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RESEARCH ARTICLE

Supplementation of Honey from Different Bee Species and proteins source to Enhance the Silk Reeling Characters of Silkworm, *Bombyx mori* L.

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ABSTRACT

The study was carried out to evaluate the impact of different concentrations of honey from various bee species and protein sources on the silk reeling characters of *Bombyx mori* L. The silkworm larvae were fed on the mulberry leaves treated with honey and protein sources from the third instar to the spinning stage. Among the treatments tested, stingless bee honey recorded significantly the highest silk filament weight (384.52 mg), silk filament length (1321.15 m), and non-broken filament length (398.92 m) over all other treatments. The same honey also drastically brought down the thickness of filament and renditta to desirable level of 2.50 and 6.33, respectively from 2.63 and 7.42 in the control. Among the various concentrations evaluated, it was proved from the results of present experiments that stingless bee honey at 5 per cent performed well in improving the silk reeling related characters of silkworm.

Keywords: Honey; Protein sources; Stingless bee; Silk reeling; *Bombyx mori;* Filament length.

INTRODUCTION

Sericulture is an agro-based rural industry opportunity that provides employment opportunities to more than nine million people in India. It provides a very good monthly income to resource poor marginal and small farmers. Silkworm, *Bombyx mori* L. is an insect, which possesses the ability to convert the plant proteins into the animal proteins efficiently for producing the continuous and lustrous silk filament. *B. mori* consumes only the leaves of mulberry (*Morus* sp.) to complete its life cycle. Hence, the nutritional qualities of mulberry decide the silk secreting capacity of the larvae. The nutritional status of mulberry leaves relies on variety and nutrient management practices. Insufficient quantity of nutrients in mulberry leaves more often results in silkworm crop failure, leading to severe economic loss for sericulturalists. This situation warrants the enrichment of mulberry leaves with various essential nutrients to increase the cocoon production and silk quality.

Various research works conducted over the period of time on the effects of supplementation of different essential nutrients like amino acids, minerals, vitamins, *etc.* on silkworm showed significant

improvement in the cocoon productivity and raw silk quality (Saha *et al.,* 1994; Saha and Khan, 1997; Rahmathulla *et al.,* 2007; Radjabi *et al.,* 2010; Murugesh, 2020).

Honey, an exogenous modulator, is a natural sweetener and multi-factorial nutrient produced by honey bees, which has attracted the attention of research works worldwide (Council of European Union, 2002). The honey is rich in carbohydrates, proteins, minerals, amino acids vitamins and enzymes (Ball, 2007). The supplementation of honey to silkworm larvae during different instars significantly improved the larval, cocoon as well as silk reeling related traits of silkworm (Gad, 2013; Saad *et al.,* 2014; Hamzah *et al.,* 2016; Thulasi and Sivaprasad, 2015; Alagumanikumaran and Prema, 2016; Tamilselvi *et al.*, 2020). But, the comparative studies on the influence of honey from different bee species on the silk reeling-related characters are not available. Hence, the experiments were undertaken to assess the influence of different honey and protein sources on silk reeling-associated characteristics of the mulberry silkworm.

MATERIALS AND METHODS

i) Silkworm rearing

The popular bivoltine silkworm hybrid, (CSR2×CSR27) × (CSR6×CSR26) was used for the experiments. The study was conducted at the Department of Sericulture, Forest College and Research INstitutre, Mettupalayam. The complete disinfection of silkworm rearing house and rearing equipment was done by spraying 0.05 % Asthra solution at the rate of 1.5 litres/ sq. m. area using rocker sprayer (Sivaprasad *et al.,* 2015). The young-age worms were purchased after the second moult from the commercial Chawki Rearing Centre, rearing was taken up under ideal environmental condition (26±2°C temperature & 80±5% humidity) by feeding the well grown mulberry leaves (variety G4) and mounting of mature worms were performed using plastic mountages (Saha *et al*., 2022).

ii) Application of exogenous modulators

Various concentrations namely 1, 2, 3, 4, 5 and 6 per cent of honey and protein sources were prepared by dissolving them in distilled water. Known quantity of fresh mulberry leaves was separately sprayed with honey from five bee species namely Indian bee honey, Italian bee honey, stingless bee honey, rock bee honey and little bee honey, and four protein sources such as, redgram flour, horse gram flour, soya flour and pollen powder. The larval batch reared by feeding on mulberry leaves sprayed with distilled water, and control without any treatment was maintained during the experiments for comparison. The exogenous modulators sprayed leaves were shade dried to remove the excess moisture and fed once daily in the morning during third, fourth and fifth instars. Each treatment was replicated thrice with 50 larvae per replication. The silkworm larvae were protected from the infestation of ants by placing the legs of rearing stands in the ant wells.

iii) Observations recorded

The observation on various silk reeling-related characters, viz., silk filament weight, silk filament length, non-broken filament length, denier, and vendetta, were recorded following standard procedure (Chattopadhyay *et al.*, 2018). The data collected from the experiments were statistically analysed using Factorial Completely Randomised Design (FCRD) as described by Panse and Sukhatme (1957).

RESULTS AND DISCUSSION

The experimental result proved that there were notable changes in the silk reeling-related characteristics of silkworms due to the enrichment of various honey and protein sources.

1. Silk filament weight

Significantly maximum silk filament weight was obtained in the larval batch treated with stingless bee honey (384.52 mg), which was found to be statistically on par with soya flour (376.91 mg) (Fig. 1). This was followed by little bee honey (357.05 mg), Italian bee honey (356.79 mg), rock bee honey (353.70 mg) and horse gram flour (352.85 mg), which showed statistical parity with each other. The lowest silk filament weight was registered in the control (289.61 mg). With respect to concentrations, the highest silk filament weight was recorded at 5 percent (349.75 mg), which was found to be on par with 6 (348.17 mg) and 4 (346.77 mg) percent. The next better concentrations were 3 (344.52 mg) and 2 (342.21 mg) per cent. The least silk filament weight was registered in 1 per cent (339.97 mg). In the interaction between treatments and concentrations, the highest silk filament weight was observed in 5 per cent stingless bee honey (394.27 mg) which showed statistical parity with 4 (387.14 mg), 6 (386.18 mg) and 3 (384.75 mg) per cent stingless bee honey, and 5 per cent soya flour (382.28 mg).

The increase in silk filament weight might be due to the enhanced weight and protein content in the silk gland of honey treated larvae as reported by Madhavi *et al.* (2018). The present results were strengthened by the findings of Khedr *et al.* (2013), who recorded improved silk filament length of 1085.01 m, when silkworm larvae were fed on mulberry leaves enriched with honey at 5 per cent concentration. The present observations also fall in line with the findings of Saad *et al.* (2014), who observed that 5 per cent camphor honey significantly enhanced the silk filament length (1051.40 m) over the control (837.10 m).

2. Silk filament length

The silk filament length varied significantly between the treatments and concentrations when the silkworm larvae were reared on mulberry leaves treated with different exogenous modulators (Table 1). Among the various treatments, stingless bee honey recorded the highest filament length of 1321.15 m which was found to be on par with soya flour (1312.15 m). The next better treatments were rock bee honey (1278.38 m), Italian bee honey (1273.40 m), horse gram flour (1262.37 m), pollen powder (1251.31 m) and little bee honey (1235.13 m) which were found to be on par with each other. The lowest silk filament length was registered in control (1198.75 m). Among the concentrations evaluated, silk filament length was maximum in 5 per cent (1274.07 m) followed by 6 (1267.48 m) and 4 (1262.88) per cent, which did not exhibit statistical difference. One per cent registered the lowest silk filament length of 1256.12 m. In the interaction between treatments and concentrations, 5 per cent stingless bee honey registered the highest silk filament length (1350.76 m) followed by 6 (1334.35 m) and 4 (1333.71 m) per cent stingless bee honey, and 5 per cent soya flour (1331.46 m), which were found to be statistically on par among themselves.

The enhanced silk filament length might be due to the increased fibroin and sericin content in cocoon as reported by Alagumanikumaran and Prema (2016). The present observations are supported with the finding of Saad *et al.* (2014), who found that 5 per cent camphor honey statistically increased the silk filament weight (280.31 mg). Further, the findings of Khedr *et al.* (2013) showing that fortification of mulberry leaves with 1 per cent honey and feeding to bivoltine silkworm hybrid significantly enhanced the silk filament weight to 257.51mg from 255.42 mg in the control, also substantiate the present observations.

3. Non-broken filament length (NBFL)

The NBFL varied significantly among the different treatments when the silkworm larvae were fed on honey enriched mulberry leaves (Table 2). Significantly highest NBFL of 398.92 m was observed in stingless bee honey over the control, which was followed by soya flour (371.23 m). The next better treatments were rock bee honey (320.30 m), Italian bee honey (319.88 m), little bee honey (311.58 m) and Indian bee honey (306.90 m). The lowest NBFL was registered in control (240.84 m). Among the various concentrations studied, statistically higher NBFL was recorded in 5 per cent (304.10 m) than all other concentrations. The concentrations such as 6 (300.38 m) and 4 (298.42 m) per cent were found to be statistically on par with each other. The lowest NBFL was observed in 1 per cent concentration (290.69 m). The interaction between treatments and concentrations showed that NBFL was more in 5 per cent stingless bee honey (426.25 m) followed by 6 per cent stingless bee honey (410.78 m), which were found to be on par with each other. The next better treatments were 4 (405.57 m) and 3 (398.10 m) per cent stingless bee honey (410.78 m) and, 5 (397.82 m) and 6 (383.96 m) per cent soya flour, which showed statistical parity among themselves. This result is strengthened by the finding of Alagumanikumaran and Prema (2016), who recorded that supplementation of honey at 25 percent to silkworm larvae from the first day of the fifth instar up to maturation, significantly increased the NBFL.

4. Denier

The results revealed that supplementation of honey and protein sources had significant positive impact in thickness of silk filament (Fig. 2). Among the different treatments, statistically lowest denier was noticed in stingless bee honey (2.50), which was found to be statistically on par with soya flour (2.52), Indian bee honey (2.53) and rock bee honey (2.53). The highest denier was observed in the control (2.63). Among the concentrations evaluated, 5 percent showed the statistically lowest denier (2.52), which was found to be on par with 6 percent (2.54). The highest denier was recorded in 1 per cent (2.57).

In the interaction between treatments and concentrations, minimum denier was registered in 5 per cent stingless bee honey (2.46), which exhibited statistical parity with 6 (2.48) and 4 (2.50) per cent stingless bee honey, 5 per cent soya flour (2.48) and 5 per cent rock bee honey (2.49). The supplementation of 2 per cent honey to silkworm larvae during fourth and fifth instars significantly reduced the thickness of silk filament compared to the control (Thulasi and Sivaprasad, 2015). Ravikumar and Anilkumar (2016) showed that supplementation of 6 per cent folic acid to silkworm larvae significantly reduced the thickness of silk filament (2.34) over the control (2.39). Enrichment of MR2 mulberry leaves with 25 per cent lysine significantly reduced the thickness of silk filament by 2.81 per cent over the control (Meeramaideen *et al.,* 2017). These findings more or less fall in line with the present observations.

5. Renditta

Notable variations were recorded in renditta, when the silkworm was fed with different exogenous modulators as compared to control (Fig. 3). Among the treatments studied, minimum renditta was found in stingless bee honey (6.33), which was found to be statistically on par with soya flour (6.40). The next better treatments were little bee honey (6.64), Italian bee honey (6.67), rock bee honey (6.68) and horse gram flour (6.68), which were found to be statistically on par with each other. Among the concentrations tested, significantly minimum renditta was observed in 5 per cent (6.71) over all other concentrations. This was

followed by 6 (6.76) and 4 (6.77) per cent, which exhibited statistical parity among themselves. The highest renditta of 6.88 was registered in 1 per cent.

In the interaction between treatments and concentrations, significantly the lowest renditta of 6.18 was observed in 5 per cent stingless bee honey, which was found to be on par with 6 (6.24) and 4 (6.30) per cent stingless bee honey, and 5 (6.31) per cent soya flour. This is in parity with the findings of Thulasi and Sivaprasad (2015), who found that 2 per cent honey significantly reduced the renditta to 5.07 from 5.20 in the control. Further, the present observations also got strengthened with the findings of Ravikumar and Anilkumar (2016), who showed that supplementation of 6 per cent folic acid to silkworm larvae significantly reduced the renditta over the control.

CONCLUSION

The present experiments showed that fortification of mulberry with stingless bee honey at 5 per cent concentration and feeding to silkworm larvae from third instar to spinning stage daily once in the morning significantly enhanced the silk reeling related characters namely silk filament weight, silk filament length and non-broken filament length. It also increased the raw silk quality and recovery by significantly reducing the denier and renditta, respectively, over all other treatments.

REFERENCES

Alagumanikumaran, N. and P. Prema. 2016. Studies on evaluation and improvements of growth and economic parameters of *Bombyx mori* L. influence under MR2 leaves fortified with natural honey. *International Journal of Current Science Research,* **2(6)** : 757-765. (DOI: 10.33545/26174693.2024.v8.i10Sn.2665)

Chattopadhyay, D., Munshi, R. and Chakravorty, D. (2018). Studies on distribution of filament length and non-broken filament length for tropical tasar and muga silk cocoons vis-à-vis mulberry silk cocoons. *The Journal of Textile Institute*, **109** (9): 1202-1207. (https://doi.org/10.1080/00405000.2017.1422307).

Council of European Union. 2002. Council Directive 2001/110/Ec of 20 December 2001 relating to honey. *Offic. J. the. Europ. Comm.,* LI0.47-52. (DOI: 10.3390/molecules17078561).

Ball, D.W. 2007. The chemical composition of honey. *Journal of chemical education,* **84(10)** : 1643. (DOI; 10.1021/ed084p1643).

Gad, A.A. 2013. Biological and Physiological Effects of Some Honey Bee Products and Its Mixtures as Nutritional Additives on Two Strains of The Mulberry Silkworm *Bombyx mori*. *Alexandria Journal Agriculture Researcher,* **58(1)** : 47-52. (DOI: 10.21608/jppp.2021.83373.1025).

Hamzah, M., Kamel, F., Gomaa Abo-Laban and M.M. Nabil. 2016. The effect of mulberry leaves enrichment with different nutritional supplements on biological aspects and economic traits of silkworm, *Bombyx mori* L.. *Annals of Agric. Sci., Moshtohor,* **54(4)** : 977-982. (DOI: 10.1016/j.heliyon.2023.e21053)

Khedr, M.M.A., Samah, N., Shafiey, E.I. and H.M. Mead. 2013. Influence of fortification of mulberry leaves with natural and synthetic multivitamins on growth and development of *Bombyx mori* L. *Journal of Plant Protection and Pathology,* **4(1)** : 111 – 123. (DOI: 10.21608/jppp.2013.87265)

Madhavi, R., Arivoli, S. and S. Sivaprasad. 2018. Determination of minimum effective concentration of honey that optimizes larval growth and silk production in the silkworm, *Bombyx mori*. *International Journal of Green and Herbal Chemistry*, **7(3)** : 477-488. (DOI: 10.36106/IJAR).

Meeramaideen, M., Rajasekar, P., Sumathi, P.G. and P. Ganesh Prabu. 2017. Studies on the morphometric and economic parameters analysis of silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae) fed with amino acid (Lysine) treated MR2 mulberry leaves. *International Journal of Modern Research and Reviews,* **5(1)** : 1468-1473. (DOI: 10.2017/IJRCS/202401030)

Murugesh, K.A. 2020. Minerals as exogenous modulators in enhancing silk productivity of silkworm, *Bombyx mori* L. *International Journal of Chemical Studies.* **8(6)** : 241-245. (DOI: 10.22271/chemi.2020.v8.i6d.11161)

Panse, V.G. and P.V. Sukhatme. 1957. Statistical methods for agricultural workers. ICAR publication, p.328.

Radjabi, R., Ebadi, R., Mirhoseini, S.Z. and S. Nair. 2010. Effect of leaves enrichment with amino acid supplementary nutrients on *Bombyx mori* L. *Acad. J. of Ent*., **3(1)** : 45-51. (https://doi.org/10.1016/j.heliyon.2023.e21053)

Rahmathulla, V.K., Das, P., Ramesh, M. and R.K. Rajan. 2007. Growth rate pattern and economic traits of silkworm, *Bombyx mori*. L. under the influence of folic acid administration. *J. Appl. Sci. Environ. Manage*. **11(4)** : 81–84. (DOI: https://doi.org/10.22271/23940522.2021.v8.i1a.783).

Ravikumar, R, and M.N. Anilkumar. 2016. Folic acid as a nutrient supplement with mulberry leaf and its impact on the economic traits of silkworm (*Bombyx mori*, L.). *Int. J. Adv. Res.,* **4(12)** : 1159-1165. (DOI: 10.21474/IJAR01/2506).

Saad, M.S.I, Hassan, E.M. and I.A.I. Saad. 2014. Comparative study of the effect of camphor honey bee and camphor oil on some biological and productivity characters of mulberry silkworm, *Bombyx mori* L. *Journal of Plant Protection and Pathology,* **5(5)** : 651-658. (DOI: 10.21608/jppp.2014.87970).

Saha, S., Kumar, P., Raj, S., Choudhury, B.M. and Sentisuba. 2022. Sericulture: management and practices of mulberry silkworm. *International Journal of Pharmaceutical Research and Applications*, **7** (2) : 35-46. (DOI: 10.35629/7781-07023546).

Saha, A.K., Rahman, M.S., Saha, B.N. and M. Uddin. 1994. Effect of proline and leucine on the growth and development of silkworm *Bombyx mori L. Univ. j. zool.,* Rajshahi Univ. 13 : 75–79. (DOI: 10.5812/ijpr-133552).

Saha, B.N. and A.R. Khan. 1997. The nutritive effects of Sinafort (R) on *Bombyx mori* L. *Entomon*, **22(1)** : 29 - 34. (http://journals.sfu.ca/bd/index.php/UJZRU).

Sivaprasad, V., Himantharaj, M.T., Verma, S. and Mogiti, T. 2015. Commercial Chawki Rearing, Central Sericultural Research & Training Institute, CSB, Mysuru, p.13.

Tamilselvi, V. Murugesh, K.A., Mangammal, P. and S.V. Krishnamoorthy. 2020. Effect of different honey and protein sources on economic characters of silkworm *Bombyx mori* L. *International Journal of Chemical Studies*. **8(4)** : 328-331. (DOI: https://doi.org/10.22271/chemi.2020.v8.i4f.10051).

Thulasi, N. and S. Sivaprasad. 2015. Larval growth, silk production and economic traits of *Bombyx mori* under the influence of honey-enriched mulberry diet. *Journal of Applied and Natural Science,* **7(1)** : 286-292. (DOI: https://doi.org/10.31018/jans.v7i1.603).

TABLES

Table 1. Influence of various honey and protein sources on silk filament length (m) of *B. mori*

Treatments	Concentrations						
	1%	2%	3%	4%	5%	6%	Mean
Italian bee honey	1273.40	1274.41	1276.71	1276.51	1293.17	1281.49	**1273.40**
Rock bee honey	1278.38	1278.37	1279.31	1280.10	1294.45	1281.31	**1278.38**
Little bee honey	1235.13	1236.28	1238.37	1240.25	1265.38	1262.51	**1235.13**
Indian bee honey	1214.35	1214.98	1223.15	1228.23	1243.91	1241.15	**1214.35**
Stingless bee honey	1321.15	1323.25	1324.23	1328.15	1331.46	1328.89	**1321.15**
Red gram flour	1237.19	1238.45	1239.35	1240.78	1257.31	1241.70	**1237.19**
Horse gram flour	1262.37	1264.15	1265.13	1267.28	1269.25	1268.31	**1262.37**
Soya flour	1312.15	1312.31	1329.31	1333.71	1350.76	1334.35	**1312.15**
Pollen powder	1251.31	1252.16	1252.23	1254.78	1257.12	1256.13	**1251.31**
Distilled water	1233.15	1234.78	1235.67	1237.19	1241.36	1238.54	**1233.15**
Control	1198.75	1199.36	1203.82	1204.69	1210.59	1207.95	**1198.75**
Mean	**1256.12**	**1257.14**	**1260.66**	**1262.88**	**1274.07**	**1267.48**	**1256.12**
F value	**SEd** T= 10.15 C= 7.52 TC= 15.00			**CD (0.05)** T= 20.30** C= 15.05** TC= 30.00*			

Values are mean of three replications and pooled mean to two silkworm crops.
T - Treatment, C - Concentration, * Significant, ** highly significant

Table 2. Influence of various honey and protein sources on non-broken filament length (m)

Treatments	Concentrations						
	1%	2%	3%	4%	5%	6%	Mean
Italian bee honey	318.35	318.6	319.12	319.17	323.29	320.73	**319.88**
Rock bee honey	319.59	319.6	319.82	319.17	323.29	320.33	**320.30**
Little bee honey	308.78	309.07	309.59	310.06	316.34	315.63	**311.58**
Indian bee honey	303.59	303.74	305.78	307.05	310.98	310.28	**306.90**
Stingless bee honey	370.38	383.43	398.10	405.57	426.25	410.78	**399.09**
Red gram flour	247.43	247.69	247.87	248.15	251.46	248.34	**248.49**
Horse gram flour	252.47	252.83	253.02	253.45	253.85	253.66	**253.21**
Soya flour	340.38	356.08	368.40	380.71	397.82	383.96	**371.23**
Pollen powder	250.26	250.43	250.44	250.95	251.42	251.22	**250.79**
Distilled water	246.63	246.96	247.13	247.44	248.27	247.7	**247.36**
Control	239.75	239.87	240.76	240.94	242.12	241.59	**240.84**
Mean	**290.69**	**293.48**	**296.37**	**298.42**	**304.10**	**300.38**	**297.23**
F value	**SEd** T= 6.28 C= 0.75 TC= 8.07			**CD (0.05)** T= 12.84** C= 1.50** TC= 16.14**			

Values are mean of three replications and pooled mean to two silkworm crops.
T - Treatment, C - Concentration, * Significant, ** Highly significant

FIGURES

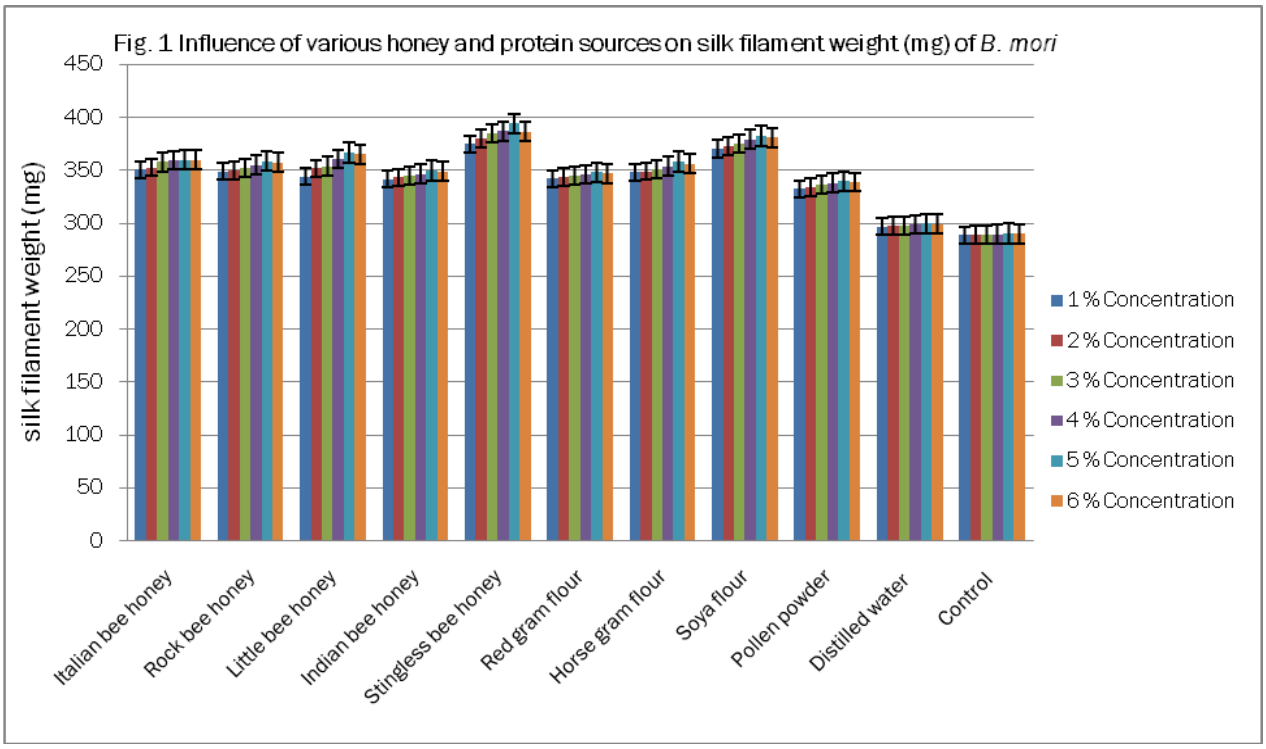
Fig. 1 Influence of various honey and protein sources on silk filament weight (mg) of B. mori
silk filament weight (mg)
450
400
350
300
250
200
150
100
50
0
Italian bee honey
Rock bee honey
Little bee honey
Indian bee honey
Stingless bee honey
Red gram flour
Horse gram flour
Soya flour
Pollen powder
Distilled water
Control
1 % Concentration
2 % Concentration
3 % Concentration
4 % Concentration
5 % Concentration
6 % Concentration

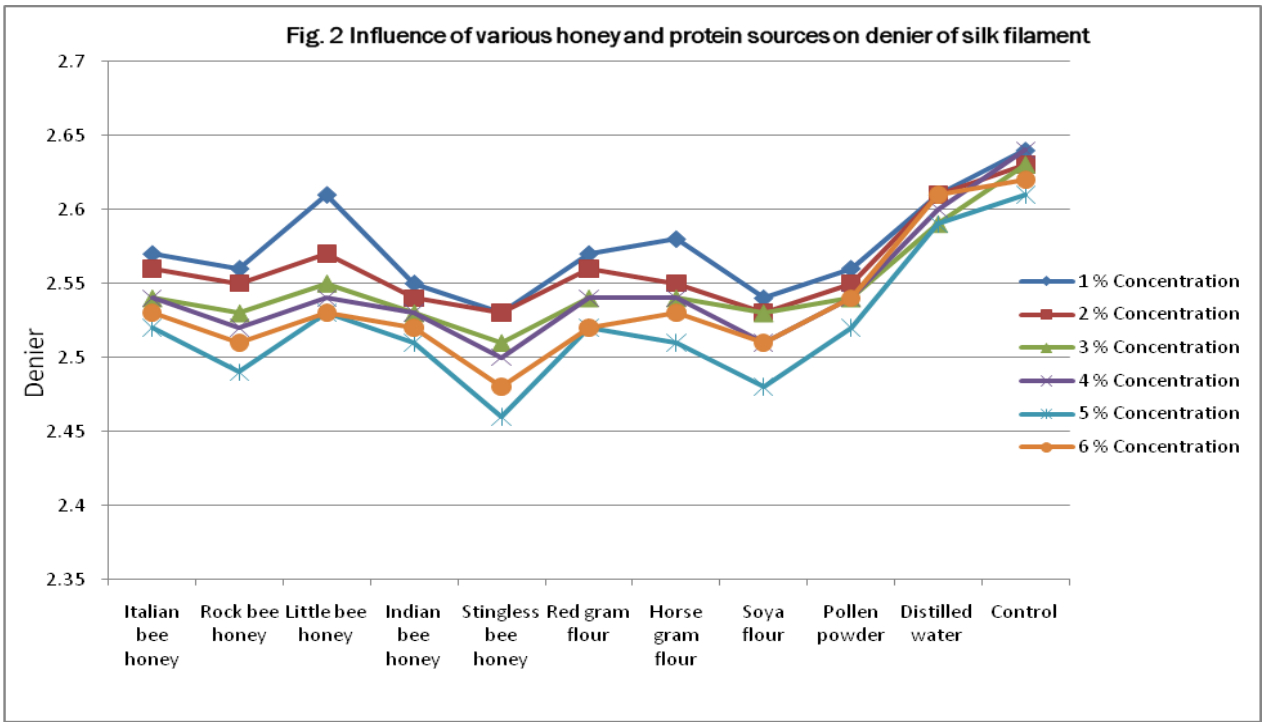
Fig. 2 Influence of various honey and protein sources on denier of silk filament
Denier
2.7
2.65
2.6
2.55
2.5
2.45
2.4
2.35
Italian bee honey
Rock bee honey
Little bee honey
Indian bee honey
Stingless bee honey
Red gram flour
Horse gram flour
Soya flour
Pollen powder
Distilled water
Control
1 % Concentration
2 % Concentration
3 % Concentration
4 % Concentration
5 % Concentration
6 % Concentration

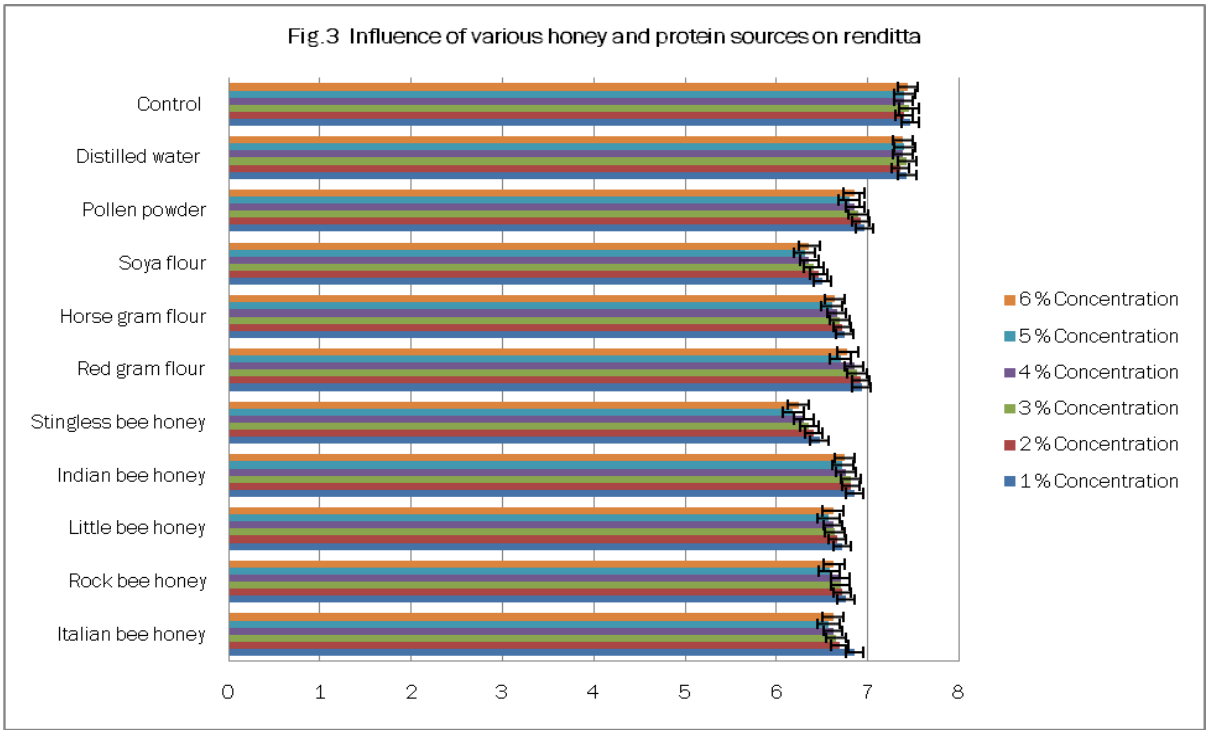
Fig.3 Influence of various honey and protein sources on renditta
Control
Distilled water
Pollen powder
Soya flour
Horse gram flour
Red gram flour
Stingless bee honey
Indian bee honey
Little bee honey
Rock bee honey
Italian bee honey
0
1
2
3
4
5
6
7
8
6 % Concentration
5 % Concentration
4 % Concentration
3 % Concentration
2 % Concentration
1 % Concentration