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Effect of secondary nutrients on rearing and cocoon parameters of bivoltine double cross hybrid mulberry silkworm (FC1 × FC2)

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Abstract
The present study on "Performance of tree mulberry applied with secondary nutrients and its influence on silkworm (*Bombyx mori* L.)" was carried out in the established V-1 tree mulberry garden at College of Sericulture, Chintamani during 2022-23. The treatments were formulated based on the recommendation for sulphur application in mulberry and proposed the recommendation for calcium, magnesium and sulphur in the ratio of 3:1:0.9, respectively. The rearing parameters *viz*., the mean larval weight (48.23 g/10 larvae), larval duration (7.26 days) and ERR (97.05%) was observed in the batches of silkworms fed with mulberry leaves of trees that received RDF + soil application of Ca:Mg:S:: 82.5:27.5:25 kg ha-1 yr^{-1}. Also, the cocoon weight (2.54 g/cocoon), pupal weight (1.97g), shell weight (0.55 g/cocoon), cocoon shell ratio (21.66%), cocoon filament length (1356.57m), non-breakable filament length (992.27m), filament weight (0.43g) and filament Denier (2.88) were found significantly higher in the same treatment.

Keywords: *Bombyx mori*, tree mulberry, secondary nutrients, V-1. cocoons

Introduction
Mulberry, as the exclusive host plant for *Bombyx mori* L. and a pivotal economic crop cultivated specifically for silkworm rearing, plays a vital role in the sericulture industry. It accounts for 38.20% of the successfully produced cocoons. Maintaining mulberry in optimal condition necessitates precise management, including the application of appropriate quantities of both organic and inorganic fertilizers. This is particularly important because mulberry is a perennial crop that undergoes periodic pruning (Rahman and Islam 2020; Vijay and Susikaran, 2023) [7, 14]. Mulberry, belonging to the *Morus* spp., is characterized by its fast growth, woody nature, and perennial status, often pruned to take on the form of bushes or dwarf trees. The quality of mulberry leaves is of paramount importance as they serve as the primary food source for silkworms (*Bombyx mori* L.), directly influencing the sustainability and profitability of the sericulture sector. The limited availability of all the nutrients leads to competition among mulberry plants for essential nutrients. This competition, in turn, negatively impacts yield, as observed in the study by Setua *et al*. (2011) [8]. To address these dynamics, the current research was undertaken to evaluate the effects of different levels of secondary nutrients influence on cocoons characters contributing to yield.

Materials and Methods
The experiment was conducted during 2022-2023, at the Department of Sericulture, University of Agricultural Sciences, Bengaluru and College of Sericulture, Chintamani. The experimental site was geographically located in the Eastern Dry Zone (Zone-5) of Karnataka and lies between 13.3354° N, 78.0824° E at an altitude of 865 m above the mean sea level and it receives average rainfall of 400 to 750 mm annually. The soil of the experimental plot was sandy clay loam in texture and prior to laying out of the experiment, composite soil samples were randomly drawn from 0 to 30 cm depth in the experimental site from each replication before imposing treatments and analyzed for chemical properties.

The treatments were formulated based on the recommendation for sulphur application in mulberry and proposed the recommendation for calcium, magnesium and sulphur in the ratio of 3:1:0.9, respectively. The field trial was laid out in a Randomized Complete Block Design (RCBD) with six treatments and four replications each *viz.,* T1: RDF+ Soil application of Ca:Mg:S :: 33:11:10 kg ha^{-1} yr^{-1}, T2: RDF+ Soil application of Ca:Mg:S :: 49.5:16.5:15 kg ha^{-1} yr^{-1}, T3: RDF+ Soil application of Ca:Mg:S :: 66:22:20 kg ha^{-1} yr^{-1}, T4: RDF+ Soil application of Ca:Mg:S :: 82.5:27.5:25 kg ha^{-1} yr^{-1}, T5: RDF+ Soil application of Ca:Mg:S :: 99:33:30 kg ha^{-1} yr^{-1}, T6: Control (recommended dose of fertilizer + FYM).The net plot size was 144 m^2 with the spacing of 8 ft X 8 ft (between rows and plants).

Cocoon parameters

Cocoon weight (g)

Ten cocoons were randomly selected from each replication of every treatment and weight was recorded on the sixth day of mounting.

Cocoon shell weight (g)

After taking the cocoon weight the same cocoons were cut to remove the pupae and the cocoon shell weight was recorded for each replication and the average was calculated to get the mean shell weight.

Pupal weight (g)

Ten pupae obtained by cutting the ten cocoons were weighed in each replication and the average was calculated to obtain the mean pupal weight.

Shell ratio (%)

The shell ratio was calculated by using the formula:

Shell ratio (%) = Cocoon shell weight (g) /Cocoon weight (g) × 100

Effective rate of rearing (ERR) (%)

The number of cocoons harvested at the end of rearing in each replication of every treatment was documented and the ERR was calculated by using the formula:

ERR (%) = Number of cocoons harvested /Total number of worms brushed × 100

Reeling Parameters

1. Average filament length (m)

Three cocoons from each replication of the treatments was randomly drawn, cooked and reeled separately on an eprouvette. The filament length was determined by,

L = R × 1.125m

where,

L-Total filament length (m); R-Number of revolutions; 1.125 m-Circumference of the eprouvette reel

Non-breakable filament length (NBFL) (m)

The non-breakable filament length was calculated using the formula:

NBFL (m) = Total filament length (m) /1 + Number of breaks

Filament weight

It is the weight of reeled silk filament from the eprouvette with a wheel circumference of 1.125 m and is expressed in grams.

Filament denier

The filament denier was calculated using the formula:

Denier = Weight of the filament (g) /Filament length (m) × 9000

Statistical analysis

The data collected from the experimental field, laboratory analysis and silkworm rearing were analyzed statistically by using RCBD for testing the significance by Fisher's method of analysis of variance (Snedecor and Cochran, 1979). The level of significance used in the F-test was $P \leq 0.05$. The critical difference (CD) values were computed to compare the significant differences between the treatments.

Results and Discussion

Effect of varied levels of secondary nutrients on rearing and cocoon parameters of bivoltine double cross hybrid mulberry silkworm (FC1 ×FC2)

Larval parameter

The effect of different levels of secondary nutrients on the *Bombyx mori* L. larval parameters on feeding with the mulberry leaves raised with different levels of secondary nutrients was recorded based on the results of silkworm rearing conducted during the first and second harvest after imposition of the treatments. The observations recorded include fifth instar larval duration, fifth instar larval weight and effective rate of rearing.

Larval duration

Among the different treatment levels of secondary nutrients, the fifth instar larval duration was found to be unwaveringly non-significant in both the crops among the treatments. However, the mean of fifth instar larval duration was found to be least in T_4 (7.26 days) followed by T_3 (7.42 days), T_2 (7.54 days), T_1 (7.62 days) and T_5 (7.73 days). The highest was found in T_6 (7.94%). The recorded observations are presented in Table 1.

Larval weight

Among the different treatment levels of secondary nutrients, the fifth instar larval weight was found to be significantly varied in both the crops among the treatments. However, the mean of fifth instar larval weight was found to be maximum in T_4 (48.23 g) on par with T_3 (46.31 g), T_2 (45.22 g) and T_1 (44.10 g) followed by T_5 (44.33 g). The minimum larval weight was found in control (40.74 g). The recorded observations are presented in Table 1.

Effective rate of rearing (ERR)

Among the different treatment levels of secondary nutrients, the effective rate of rearing was found to be non-significant in both the crops among the treatments. However, the mean effective rate of rearing in both crops ranged from 92.45% to 97.05% (Table 1).

Table 1: Growth performance of bivoltine double hybrid silkworm (FC1×FC2)-v instar fed on leaves from tree mulberry applied with different levels of secondary nutrients

Treatments	Larval duration (days)			Larval weight (g/10 larvae)			Effective Rate of Rearing (ERR) (%)		
	Crop-I	Crop-II	Mean	Crop-I	Crop-II	Mean	Crop-I	Crop-II	Mean
T_1: RDF+ Soil application of Ca:Mg:S :: 33:11:10	7.86	7.39	7.62	41.90	46.30	44.10	94.28	95.38	94.83
T_2: RDF+ Soil application of Ca:Mg:S :: 49.5:16.5:15	7.79	7.29	7.54	43.20	47.24	45.22	95.15	95.52	95.33
T_3: RDF+ Soil application of Ca:Mg:S :: 66:22:20	7.64	7.20	7.42	44.10	48.53	46.31	96.04	96.43	96.23
T_4: RDF+ Soil application of Ca:Mg:S :: 82.5:27.5:25	7.36	7.17	7.26	46.80	49.67	48.23	96.42	97.68	97.05
T_5: RDF+ Soil application of Ca:Mg:S :: 99:33:30	8.01	7.45	7.73	43.50	45.16	44.33	93.26	93.87	93.56
T_6: Control (recommended dose of fertilizer + FYM)	8.34	7.54	7.94	39.50	41.99	40.74	92.89	92.01	92.45
F-Test	NS	NS	NS	*	*	*	NS	NS	NS
S.E.M±	-	-	-	1.268	1.364	1.197	-	-	-
CD @ 5%	-	-	-	3.798	4.084	3.611	-	-	-
CV %	6.334	6.223	5.635	5.877	5.87	5.345	6.083	6.047	5.624

Note: *Significant at 5%, NS= Non-significant, RDF-Recommended dose fertilizers, Crop-I (Dec-Feb), Crop-II (March-May)

Cocoon parameters

Cocoon weight (g): Significant influence was exerted in cocoon weight when bivoltine double hybrid silkworms were reared on tree mulberry leaves treated with different levels of secondary nutrients. The mean of both crops revealed that the maximum cocoon weight was recorded in T_4 (2.54 g) (RDF+ Soil application of Ca:Mg:S:: 82.5:27.5:25 kg ha^{-1} yr^{-1}) which was on par with T_3 (2.46 g) (RDF+ Soil application of Ca:Mg:S :: 66:22:20 kg ha^{-1} yr^{-1}) followed by T_2 (2.35 g), T_1 (2.29 g) and T_5 (2.23 g). The minimum cocoon weight was found in the untreated check T_6 (recommended dose of fertilizer + FYM) (2.08 g). The recorded observations are presented in Table 2.

Pupal weight (g): Significantly higher pupal weight was recorded in the cocoons spun by bivoltine double hybrid silkworm (FC1 ×FC2) when reared on tree mulberry leaves produced with different levels of secondary nutrients. The mean of both crops revealed that the maximum pupal weight was recorded in T_4 (1.97 g) (RDF+ Soil application of Ca:Mg:S:: 82.5:27.5:25 kg ha^{-1} yr^{-1}) which was on par with T_3 (1.93 g), T_2 (1.86 g) and T_1 (1.82 g) followed by T_5 (1.77 g). The minimum pupal weight was found in control T_6 (recommended dose of fertilizer + FYM) (1.69 g), (Table 2).

Cocoon shell weight (g): The mean shell weight of cocoons spun by bivoltine double hybrid silkworm (FC1 X FC2) in both crops was significant among different treatments.

Table 2: Effect of feeding leaves of tree mulberry applied with different levels of secondary nutrients on cocoon characters of bivoltine double hybrid silkworm

Treatments	Cocoon weight (g)			Pupal weight (g)			Cocoon shell weight (g)			Cocoon shell ratio (%)		
	Crop-I	Crop-II	Mean	Crop-I	Crop-II	Mean	Crop-I	Crop-II	Mean	Crop-I	Crop-II	Mean
T_1: RDF+ Soil application of Ca:Mg:S :: 33:11:10	2.27	2.31	2.29	1.80	1.85	1.82	0.46	0.48	0.47	20.26	20.77	20.51
T_2: RDF+ Soil application of Ca:Mg:S :: 49.5:16.5:15	2.36	2.34	2.35	1.84	1.88	1.86	0.47	0.49	0.48	20.67	20.94	20.80
T_3: RDF+ Soil application of Ca:Mg:S :: 66:22:20	2.45	2.48	2.46	1.93	1.94	1.93	0.51	0.53	0.52	20.83	21.37	21.10
T_4: RDF+ Soil application of Ca:Mg:S :: 82.5:27.5:25	2.51	2.57	2.54	1.96	1.98	1.97	0.54	0.56	0.55	21.54	21.78	21.66
T_5: RDF+ Soil application of Ca:Mg:S :: 99:33:30	2.21	2.25	2.23	1.76	1.79	1.77	0.44	0.46	0.45	20.28	20.44	20.36
T_6: Control (recommended dose of fertilizer + FYM)	2.06	2.10	2.08	1.68	1.71	1.69	0.41	0.43	0.42	20.19	20.47	20.33
F-Test	*	*	*	*	*	*	*	*	*	NS	NS	NS
SE(m)±	0.067	0.068	0.061	0.054	0.055	0.049	0.014	0.014	0.012	-	-	-
CD @ 5%	0.202	0.205	0.185	0.162	0.165	0.149	0.041	0.043	0.038	-	-	-
CV %	5.830	5.843	5.303	5.902	5.914	5.376	5.764	5.778	5.235	6.064	6.065	5.573

Note: *Significant at 5%, NS= Non-significant, RDF-Recommended dose fertilizers, Crop-I (Dec-Feb), Crop-II (March-May)

Silkworms fed with leaves harvested from the treatment with RDF+ Soil application of Ca:Mg:S:: 82.5:27.5:25 kg ha^{-1} yr^{-1} (T_4) exhibited significantly higher shell weight of 0.55 g, on par with T_3 (0.52 g) followed by T_2 (0.48 g), T_1 (0.47 g) and T_5 (0.45 g). On the other hand, the cocoons harvested from treatment T_6 (recommended dose of fertilizer + FYM) registered the lowest shell weight of 0.42 g. The recorded observations are presented in Table 2.

Cocoon shell ratio (%): The cocoon shell ratio did not differ significantly among the treatments of the experiment. However, the mean per cent cocoon shell ratio of both crops was maximum in T_4 (21.66%) (RDF+ Soil application of Ca:Mg:S:: 82.5:27.5:25 kg ha^{-1} yr^{-1}) on par with T_3 (21.10%), T_2 (20.80%), T_1 (20.51%) and T_5 (20.36%). The per cent cocoon shell ratio was least in control T_6 (recommended dose of fertilizer + FYM) (20.33%). The recorded observations are presented in Table 2.

Reeling parameters: There was a significant difference among the treatments with respect to cocoon filament length in both the crops with the application of different secondary nutrients on tree mulberry. Significantly longest filament length was recorded in the cocoons spun by the silkworms fed on leaves harvested from treatment T_4 (1356.57 m) followed by T_3 (1258.10 m), T_2 (1175.03 m), T_1 (1160.48 m) and T_5 (1112.92 m). However, shortest filament length was noticed in the batches of cocoons obtained from the silkworms fed with leaves obtained from absolute control (T_6) (1077.34 m). The non-breakable filament length (NBFL) was found to be unwavering among the treatments. Significantly higher filament weight was recorded in the cocoons spun by the silkworms fed on leaves harvested from treatment T_4 (0.43 g) which received RDF+ Soil application of Ca:Mg:S :: 82.5:27.5:25 followed by T_3 (0.40 g), T_2 (0.37 g), T_1 (0.36 g) and T_5 (0.35 g). While significantly least filament weight was obtained in cocoons of silkworms fed with leaves of control (T_6) with 0.34 g. The filament denier was non-significant and found to be unwavering among the treatments (Table 3).

Table 3: Effect of feeding leaves of tree mulberry applied with different levels of secondary nutrients on commercial cocoon characters of bivoltine double cross hybrid silkworm.

Treatments	Cocoon filament length (m)			Non-breakable filament length (m)			Filament weight (g)			Filament denier		
	Crop-I	Crop-II	Mean	Crop-I	Crop-II	Mean	Crop-I	Crop-II	Mean	Crop-I	Crop-II	Mean
T_1: RDF+ Soil application of Ca:Mg:S :: 33:11:10	1149.14	1171.81	1160.48	959.23	961.27	960.25	0.34	0.38	0.36	2.66	2.91	2.78
T_2: RDF+ Soil application of Ca:Mg:S :: 49.5:16.5:15	1162.03	1188.03	1175.03	963.76	969.18	966.47	0.36	0.39	0.37	2.78	2.95	2.86
T_3: RDF+ Soil application of Ca:Mg:S :: 66:22:20	1177.46	1338.74	1258.10	973.16	981.04	977.10	0.38	0.43	0.40	2.90	2.89	2.89
T_4: RDF+ Soil application of Ca:Mg:S :: 82.5:27.5:25	1345.78	1367.36	1356.57	986.42	998.13	992.27	0.43	0.44	0.43	2.87	2.89	2.88
T_5: RDF+ Soil application of Ca:Mg:S :: 99:33:30	1068.42	1157.42	1112.92	956.37	960.41	958.39	0.34	0.36	0.35	2.86	2.79	2.82
T_6: Control (recommended dose of fertilizer + FYM)	994.20	1160.47	1077.34	947.76	958.46	953.11	0.33	0.35	0.34	2.98	2.71	2.84
F-Test	*	*	*	NS	NS	NS	*	*	*	NS	NS	NS
SE(m)±	33.159	36.57	31.68	-	-	-	0.011	0.011	0.009	-	-	-
CD @ 5%	99.284	109.498	95.494	-	-	-	0.032	0.034	0.033	-	-	-
CV %	5.769	5.943	5.324	6.085	6.092	5.595	5.835	5.827	5.295	6.235	6.013	5.569

**Note: ***Significant at 5%, NS= Non-significant, RDF-Recommended dose fertilizers, Crop-I (Dec-Feb), Crop-II (March-May)

The silkworm larval, cocoon and reeling parameters were significant for larval weight, single cocoon weight, shell weight, pupal weight and average filament length except for larval duration, cocoon shell ratio, ERR, Non-breakable filament length and denier. The shoot harvest method leads to quick depletion of soil nutrients in the mulberry garden. Application of calcium, magnesium and sulphur-containing fertilizers has not so far been covered under the package of practices for mulberry cultivation (Jolly, 1987) [4]. Good cocoon yield is known to be mainly influenced by the nutrient status of the mulberry leaf which in turn depends on the cultivation practices followed in respect of agronomic inputs (Bongale and Chaluvachari, l993) [1]. It is interesting to note that about 34 per cent increase in leaf yield was observed when secondary nutrients were used and it also led to improvement in cocoon weight, shell weight, pupal weight and filament length of cocoons (Subbarayappa, 1988) [12]. Due to intensive cultivation, in addition to NPK, supply of Ca, Mg and S should also be given importance in nutrition of mulberry to obtain maximum quality leaf as it is a perennial crop yielding good returns to farmers throughout the year in tropical countries (Shankar, 1990) [9]. Hence, systematic budgeting in respect of Ca, Mg and S removal and supplementation in mulberry cultivation is suggested (Shivaprakash *et al*., 2009) [11]. Muhammad *et al*. (1998) [5] reported the food consumption, coefficient of utilization, maximum larval body weight, body length and cocoon yield are maximum and attributed to the role of mineral nutrients with calcium (1.0%) supplemented through mulberry leaves. Mulberry leaf quality has a significant impact on silkworm growth and development, as well as the quality of cocoons produced. The nutritional value of mulberry leaves is critical for increasing and enhancing cocoon quality (Dasgupta, 1961) [2].

The addition of secondary nutrients (Ca_{230}, S_{150}, $Mg_{5.5}$) units per hectare and micronutrients to the mulberry field led to an increase in silkworm pupation rate by 6.81-13.67 per cent and an increase in cocoon yield (Greiss *et al*., 2001) [3]. Tewary *et al*. (2008) [13] reported the weight of ten mature larvae (31.50 g), larval period (27 days), single cocoon weight (1.56 g), single shell weight (0.27 g), shell ratio (17.00%) and cocoon yield (44.15 kg/100 DFLs) when fed on leaves from tree mulberry. The effect of leaf macro-mineral composition in mulberry reproduction potential of silkworms showed a positive correlation between the presence of Ca, Mg, S in leaves and silkworm parameters (Shifa *et al*., 2020) [10]. Murugesh *et al*. (2021) [6] revealed enhancement of larval and cocoon parameters by supplementation of magnesium sulphate with foliar spray of 200 and 100 ppm.

Conclusion

Among the larval parameters, the mean larval weight (48.23 g/10 larvae) was higher in the silkworms fed with leaves harvested from mulberry trees treated with RDF+ Soil application of Ca:Mg:S :: 82.5:27.5:25 kg ha-1 yr^{-1}, whereas mean larval duration recorded was shorter in batches of silkworms fed on leaves from the trees treated with RDF+ Soil application of Ca:Mg:S :: 82.5:27.5:25 kg ha-1 yr^{-1} (7.26 days). The mean ERR (97.05%) and lower mortality was observed in the same treatment. The mean cocoon and silk reeling parameters *viz*., cocoon weight (2.54 g), pupal weight (1.97 g), cocoon shell weight (0.55 g), cocoon shell ratio (21.66%), cocoon filament length (1356.57 m), non-breakable filament length (992.27 m), filament weight (0.43 g) and filament denier (2.88) were found significantly higher in silkworms fed with leaves of RDF+ Soil application of Ca:Mg:S :: 82.5:27.5:25 kg ha-1 yr^{-1} treated plot followed by the treatment with RDF+ Soil application of Ca:Mg:S :: 66:22:20 kg ha^{-1} yr^{-1}.

Authors' contributions

This work was carried out in collaboration among all authors. Author Harish Reddy C. executed the research, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author Ramakrishna Naika and Naveen, D. V planned the study and arranged for the fertilizer. Author Dukare Pradip Gulabrao, supported in silkworm rearing, managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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