



IMPACT OF FORTIFICATION OF CHLOROGENIC ACID RICH BOTANICAL ON SILKWORM GROWTH AND COCOON PARAMETERS OF SILKWORM *BOMBYX MORIL*.

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Abstract

Mulberry leaves supplemented with four different grade coffee and tea extracts with different concentration were orally fed to V instar silkworm hybrid (PM×CSR₂). Among them oral supplementation of 5 per cent green coffee bean extract resulted in significant increase in growth parameters such as larval length, larval weight, silk gland weight (7.42cm, 41.55g/10 larvae, 0.93g/10 larvae respectively) and cocoon parameters such as cocoon weight (18.49g/10 cocoon), pupal weight (16.16g/10 pupae), shell weight (4.31g/10 shell), ERR (96.33%), total cocoon yield (165.36g), shell ratio (20.11%) silk filament length (999.67m/10 cocoon), silk filament weight (0.29g), silk productivity (5.89cg/day), fibrion (78.74%) and denier (2.68) whereas reduction in fifth instar duration (164.70h), disease incidence (2.33%) and sericin content (21.26%) as compared to absolute control.

Key words : Silkworm, mulberry, green coffee bean, growth, cocoon.

Introduction

Mulberry silkworm is the fully domesticated among all silk producing insects (Eri silkworm, Muga silkworm and Tassar silkworm), the increment in the raw silk production is brought by improving the mulberry leaf quality because it's being a monophagous insect derives almost all the nutrient required for its growth from mulberry leaf itself. Eventhough, mulberry leaf provides almost all of these nutrients required during larval development, the variation in quantity of these nutrients in the mulberry leaves is the limiting factor that hinders production of silk in mulberry silkworm. There is significant seasonal variation occurs in the nutritional value and composition of mulberry leaves depending on different factors *viz.*, weather, pest and disease incidence, mulberry varieties, fertilizer used and method of pruning. Thus, demand of larval nutrition is not met completely, especially during fifth instar for producing good quality and quantity of silk. Seki and Oshikane (1959) observed that good quality cocoons can be obtained when silkworm larvae are fed on nutritionally enriched leaves that would improve the silk production.

Coffee powder contains nutrients and different chemicals, which includes carbohydrates, lipid, vitamins, minerals, alkaloids, nitrogenous compound and phenolic compounds. These chemicals in coffee do not act independently within body, they interact with each other in innumerable different ways, most of which are yet to be discovered. In the plant kingdom coffee bean have maximum content of chlorogenic acid. According to Kato and Yamada (1966), when silkworm were reared on diet containing coffee powder, the results were better for the variety of coffee bean, which had higher content of chlorogenic acid. It is the ester of quinic acid and caffeic acid, growth promoting action is present in caffeic acid and absent in quanic acid. The most popular polyphenolic acid called gallic acid also had a growth promoting action in mulberry silkworm. Green tea and black tea are also beverages which have chlorogenic acid, vitamins (ascorbic acid), and minerals *viz.*, potassium, aluminium and manganese (Graham, 1984). Patil and Chandrashekhar (2013) tried that fortification of mulberry leaves with 5 per cent roasted coffee powder extract to silkworm, there was uniform and good growth of silkworm. Present study was undertaken to find out fortification of effective

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concentration of different grade coffee and tea extract to the mulberry silkworm and its cocoon parameters are required for the commercial use.

Materials and Methods

The studies were conducted at Ericulture Laboratory, Department of Agriculture Entomology, University of Agricultural Sciences, Dharwad. The chawki worms of silkworm hybrid PM×CSR₂ were procured from private chawki centre of Ranebennur area, Haveri district, Karnataka, India and used for the study. From III instar to IV instar, the mass rearing of silkworm was carried out in the laboratory by feeding V-1 mulberry leaves three times a day. III and IV instar worms were fed with chopped leaves. Whereas entire leaves to V instar silkworms. During rearing, immediately after fourth moult fifth instar worms were grouped into three batches of 100 larvae for each treatment. Green coffee beans were obtained from Plantation crops unit, Department of Horticulture, University of Agricultural Sciences, Dharwad. They are sun dried for about 10 days and grounded to make fine powder using an electrically operated mixer grinder. 7.5g and 5g of green coffee bean powder dissolved in 100 ml of water in separate flask to make decoction of 7.5 and 5 per cent of extract respectively everyday. Similarly, commercially available different grade coffee and tea powder were procured *i.e.*, filter coffee powder, green tea powder and black tea powder from MORE shop at Dharwad. Known quantity of black tea powder *i.e.*, 7.5 and 5g dissolved in 100ml of water separately and boiled through electric water heater, decoction was prepared freshly everyday as per the treatment *i.e.*, 7.5 and 5 per cent, respectively. The procedure followed for preparing decoction of filter coffee powder and green tea powder at 7.5 and 5 per cent was same as that of black tea decoction. The mulberry leaves of V-1 variety were dipped separately in coffee and tea extracts of 5 per cent and 7.5 per cent, shade dried and fed to V instar silkworm daily once in the evening as per the treatments and remaining two feeds with normal leaves without any treatments till cocoon spinning. The growth parameters such as, V instar duration, mature larval weight, silk gland weight, V instar larval length and cocoon parameters such as cocoon weight, pupal weight, shell weight, total cocoon yield were recorded and statistically analyzed. Certain parameters were calculated using following formula :

$$\text{ERR} = \frac{\text{Number of cocoons harvested}}{\text{Number of worms per treatment}} \times 100$$

$$\text{Disease (\%)} = \frac{\text{No. of worms dead due to disease}}{\text{Number of worms per treatment}} \times 100$$

$$\text{Shell ratio} = \frac{\text{Shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

$$\text{Silk productivity (cg/day)} = \frac{\text{Weight of shell (g)}}{\text{5th instar larval duration (days)}}$$

Silk filament length (m) = No. of revolutions on epprouvette × Circumference of wheel (1.125m).

Silk filament weight (g) : The reeled silk from five cocoon was dried by keeping in hot air oven at 50° C and weight was recorded by weighing in electronic balance.

$$\text{Fibroin (\%)} = \frac{\text{Weight of fibroin (g)}}{\text{Weight of shell (g)}} \times 100$$

$$\text{Sericin (\%)} = 100 - \text{Fibroin (\%)}$$

$$\text{Denier} = \frac{\text{Cocoon filament weight}}{\text{Filament length of cocoon}} \times 9000$$

Results and Discussion

Coffee and tea extract show tendency to improve growth, cocoon and silk parameters. The result on impact of coffee and tea extract on growth parameters of silkworm are presented in table 1. Results showed that supplementation of five per cent green coffee bean extract induced the growth parameters such as larval weight, larval length and silk gland weight (41.55g, 7.42cm and 0.93g, respectively) and also ERR (96.33%). Which was on par with black tea extract at five per cent and green coffee bean extract at 7.5 per cent. Whereas, lowest was recorded in absolute control (31.26g, 5.74cm, 0.74g and 88.67%, respectively). It might be attributed to stimulatory effect and enhanced food consumption by silkworm at optimal concentration of feed additive. Also feed supplement contains carbohydrates, vitamins, minerals and phenolic compounds that strongly influence the larval growth at certain concentration. Meanwhile, reduction in fifth instar duration and disease incidence (164.70h and 2.33%, respectively) was recorded by supplementation of same concentration of green coffee bean extract as compared to absolute control. The lower disease incidence and reduced larval duration in the treated silkworms might be due to the influence of phenolic compound such as chlorogenic acid present in coffee, which has antioxidant property, boost the tissues and making them robust and thus imparts healthiness to larvae and satisfy nutritional requirement in lesser duration.

Table 1 : Effect of fortification of coffee and tea extract to V instar silkworm *B. mori* on its growth parameters, during *kharif* 2013-14.

Treatments	Conc.	Fifth instar duration (h)*	Larval length (cm)*	Larval weight (g/10 larvae)*	Silk gland weight (g/10 larvae)*	ERR (%)**	Disease (%)**
T ₁ - Green coffee bean extract	5%	164.70(12.85) ^d	7.42(2.81) ^a	41.55(6.48) ^a	0.93(1.20) ^a	96.33(78.98) ^a	2.33(8.74) ^f
T ₂ - Green coffee bean extract	7.5%	165.83(12.90) ^{cd}	6.96(2.72) ^{ab}	39.30(6.31) ^{abc}	0.90(1.18) ^{ab}	95.33(77.54) ^{ab}	3.46(10.64) ^{ef}
T ₃ - Filter coffee solution	5%	172.77(13.16) ^{ab}	5.98(2.54) ^{de}	34.04(5.88) ^d	0.78(1.13) ^d	89.67(71.25) ^e	9.67(18.11) ^a
T ₄ - Filter coffee solution	7.5%	172.99(13.17) ^{ab}	5.86(2.52) ^{de}	33.02(5.79) ^{de}	0.75(1.12) ^e	89.00(70.64) ^e	10.00(18.43) ^a
T ₅ - Green tea extract	5%	168.57(13.00) ^{bcd}	6.24(2.60) ^{bcd}	37.74(6.18) ^{bc}	0.85(1.16) ^c	94.33(76.24) ^{bc}	4.02(11.66) ^{de}
T ₆ - Green tea extract	7.5%	169.95(13.06) ^{abc}	6.09(2.57) ^{cde}	37.25(6.14) ^c	0.84(1.16) ^c	93.67(75.43) ^c	5.00(12.88) ^{bc}
T ₇ - Black tea extract	5%	165.27(12.88) ^{cd}	7.10(2.76) ^a	39.89(6.35) ^{ab}	0.91(1.19) ^{ab}	95.67(78.06) ^a	3.00(9.97) ^{ef}
T ₈ - Black tea extract	7.5%	167.83(12.97) ^{bcd}	6.41(2.66) ^{bc}	38.48(6.24) ^{bc}	0.89(1.18) ^b	94.65(76.52) ^{bc}	3.67(11.02) ^{de}
T ₉ - Water control		174.23(13.22) ^a	5.99(2.55) ^{cde}	32.91(5.78) ^{de}	0.78(1.13) ^d	92.00(72.92) ^d	5.67(13.76) ^b
T ₁₀ - Absolute control		174.57(13.23) ^a	5.74(2.50) ^e	31.26(5.64) ^e	0.74(1.11) ^e	88.67(70.33) ^e	10.33(18.75) ^a
S Em±	0.06	0.03	0.06	0.01	0.01	0.57	
C. D. (P=0.01)	0.20	0.11	0.23	0.03	0.03	2.16	

*Figures in the parentheses are $\sqrt{(X + 0.5)}$ transformed values, ** Figures in the parentheses are arc sign transformed values. In vertical columns means followed by similar letters do not differ statistically (CD=0.01) by DMRT.

Table 2 : Effect of fortification of coffee and tea extract to V instar silkworm *B. mori* on cocoon parameters, during *kharif* (2013-14).

Treatments	Conc.	Cocoon weight (g/10 cocoon)*	Pupal weight (g/10 pupae)*	Shell weight (g/10 shell)*	Total cocoon yield (g)*	Shell ratio (%)**
T ₁ - Green coffee bean extract	5%	18.49(4.36) ^a	16.16(4.08) ^a	4.31(2.19) ^a	165.36(12.88) ^a	20.11(26.64) ^a
T ₂ - Green coffee bean extract	7.5%	17.83(4.28) ^{ab}	15.28(3.97) ^{ab}	3.82(2.07) ^{ab}	162.50(12.77) ^{ab}	19.12(25.93) ^{ab}
T ₃ - Filter coffee solution	5%	13.81(3.78) ^d	12.13(3.55) ^d	2.65(1.78) ^c	112.97(10.67) ^e	17.52(24.82) ^{cd}
T ₄ - Filter coffee solution	7.5%	13.50(3.74) ^d	12.04(3.54) ^d	2.51(1.73) ^c	110.77(10.55) ^e	16.96(24.32) ^{de}
T ₅ - Green tea extract	5%	16.98(4.26) ^{bc}	14.35(3.85) ^{bc}	3.24(1.93) ^{abc}	157.17(12.56) ^{bc}	18.11(25.18) ^{bcd}
T ₆ - Green tea extract	7.5%	16.06(4.07) ^{bcd}	13.77(3.78) ^c	3.10(1.90) ^{bc}	154.55(12.45) ^c	17.89(25.05) ^c
T ₇ - Black tea extract	5%	18.06(4.31) ^{ab}	15.77(4.03) ^{ab}	3.87(2.08) ^{ab}	163.23(12.81) ^{ab}	19.49(26.05) ^{ab}
T ₈ - Black tea extract	7.5%	17.12(4.19) ^b	14.37(3.86) ^{bc}	3.30(1.94) ^{abc}	157.45(12.56) ^{bc}	18.39(25.38) ^{bcd}
T ₉ - Water control		14.86(3.92) ^{cd}	13.27(3.71) ^{cd}	3.03(1.88) ^{bc}	151.59(12.33) ^c	17.42(24.67) ^{cd}
T ₁₀ - Absolute control		13.31(3.71) ^d	11.97(3.53) ^d	2.29(1.67) ^c	108.00(10.42) ^e	15.67(23.32) ^e
S Em±	0.074	0.05	0.09	0.11	0.35	
C. D. (P=0.01)	0.30	0.20	0.36	0.43	1.39	

*Figures in the parentheses are $\sqrt{(X + 0.5)}$ transformed values, ** Figures in the parentheses are arc sign transformed values. In vertical columns means followed by similar letters do not differ statistically (CD=0.01) by DMRT.

Table 3 : Effect of fortification of different concentration of coffee and tea extract to V instar silkworm *B. mori* on silk parameters, during *kharij* (2013-14).

Treatments	Conc.	Silk filament length (m/10 cocoon)*	Silk filament weight(g)*	Silk productivity (cg/day)*	Sericin (%)**	Fibrion (%)**	Denier**
T ₁ - Green coffee bean extract	5%	999.67(31.63) ^y	0.29(0.89) ^a	5.89(2.53) ^a	21.26(27.46) ^f	78.74(62.54) ^y	2.68(1.78) ^a
T ₂ - Green coffee bean extract	7.5%	993.23(30.92) ^y	0.25(0.85) ^{abc}	5.27(2.40) ^{ab}	23.09(28.72) ^e	76.91(61.28) ^b	2.40(1.70) ^{ab}
T ₃ - Filter coffee solution	5%	849.13(29.15) ^e	0.12(0.78) ^f	3.47(1.99) ^{cd}	25.50(30.33) ^e	74.37(59.25) ^e	1.32(1.34) ^d
T ₄ - Filter coffee solution	7.5%	838.31(28.96) ^e	0.12(0.79) ^{ef}	3.15(1.91) ^d	26.03(30.87) ^{bc}	73.77(59.19) ^e	1.23(1.31) ^d
T ₅ - Green tea extract	5%	953.40(30.89) ^e	0.20(0.84) ^{bcd}	4.63(2.26) ^b	24.48(29.65) ^d	75.66(60.44) ^e	1.86(1.53) ^{bc}
T ₆ - Green tea extract	7.5%	931.37(30.53) ^d	0.18(0.83) ^{cd}	4.40(2.21) ^{bc}	24.63(29.75) ^d	75.52(60.35) ^e	1.65(1.47) ^{cd}
T ₇ - Black tea extract	5%	995.71(31.56) ^y	0.26(0.87) ^{ab}	5.57(2.46) ^{ab}	21.38(27.54) ^f	78.62(62.46) ^y	2.10(1.61) ^{abc}
T ₈ - Black tea extract	7.5%	951.17(30.85) ^e	0.19(0.83) ^{bcd}	5.03(2.35) ^b	23.07(28.71) ^e	76.93(61.29) ^b	1.86(1.53) ^{bc}
T ₉ - Water control		847.03(29.11) ^e	0.15(0.81) ^{def}	3.23(1.93) ^d	25.34(30.26) ^c	74.50(59.67) ^{de}	1.65(1.46) ^{cd}
T ₁₀ - Absolute control		820.93(28.66) ^f	0.11(0.78) ^f	3.07(1.88) ^d	26.17(30.98) ^{bc}	73.23(58.84) ^e	1.17(1.29) ^d
S Em±		0.08	0.01	0.09	0.10	0.10	0.06
C. D. (P=0.01)		0.32	0.4	0.35	0.39	0.39	0.23

*Figures in the parentheses are $\sqrt{(X + 0.5)}$ transformed values, ** Figures in the parentheses are arc sign transformed values. In vertical columns means followed by similar letters do not differ statistically (CD=0.01) by DMRT.

The results from the table 2 indicated that cocoon parameters were significantly superior by supplementation of different grade coffee and tea extract. Among the different grade coffee and tea extract, five per cent green coffee bean extract recorded significantly higher cocoon parameters such as cocoon weight, pupal weight, shell weight, total cocoon yield and shell ratio (18.49g, 16.16g, 4.31g, 165.36g and 20.11%, respectively) as compared to absolute control. These improvements in cocoon parameters might be due to nutritional composition of green coffee bean extract especially chlorogenic acid. Supplementation of chlorogenic acid rich botanical such as coffee and tea extract had positive influence on silk parameters as presented in table 3. Results revealed that silk filament length, silk filament weight, silk productivity were increased by supplementation of five per cent green coffee bean extract (999.67m, 0.29g and 5.89cg/day). Among silk protein fibrion content was increased (78.74%) and sericin content was reduced (21.26%) by supplementation. Similar trend noticed in denier. The current results are in agreement with Reshma (1997) and Krishnaprasad *et al.* (2000) observed that supplementation of 1:4 concentration of potato leaf extract to fifth instar silkworm resulted in significant elevation in mature larval weight (2.66g), cocoon weight (1.68g), pupal weight (1.42g), shell weight (0.26g) shell ratio and filament length (650m) in comparison with control it may be attributed to richness of chlorogenic acid in potato. Similarly, Patil and Chandrashekhar (2013) reported uniform and good growth of silkworm when fed with five per cent roasted coffee bean extract. Kato and Yamada (1996) recorded increased larval duration and survivability by the fortification of cotton seed oil in combination with chlorogenic acid. Jeypaul *et al.* (2003) who studied that influence of *Coffea arabica* leaf extract on cocoon weight, shell weight, pupal weight and shell ratio, in which 1:25 concentration was found effective. Thulasi *et al.* (2014), who reported that supplementation of alfalfa induced larval weight and silk gland weight.

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