performance of the worms on the mulberry varieties. As a result the following formulae were adopted for estimation when appropriate.

The following formulae, which were adopted by Singh and Benchamin (2002) used for data on rearing performance:-

$$\text{Hatching percent} = \frac{(\text{Number of normal eggs - number of non hatched eggs}) * 100}{\text{Number of normal eggs}}$$

$$\text{Effective rate of rearing (ERR)} = \frac{\text{(No. of larvae spinning cocoon * 100)}}{\text{No. of larvae brushed}}$$

Data Analysis

Finally, the data for significance determination were analyzed statistically (one-way CRD) by using SAS software at 5% level of significance. Moreover, correlation coefficients of minerals of leaves of the mulberry varieties with their corresponding rearing performance and cocoon traits of silkworms was carried out to know their inter-relationship similarly by SAS software at 5% level of significance (SAS Institute Inc,1999-2000) .

RESULTS

Substantial variability has been obtained in mineral composition of mulberry varieties and these differences also caused to variability in rearing performance of mulberry silkworms when served as feed sources.

Micro nutrient composition of different mulberry varieties

All mulberry varieties differed significantly from one another in respect to micronutrients (sodium, copper, iron, zinc, manganese and molybdenum) considered in this study. Mean values of foliar compositions of the varieties are presented in table 1.

A. Sodium

The sodium concentration has also shown significance difference among mulberry varieties. The minimum concentration was obtained in K-2 (78.65 mg/kg) while the maximum concentration was in local check (172.533 mg/kg).

B. Copper

Copper concentration has also shown significant difference among mulberry varieties. The lowest record was from K-2 (1.34 mg/kg). However, the highest was obtained from local check (2.68 mg/kg) followed by S-13 (2.41333 mg/kg).

C. Iron

The highest iron concentration of mulberry varieties was obtained from Nekemte coll (293.77 mg/kg) followed by local check (288.07 mg/kg). However, the lowest records were obtained from K-2 (201.503 mg/kg).

D. Zinc

The lowest zinc concentration of leaves of mulberry varieties was recorded from Nekemte coll (6.66333 mg/kg) while the highest was from M-4 (10.63667 mg/kg) followed by S-13 (10.14333 mg/kg).

E. Manganese

The manganese concentration of varieties of mulberry has also depicted significant variability, which shows lowest in K-2 (32.3767 mg/kg) and highest in S-13 (53.4167 mg/kg).

F. Molybdenum

The concentration of molybdenum in leaves of mulberry has also witnessed significant variability among varieties, ranging from 0.325 mg/kg in Jimma coll to 1.62867 mg/kg in S-13.

Table 1: - Micronutrient composition of different mulberry varieties

Mulberry varieties	Na (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Mo (mg/kg)
Nekemte coll	112.363e	1.97333c	293.770a	6.66333f	34.5667e	0.39667d
Jimma coll	122.783d	1.65000d	281.033c	7.14667e	40.1533c	0.32500e
M-4	136.273c	1.98667c	259.517d	10.63667a	52.5500b	0.98417b
K-2	78.650f	1.34000e	201.503f	8.19000d	32.3767f	0.62733c
S-13	144.753b	2.41333b	231.720e	10.14333b	53.4167a	1.62867a
Local	172.533a	2.68000a	288.070b	9.62333c	35.6567d	0.44223d
SE	7.03490178	0.10816897	8.05378062	0.36390236	2.05751659	0.11052111
CV (%)	1.143261	0.019579	0.996144	0.473622	0.803212	4.972816
Pr	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Note: - Means with the same letter are not significantly different at Pr > 0.05.

Effect of different mulberry varieties on rearing performance

In this experiment, all the characters of mulberry silkworm rearing like hatching percentage, larval weight, single cocoon weight, single shell weight, fecundity and effective rate of rearing (ERR) showed significant differences when mulberry silkworm fed with different mulberry varieties. The results of silkworm rearing characters fed on different mulberry accessions are summarized in the table 3.

A. Fecundity

Silkworm fed on S-13 showed significantly higher fecundity (305.467) followed by M-4 (289.867). The local check (259.533) was lower performing compared to the rest mulberry varieties for fecundity of mulberry silkworms.

B. Percentage hatching of eggs

Hatching rate of silkworm eggs in to larva showed significant variation when a silkworm fed on different mulberry varieties ranging from 62.02 % to 75.33 %. The maximum hatching was recorded in M-4 (75.33 %) followed by K-2 (70.7033 %) and S-13 (70.00 %) (see Table 1).

C. Larval weight

Matured worms fed on S-13 recorded significantly higher larval weight (2.5303 g) and 8.17g respectively. However, the least larval weight was obtained from worms fed on local check (2.00633 g).

D. Effective rate of rearing (ERR)

ERR has also revealed a significant difference when mulberry silkworm fed on different mulberry varieties. Mulberry silkworm fed on S-13 (81.50 %) recorded higher ERR closely followed by M-4 (77.0 %) and Jimma (74.02 %). The least ERR was obtained from local check (68.833 %) (see Table 1).

E. Cocoon weight and shell weight

In accordance to yield, maximum single cocoon weight was recorded in those larvae which were fed on the leaves of S-13 (1.11167 g). The minimum single cocoon weight (0.86567 g) was recorded from larvae fed on local check. Similarly, single shell weight of a cocoon revealed significant variation when fed with the different mulberry accession. S-13 revealed significantly higher shell weight (0.187 g) but local check showed the least shell weight (0.136 g).

Table 2: - Effects of mulberry varieties on the performance of a bivoltine mulberry silkworms

Treatment	Fecundity (%)	Hatching percent	Larval weight (gram)	ERR (%)	Cocoon weight (gram)	Shell weight (gram)
K2	279.933c	70.7033b	2.17700b	75.56667c	1.03000b	0.16833ab
S13	305.467a	70.0000b	2.53033a	81.50000a	1.11167a	0.18700a
M4	289.867b	75.3300a	2.17100b	77.00000b	1.01333b	0.16333ab
Nekemte	270.533c	65.4300c	2.19200b	73.67000e	1.00133	0.15867bc
Jimma	278.200c	62.0200c	2.23300b	74.02000d	1.00600b	0.16133bc
Local	259.533d	65.0000c	2.00633c	68.83333f	0.86567c	0.13600c
SE	3.67579869	1.07154353	0.04213927	0.92527588	0.01868696	0.00461825
CV (%)	1.973116	0.637592	4.154814	0.157055	3.194023	8.825915
Pr	<.0001	<.0001	0.0005	<.0001	<.0001	0.0238

Note: - Means followed by the same letter within a column are not significantly different from each other at 5% level of probability (LSD mean comparison test)

Relationship of micronutrients of leaves of mulberry varieties with mulberry silkworm rearing performance

The foliar mineral composition of mulberry varieties resulted in significantly important inter-relationship with performance of mulberry silkworms when their leaves served as feeds. This relationship between leaf composition values and important mulberry silkworm traits have been worked out through correlation analysis and the results are summarized in table 3.

Sodium and Copper
Sodium and copper composition of mulberry leaves had shown no significant correlation with the studied silkworm parameter.

Iron
Iron had showed a significantly negative correlation with most of the studied parameters including egg fecundity (r=-0.54971) and hatchability (r=-0.58335), larval effective rate of rearing (r=-0.59971), cocoon weight (r=-0.55984) and shell weight (r=-0.49580).

Zinc
Zinc showed a significant positive correlation only with egg hatchability (r=0.68995). It had no significant correlation with other silkworm parameters.

Manganese and Molybdenum
Manganese and molybdenum showed a positive significant correlation with almost all studied parameters of the silkworm. Manganese was positively correlated with egg fecundity (r=0.77777), hatchability (r=0.55023), larval weight (r=0.56392), effective rate of rearing (r=0.72625) and cocoon weight (r=0.50028). Similarly, molybdenum was positively correlated with egg fecundity (r=0.84504), hatchability (r=0.62311), larval weight (r=0.56392), effective rate of rearing (r=0.85376), cocoon weight (r=0.65160) and shell weight (r=0.57690).

Table 3: - Relationship of micronutrients with mulberry silkworm rearing parameters

Minerals	Silkworm traits					
	Fecundity	Egg hatchability	Larval weight	Effective rate of rearing	Cocoon weight	Shell weight
Na	-0.09065	-0.1312	-0.06613	-0.2274	-0.41726	-0.28029
Cu	-0.06199	-0.07823	0.02245	-0.14981	-0.32754	-0.21233
Fe	-0.54971*	-0.58335*	-0.36992	-0.59971*	-0.55984*	-0.49580*
Zn	0.43365	0.68995*	0.13967	0.33452	0.04971	0.11432
Mn	0.77777*	0.55023*	0.56392*	0.72625*	0.50028*	0.45255
Mo	0.84504*	0.62311*	0.69790*	0.85376*	0.65160*	0.57690*

* Significant at Pr $\leq$ 0.05

DISCUSSION AND CONCLUSION

It can be realized from the results of the present investigation that mulberry varieties (K-2, S-13, M-4, Nekemte coll, Jimma Coll and local check) showed wide variation in their qualitative traits with respect to minerals from such studies of the first type in Ethiopia. Consequently, since accumulation of nutrients in insect greatly influenced by the nutritional richness of the host plants, the micronutrient compositions of leaves (namely sodium, copper, iron, zinc, manganese and molybdenum) of mulberry varieties have been studied and they revealed very significant differences among mulberry varieties. Similarly, studies on different mulberry varieties have been conducted in abroad by several authors and they have found out significant differences among different varieties (Jayaramaiah and Sannappa, 1998; Scriber and Slansky, 1981).

Furthermore, the inter-relationship of performance of mulberry silkworms and the micro-nutrient composition of leaves of mulberry varieties have been analyzed. The results suggest that different traits of mulberry silkworm have significantly different relationship with various biochemical constituents of mulberry leaves. As a result, cocoon traits such as cocoon weight and shell weight found to have significant positive relationship with manganese and molybdenum but negatively correlated with iron composition. In addition, larval effective rate of rearing showed a significant positive correlation or interaction with manganese and molybdenum. Moreover, grainage parameters like egg fecundity and hatching showed significant positive correlation with manganese, molybdenum and zinc composition of mulberry leaves. Therefore, the significant positive relationship obtained from foliar mineral composition (manganese, molybdenum and zinc contents) and silkworm performance indicators was found to be very important for consideration of mulberry varieties as obtained

Jayaramaiah and Sannappa (1998) and Murthy *et al* (2013). However, sodium and copper composition of mulberry had been found not to affect the studied parameters of silkworms but iron composition of the leaves was negatively interacted with the silkworm growth and development. It could be because the varieties have contained enough or higher composition of sodium, copper and iron compared to the needs of silkworms and so they will be interfering with the roles of other important nutrients. As a result, it can be concluded that among micronutrients studied, the presence of higher managanese, molybdenum and zinc contents and lesser iron content will be helpful to gain better mulberry silkworm growth and yield. As a result, these micronutrients will be considered as basic parameters for evaluation of mulberry varieties for mulberry silkworms rearing in the future.

Hence, the present study reveals that the selection of mulberry varieties for rearing mulberry silkworm inconsideration with foliar micro nutrient constituents of the mulberry varieties as well as rearing performance is very important in order to get improvements in larval development, cocoon production and grainage parameters. However, more research should also be carried out to support the current findings in consideration with varying period of leaf picking and nutrient analysis, leaf proximate composition, field performances of mulberry varieties and in relation feeding efficiency of mulberry silkworms when using these varieties as feed sources.

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