Screening of mulberry varieties for larval growth, cocoon yield, and quality through silkworm rearing

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Abstract

Mulberry (Morus spp.) production is a vital activity in sericulture along with rearing of silkworms (Bombyx mori L.) for silk production. The Sericulture Research and Development Institute (SRDI) conducted a study that evaluated 11 mulberry varieties, including nine newly-evolved mulberry hybrids and two check varieties. These mulberry varieties were fed to silkworm hybrid, DMMMSU 115 x 100. The rearing performance of DMMMSU 115 x 100 was determined based on the silkworm economic traits such as weight of 10 matured larvae, cocoon weight, shell weight, shell percentage, cocoon yield, reelability percentage, filament length and filament size. Six silkworm rearing trials were conducted with 50 silkworm larvae per treatment in three replicates. The data were analyzed in a one-way Analysis of Variance. Significant differences were tested further using Tukey's Honest Significant Difference. Mulberry varieties that garnered an evaluation index value of higher than 50 were identified and selected. Results of the study revealed significant variations among the mulberry varieties based on the parameters gathered except on the reelability of cocoons, filament length and size. Matured silkworm larvae fed with Alf 55 were significantly heavier and higher cocoon yield compared to those fed with Alfonso and S₅₄. Alf 32, Alf 55, Alf 58, Bat 37 and Bat 73 were identified as potential varieties and highly recommended as feed for the silkworm hybrid, DMMMSU 115 x DMMMSU 100.

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Introduction

Mulberry varieties differ in various characters and the impact of a particular mulberry variety can be evaluated based on the growth and development, survival, and finally the produced cocoons which directly determines the silk productivity which cocoon is an important commercial and economic product of silkworm rearing (Alipanah, 2020).

Silkworms convert the protein content of the mulberry leaf

into silk (Seshagiri *et al.*, 2009) by consuming about 20 grams of mulberry leaf throughout its larval life and about 90% of the total leaf requirements are consumed by the fourth and the fifth instar larvae (Sajgotra *et al.*, 2018) and when fed with quality leaves produces quality cocoons (Raja and Kumar, 2016), which is why evaluation of the different mulberry varieties for rearing performance is imperative (Yogananda-Murthy *et al.*, 2013a). Previous evaluation on the screening of the different mulberry varieties through silkworm rearing

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made use of the Batac variety as control variety. It was the first local variety used by sericulture farmers with an average cocoon yield of about 30 kgs/20,000 worms reared. The evaluation registered an average cocoon yield of 31 kgs/20,000 worms reared when silkworms were fed with Alfonso to increase cocoon yield, so continuous evolvement of high-yielding mulberry varieties should be done through rearing performance (Dandin and Giridhar, 2014) and further select and identify promising varieties. Thus, the present study focused on the evaluation of the 11 mulberry varieties through silkworm rearing based on their economic traits, i.e. (a) weight of ten matured larvae; (b) weight of single cocoon; (c) weight of single shell; (d) cocoon shell percentage; (e) cocoon yield; (f) filament length; (g) filament size; and (h) reelability.

Materials and Methods

Materials and Procedures

Mulberry Farm Establishment

The varieties were planted in 100 m² area at the experimental farm of the Sericulture Research and Development Institute (SRDI), Bacnotan, La Union, Philippines. This was equally divided into three equal blocks, and further subdivided into 11 equal plots in Randomized Complete Block Design (RCBD). Each block was planted with the nine mulberry hybrids and two varieties spaced at 1 m between rows and 50 cm between plants. Package of Technology (POT) for mulberry production was uniformly followed.

Silkworm Rearing

Eleven layings of silkworm hybrid, DMMMSU 115 x 100 were incubated for 12 days at an optimum room temperature of 24°C and 80% relative humidity. Brushing of hatched larvae followed when 90% of the eggs hatched in each laying. Employing the silkworm rearing standards, newlyhatched larvae were transferred into a rearing tray lined with paraffin paper with the aid of a disinfected white chicken feather. Larvae were fed four times a day (5 AM, 10 AM, 3 PM and 8 PM) according to recommended leaf age and weight requirements of silkworms until maturity. Ten matured and translucent larvae were randomly selected from each treatment, weighed, then placed on a cocooning frame. Mounting was done until all the worms were matured. Six (6) days from mounting, cocoons were harvested and assessed based on the economic traits of silkworm.

Experimental Design and Data Gathered

The treatments were laid out in a Randomized Complete Block Design (RCBD) in three replications: (a) Weight of the ten matured larvae (g) was gathered by randomly selecting 10 matured larvae from each treatment in each replications; (b) weight of single cocoon (g) was determined by getting the average of randomly weighed 10 cocoons from each treatment in each replications; (c) weight of single shell (g) was determined by getting the average of randomly weighed 10 single cocoon; (d) cocoon shell percentage (%) was determined by getting the average of randomly weighed cut cocoon (pupa and cocoon shell) using the formula: weight of 10 shells/ weight of 10 cocoons x 100%; (e) cocoon yield (kg/box) was determined using the formula: survival percentage x weight of single cocoon (g) x 20,000 / 1000 g; where box refers to 20,000 larvae and survival percentage refers to the total number of silkworm larvae survived / total number of silkworm larvae reared; (f) filament length (m) was determined using the formula: R x 1.125; where R is the number of revolutions recorded by epprouvette (1.125 m circumference); (g) filament size (denier) was determined using the formula: weight of raw silk x 9000/ length of silk filament; and (g) reelability percentage (%) was determined by getting the ratio of the cocoons reeled without break and the total number of cocoons casted.

Data were analyzed adopting the method of one-way analysis of variance using the Statistical Tool for Agricultural Research (STAR). Tukey's Honest Significant Difference (HSD) was used for further testing of significance.

Selection of the Best Mulberry Hybrids

The best mulberry varieties were selected based on the evaluation index method used by Mano *et al.* (1993), as done by Tikader and Kamble (2007). Mulberry varieties with a cumulative evaluation index (E. I.) value of 50 and above were selected and derived with the formula: Evaluation Index (E.I.) = $A - B/C \ge 10 + 50$, where: A = value obtained for a trait in a breed; B = overall mean of the particular trait (experimental mean); C = standard deviation; and 10 = standard and 50 = constant.

Results

Significant Findings

Weight of ten matured larvae

Comparable weights of 10 matured silkworm larvae were observed on silkworms fed with Alf 32, Alf 58, Alf 65, Alf 74, Bat 37, and Bat 73 with means of 33.45, 32.71, 32.70, 31.91, 33.23, and 32.65 g, respectively, as compared to those fed with the check variety, Alfonso (32.71 g). Significantly lighter matured larvae were recorded in silkworms fed with Alf 23 (30.28 g) and Alf 47 (31.19g) compared to the check variety. However, significantly heavier matured larvae were recorded in silkworm fed with Alf 55 (34.92g) compared to the check variety. Silkworms fed with the check variety S54 recorded a mean weight of 31.62 g did not differ significantly with Alf 47, Alf 58, Alf 65, Alf 74, and Bat 73 with respective mean weight of 31.19, 32.71, 32.70, 31.91 and 32.65 g. However, silkworms fed with Alf 32, Alf 55, and Bat 37 have recorded significantly heavier matured larvae with respective means of 33.45, 34.92 and 33.23 g than S54 (31.62 g) while silkworms fed with Alf 23 recorded a significantly lower weight (30.28 g) as compared with the check variety, S54.

Weight of single cocoon

No significant difference was observed on the weight of single cocoon produced by silkworms fed with Alf 32 (1.71 g), Alf 55 (1.73 g) Alf 58 (1.66 g), and Bat 37 (1.70 g) compared to those fed with Alfonso (1.75 g). Silkworms fed with Alf 23 (1.47 g), Alf 47 (1.55 g), Alf 65 (1.65 g), Alf 74 (1.58 g), Bat 73 (1.66g) produced significantly lighter cocoon (1.66 g) compared with the check the variety Alfonso (1.75 g). Meanwhile, no significant differences were observed on the weight of cocoons produced by silkworms fed with all the hybrids with means ranging from 1.58 – 1.73 g, except silkworms fed with Alf 23 (1.47 g) and Alf 47 (1.55 g) which produced significantly lighter cocoons compared with check the variety S₅₄ (1.66 g).

Shell weight

Silkworms fed with Alf 32 and Bat 73 produced cocoons with shell weights of 0.32 and 0.33g, respectively, which did not differ significantly with the check variety, Alfonso with a mean of 0.34 g. On the other hand, silkworms fed with the rest of the hybrids produced cocoons with mean shell weight ranging

from 0.27 to 0.31 g, significantly lower than the check variety, Alfonso with a mean of 0.34 g. Meanwhile, the performance of the mulberry hybrids fed to silkworms compared with the check variety S₅₄ showed that silkworms fed with Alf 23 and Alf 47 produced cocoons with significantly lighter shells having respective means of 0.27 and 0.29 g. The rest of the varieties fed to silkworms produced cocoons with mean shell weights ranging from 0.31 to 0.34 g, and did not differ significantly with the check variety, S₅₄.

Cocoon shell percentage

Shell percentage varied significantly among the different mulberry varieties. Shell percentage of cocoons produced by silkworms fed with all the hybrids ranged from 18.48 to 19.83%, did not differ significantly with the two check varieties, Alfonso and S_{54} with respective mean shell percentages of 19.15 and 19.13%.

Cocoon yield/box

Silkworms fed with Alf 32, Alf 58, Alf 65, Bat 37, and Bat 73 produced cocoons with a mean yields of 32.23, 35.29, 34.82, 34.67 and 33.34 kg/box respectively, which did not differ significantly with the check variety, Alfonso (34.73 kg/box). On the other hand, silkworms fed with Alf 23, Alf 47 and Alf 74 produced significantly lower cocoon yields of 31.45, 31.70 and 32.71 kg/box, respectively. However, silkworms fed with Alf 55 have recorded a significantly higher cocoon yield with a mean of 37.25 kg/box, resulting to an increase of 7.25% over the check variety, Alfonso (34.73 kg/box). Silkworms fed with Alf 32, Alf 74, Bat 37 and Bat 73 produced cocoons with yields of 34.23, 32.71, 34.67 and 33.34 kg/box, respectively, comparable with check the variety, S₅₄ (33.29 kg/box). However, significantly higher cocoon yield was recorded in silkworms fed with Alf 55, Alf 58 and Alf 65 with respective cocoon yields/ box of 37.25, 35.29 and 34.82 compared with the check variety S_{54} (33.29 kg/ box). Result implies an increase in cocoon yield of silkworms fed with the said variety with respective increases of 11.89, 5.40 and 4.59% over the check variety S54. Meanwhile, comparable performance was observed in Alf 32, Alf 74, Bat 37 and Bat 73 with the check variety S_{54} (33.29 kg/box) with respective means of 34.23, 32.71, 34.67, and 33.34 kg/box.

Reelability percentage, filament length, and size

Silkworms fed with all the hybrids produced cocoons with

 Table 1. Performance of silkworm fed with mulberry hybrids and varieties based on the weight of ten matured larvae, single cocoon, shell weight, cocoon shell percentage, cocoon yield per box and reelability percentage*.

Mulberry Hybrid	Weight of ten matured larvae (g)	Weight of single cocoon (g)	Single shell weight (g)	Cocoon shell (%)	Cocoon yield/box (kg)	Reelability (%)
Alf 23	30.28 e	1.47 e	0.27 d	18.70 ab	18.70 ab 31.45 f	
Alf 32	33.45 b	1.71 ab	0.32abc	19.08ab	34.23 bcd	84.50 a
Alf 47	31.19 de	1.55 de	0.29 d	18. 48 b	31.70 f	86.58 a
Alf 55	34.92 a	1.73 ab	0.34 a	19.44ab	37.25 a	83.50 a
Alf 58	32.71 bc	1.66 abc	0.31 c	18.67ab	35.29 b	86.69 a
Alf 65	32.70 bc	1.65 bc	0.31 c	18.59ab	34.82 bc	84.96 a
Alf 74	31.91 cd	1.58 cd	0.31 c	19.26ab	32.71 ef	83.56 a
Bat 37	33.23 b	1.70 ab	0.31 c	18.82ab	34.67 bcd	89.41 a
Bat 73	32.65 bc	1.66 bc	0.33 ab	19.83 a	33.34 cde	87.52 a
Alfonso(check)	32.71 bc	1.75 a	0.34 a	19.15ab	34.73 bcd	85.70 a
S54 (check)	31.62 cd	1.66 bc	0.32abc	19.13ab	33.29 de	89.33 a
cv (%)	1.32	1.77	2.00	2.25	1.50	2.56

Means within a column with the same letter are not significantly different at 5% level (HSD).

*- mean of six (6) rearing trials

Table 2. Performance of silkworm fed with mulberry hybrids and
varieties based on filament size and length*

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Hybrid	Filament size (denier)	Filament length(m)
Alf 23	2.71	724.47
Alf 32	2.76	738.49
Alf 47	2.71	699.89
Alf 55	2.75	782.27
Alf 58	2.82	757.72
Alf 65	2.81	733.96
Alf 74	2.92	707.53
Bat 37	2.78	744.98
Bat 73	2.81	732.51
Alfonso (check)	2.83	769.36
S54 (check)	2.74	764.87
cv (%)	1.80	4.64

* - mean of six (6) rearing trials

a reelability percentage ranging from 83.50 to 89.41%, which did not vary significantly with the silkworms fed with the check varieties, Alfonso and S_{54} with respective reelability percentages of 85.70 and 89.33% was recorded (Table 1). No significant

differences were observed among the different mulberry hybrids with regards to size and length of filament reeled from cocoons produced by silkworms fed with all the hybrids and varieties. The different varieties of mulberry used did not influence the said filament characteristics (Table 2).

Cumulative evaluation index

Among the hybrids, five garnered above 50 evaluation index; Alf 32, Alf 55, Alf 58, Bat 37 and Bat 73 with respective E.I. of 51.38, 58.63, 52.68, 54.22 and 54.28. Alf 23, Alf 47, Alf 65, and Alf 74 recorded below 50 E.I. of 37.63, 38.57, 49.32, and 44.45 respectively failed to garner above 50 evaluation index in all the parameters except on reelability Table 3).

Discussion

One of the factors that contribute to the success of cocoon production is the mulberry leaf as feed for silkworms. The quality of mulberry leaf has a contribution of about 38% (Chundang *et al.*, 2020). Moreover, mulberry leaf quality also depends on the variety, agronomic practices, maturity, position of the leaf, and season. Feeding silkworms with

Hybrid	WTML (g)	SCW (g)	SSW (g)	CYB (kg)	CSP (%)	FL (m)	FS (denier)	R (%)	Average E. I.**
Alf 23	32.14	28.51	29.35	35.14	42.22	43.38	36.36	53.92	37.63
Alf 32	57.79	57.88	55.49	51.63	51.61	48.84	46.34	41.44	51.38
Alf 47	39.41	38.57	36.56	36.61	36.86	33.80	35.21	51.55	38.57
Alf 55	69.72	60.30	61.80	69.60	60.37	65.91	44.80	36.52	58.63*
Alf 58	51.80	51.65	48.28	57.95	41.61	56.34	61.69	52.12	52.68
Alf 65	51.76	50.04	47.38	55.17	39.73	47.08	59.77	43.68	49.32
Alf 74	45.27	42.39	46.48	42.61	55.87	36.77	49.41	36.84	44.45
Bat 37	55.99	56.48	52.79	54.26	45.34	51.37	52.09	65.42	54.22
Bat 73	51.32	50.84	58.19	46.36	70.12	46.51	59.00	56.19	54.82
Alfonso (check)	51.84	61.51	60.90	54.63	53.28	60.88	63.22	47.28	56.69
S54 (check)	42.96	51.85	52.79	46.04	53.00	59.13	42.12	65.04	51.61
Mean	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00

Table 3. Evaluation index of each mulberry hybrid and variety on the different silkworm economic trait and the average evaluation index.

*- Highest average evaluation index ** - Evaluation index

SCW - single cocoon weight

SSW - single shell weight

FS - filament size

R - reelability

CSP- cocoon shell percentage

WTML-weight of ten matured larvae

good quality mulberry leaves with the nutrients required for optimum silkworm growth larvae results in lower mortality of the larvae (Caccam and Mendoza, 2012). The percentage contribution of each factor has been developed in Japan (Bose, 1989). Nine (9) newly-evolved mulberry hybrids and two check varieties (Alfonso and S54) were evaluated based on their effects on the economic traits of the silkworm hybrid DMMMSU 115 x 100. Further, hybrids gaining an evaluation index of 50 and above identified for higher cocoon production and higher raw silk production for sericulture farmers and cocoon processors respectively. Variations observed in the performance of the silkworm point to the significant differences among the mulberry hybrids and varieties with regards to weight of ten matured larvae, weight of single cocoon, single shell weight, cocoon shell percentage and cocoon yield per box which implies that variety of mulberry has significantly affected the said parameters. On the other hand, no significant variations were observed on reelability of cocoons produced, filament length and size of silk thread extracted from cocoon produced by silkworms fed with the different hybrids and varieties. Several reports are already available on the evaluation of mulberry varieties through rearing performance conducted by various researchers (Tikader and Kamble, 2007; Radjabi, 2011). Their findings

revealed significant differences among the varieties tested based on characteristics of cocoon crop. Silkworms growth and development is adversely affected by the low moisture content of mulberry leaves as feed (Sengupta et al., 1971). Thus, leaves should contain high moisture content which has a positive influence on the growth and development of silkworm (Sudan et al., 2020). Paul et al. (1992) reported that the amount of food ingested and digested could be increase through increased moisture content of leaves as feed for silkworm which acts as olfactory and gustatory stimulant. In the present study, weight of ten matured larvae were heavier when fed with Alf 55 which may implies that the leaves of Alf 55 may contain high moisture content that makes silkworm larvae growth and development better which varies with variety Ashfaq et al. (2001). This may also be attributed to higher digestibility of food which is Alf 55 by the silkworm leading to higher weight gain during fifth instar. The present result agrees with Pakhale et al. (2014) that V-1 was observed superior among tested varieties. Likewise, with Bahar et al. (2011) which the highest performance was observed in silkworm feed with variety BSRM-34 with regards to the average weight of 10 mature larvae. Moreover, Yogananda-Murthy et al. (2013a) also found out that the weight of ten matured larvae were significantly higher when fed with S1708

leaves than the four varieties. Thangamani and Vivekananda (1984) reported the significant influence of different mulberry genotypes on the growth and development of silkworm and cocoon production. Moreover, quality of leaves fed to silkworms is important on the growth, health and development of larvae (Ray et al., 1998) with leaf quality differences may have caused the differences in the rearing performance (Kumar et al., 2014). Thus, growth and development of silkworm larvae depend on the quality of mulberry leaves (Mahadeva and Shree, 2015). Recognizing the importance of food intake of silkworm larvae wherein the production of one (1) g larval dry weight, there is a need of ingestion and digestion of food of 4.2 mg and 1.8 mg respectively (Horie et al., 1995) and the food intake of silkworm during its larval life is reflected in its larval weight (Sajgotra et al., 2018). Cocoon weight is considered an important parameter to evaluate the nutritional effect of mulberry varieties (Alipanah et al., 2020) and is used as one factor in the computation of computed cocoon yield. Cocoon weight is positively correlated with the cocoon shell which means that the heavier the cocoon, the greater is the portion of the shell. In the present study, the different varieties varied with regards to cocoon weight which implies that variety has significantly affected the cocoon weight. A single cocoon was heavier when fed with Alfonso. Kumar et al. (2014) also observed that among the varieties used as feed, feeding silkworms with S-1635 produced heavier cocoons than the rest of the varieties. The cocoon shell represents the actual silk, and an important economic silkworm trait (Rahmathulla et al., 2004). In the present study, silkworms fed with Alf 55 and Alfonso produced cocoons with the heaviest shell. Mulberry leaves contain a rich source of protein for the production of nearly 70% silk and amino acids which influences the shell weight of cocoons (Machii and Katagiri, 1991). Thus, the result may imply that the leaves of Alfonso and Alf 55 may contain higher protein than the rest of the varieties. Terefe et al. (2020) found that the shell weight of cocoons revealed significant variation when fed with different mulberry accessions. Mulberry genotypes S13 and K2 revealed significantly higher shell weights as compared to the local check variety and other treatments. Also, the findings of Yogananda-Murthy et al. (2013b) showed that shell percentage was significantly higher in cocoons produced by silkworms fed with Tr8. Cocoon shell percentage is regarded as a quality parameter that was influenced by the variety of mulberry fed to silkworms as shown by the significant variations among

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varieties. It is also an important parameter because the higher the shell percentage the more varn will be produced thus, fetch higher economic value. In the present study, the shell percentage of cocoons produced by silkworms fed with Bat 73 registered the highest cocoon shell percentage. Findings of Yogananda-Murthy et al. (2013a). revealed that shell percentage were significantly higher in cocoons produced by silkworms fed with S1708 leaves. Moreover, the quality of leaves fed to silkworms is important for the growth, development and health of larvae which will ultimately affect the quality and yield of cocoons (Ray et al., 1998). In the present study, cocoon yield of the silkworms fed with the different mulberry varieties showed significant variations, which may imply that the nutritional composition of mulberry leaves varies with variety which could influence cocoon production (Nagaraju, 2002). Saratchandra et al. (1992) stressed that the variety of mulberry can significantly affect the cocoon yield in weight. Among the hybrids, only Alf 55 when fed to silkworms produced higher cocoon yield than Alfonso and S54. The high cocoon yield of silkworms when fed with Alf 55 is attributed to the heavy matured larvae, heavy shell and cocoon, as well as high shell percentage. This validates the findings of Ghosh et al. (2000) where larval weight, cocoon and shell weight, and shell ratio is positively correlated with cocoon yield. Qader et al., (1992) accounted that a good variety of mulberry is determined by the quality of leaves. Based on the results, it can be deduced that the leaf quality of Alf 55 accounts for the better yield and cocoon characteristics of silkworms, thus, Alf 55 is a good variety of mulberry.

Reelability percentage, filament length, and size of cocoons produced by silkworms were not affected by the variety of mulberry. However, the filament length of silk recovered from cocoons produced by silkworms fed with the different varieties falls within the range of 600-1500 m which is 80% reelable (FAO, 1999). This implies that all the varieties performed comparably well. On the other hand, Kumar *et al.* (2013) found out that the different mulberry varieties had influenced the filament length (m) and size (denier) of extracted silk from cocoons produced by silkworm fed with different mulberry varieties which does not agree with the present study.

Screening of mulberry varieties through silkworm rearing involving multiple traits using evaluation index is a very appropriate tool to determine the superiority of mulberry varieties (Pandit *et al.*, 2006). All the silkworm economic traits that correspond to the variety of mulberry were computed for the evaluation index

value. The cumulative evaluation index value was derived by adding the E.I. of each trait and was divided by the number of traits. Among the hybrids, it was found out that Alf 32, Alf 55, Alf 58, Bat 37, Bat 73 garnered an average E. I. of above 50. Alf 55 garnered above 50 E.I. on weight of ten matured larvae, single cocoon and shell weight, shell percentage, cocoon yield, filament length which made it first rank. Bat 37 garnered above 50 E.I. in all the parameters except for cocoon shell percentage while on cocoon yield/box and filament length for Bat 73. Meanwhile, Alf 32 garnered above 50 E.I. in all the parameters gathered except for filament length, filament size and reelability. The present study revealed a potential effect of the selected mulberry varieties on growth and development of silkworms and further on cocoon yield and quality specifically on shell percentage which has a very high E.I. of 70.12 of the variety Bat 73. Therefore, the use of the five hybrids (Alf 32, Alf 55, 58, Bat 37 and Bat 73) as feeds guarantee a successful rearing of silkworm hybrid DM-MMSU 115 x DMMMSU 100.

Conclusions

Silkworms fed with Alf 55 produced significantly heavier matured larvae and higher cocoon yield than Alfonso and S54 which was comparable to the cocoon weight, shell weight, shell percentage and reelability. Alf 55, Alf 58 and Alf 65 recorded significantly higher cocoon yield than the check variety, S54. Mulberry variety influenced the rearing performance of silkworms and their economic traits. Therefore, selection of a variety of mulberry for silkworm rearing is imperative. Feeding DM-MMSU 115 x100 with Alf 32, Alf 55, Alf 58, Bat 37 and Bat 73 improves rearing performance and quality of cocoons. Further, feeding trials exploiting other promising multivoltine and bivoltine silkworm purelines for quality egg production should be done by egg producers. Likewise, other promising bivoltine hybrids aside from DMMMSU 115 x100 should be used and tested for quality cocoon production by sericulture farmers in the country is recommended.

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