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Effect of probiotic feed supplements to mulberry silkworm, *Bombyx mori* L. for larval growth and development parameters

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Abstract
The silkworm larvae were fed on mulberry leaves treated with four different kinds of probiotics feed supplements *viz., spirulina,* azolla, yeast and soy milk at five different concentrations (1, 2, 3, 4 and 5%) each was supplemented to silkworm hybrid, PM × CSR-2 from fourth instar onwards through mulberry leaves every day once in the morning till the spinning stage during both June-July and March-April rearings. Among the probiotics tested azolla was found to be superior for all larval parameters such as larval weight a day before spinning, fifth instar and total larval duration, effective rate of rearing and fresh weight of silk glands on the day of 50 per cent spinning followed by soy milk and yeast in comparison with control (unsupplementation) in both the rearings. Among the concentrations tried, two per cent concentration was found superior in both the rearings. The interaction of feed additives (probiotics) along with their concentrations indicated that azolla at two per cent was superior for all larval parameters studied in both the rearings.

Keywords: Probiotics, probitic concentrations, *Bombyx mori* (L.) and larval parameters

Introduction
India is the second largest producer of silk in the world with an annual mulberry silk production of 20,478 MT with an area of 2.23 lakh hectares of mulberry during 2016-17 (Anon, 2016) [3]. The silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is an important economic insect is being used as a tool to convert mulberry leaf protein into silk. Nutrition plays an important role in improving the growth and development of the mulberry silkworm like the other organisms. It is stated that silk production is dependent on the larval nutrition and nutritive value of mulberry leaves, which plays a very effective role in producing good quality cocoons (Legay, 1958) [20]. In addition to mulberry leaves, feed supplements are also given to silkworm to enhance economic characteristics (Jeyapaul *et al.,* 2003, Sheeba *et al.,* 2006) [15, 31]. Nutritional supplements include vitamins, amino acids, proteins and probiotics when added to larval feed tend to increase nutritional efficiency and economic traits of silkworm (Etebari and Matindoost, 2005; Amalarani *et al.,* 2011, Singh *et al.,* 2005) [9, 1, 32].
Probiotics derived from Latin and Greek words pro means "for" and biotic means "life" together ''for life'' and defined in many ways. Probiotics are the live microbial food supplements beneficially affecting host by improving the microbial balance and enhanced rapid cellular growth and development (Fuller, 1993) [10]. Several researchers have reported about beneficial role played by use of probiotics in humans (Chan *et al*., 1985; Brigidi *et al*., 2000) [6, 4] ruminants, aquaculture (Douillet and Langdon, 1994 and Gildberg *et al*., 1997) [8, 12] and insects (Dillon and Dillon, 2004) [7].
Spirulina is blue-green micro algae belong to the class of cyanobacteria. It contains 18 amino acids and vital vitamins like biotin, tocopherol, thiamine, riboflavin, niacin, folic acid, pyrodozoic acid, beta-carotene and vitamin B12 etc. (Kumar *et al.,* 2009 and Ganeshprabu *et al.,* 2011) [18, 11]. Fifty different minerals and trace minerals, β-carotene, chlorophyll, GLA (γ-linolenic acid), Ω-3 fatty acid, and many other nutrients found in *Spirulina*.
Among the aquatic plant species, the floating water fern *Azolla pinnata* belongs to family Azolaceae and order Pteridophyta, the leaves contain the cyanobacterium *Anabaena azollae*, which is a symbiont that fixes nitrogen from the atmosphere that the fern can use and this small plant is rich in protein (25-30%), essential amino acids (7-10%), minerals (10-15%) like Ca, P, K, Fe, Cu, Mg etc. and vitamins (*viz*., A, B12 and β-carotene) and growth promoter

intermediaries.

Soy milk is an aqueous extraction of the soybean resembling milk. In 100 ml commercial nutrient-fortified brand of soy milk drink provides 80 calories from four grams of carbohydrates, including one gram of sugar, four grams of fat and seven grams of protein. This processed soy milk contains appreciable levels of vitamins (Vit. A, Vit. B and Vit.D) in a range of 10 to 45 per cent, with calcium and magnesium also in significant content.

Impact of probiotics (*Lactobacilus, Saccharomyces cerevisiae* and other effective microorganisms) treatment on mulberry leaves to modulate the economic parameters of fifth instar larvae of *B. mori* were studied (Jeyapaul *et al*., 2003) [15]. Amalarani *et al*., 2011 [1] had stated that *Saccharomyces cerevisiae* serves as an immune-modulating agent in silkworm, *Bombyx mori*. Soy bean protein posses a high nutritive value for *B. mori* as indicated from the nutritional research conducted on defatted soy bean meal and various proteins (Ito and Arai, 1965) [14]. Patil (2010) [24] tested the azolla supplementation to fifth instar silkworms with favourable results on cocoon traits. Mulberry leaf supplemented with *Spirulina* as a feed to *B. mori* was found to be effective in enhancing the larval and cocoon characters (Mathavan *et al.,* 1984 and Venkataramana, 2003) [22, 33].

Material and methods

The present study was undertaken to know the effect of different probiotic feed supplementation to mulberry silkworm, *Bombyx mori* L. at the Department of Agricultural Entomology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka. The experiment was carried out during 2016-17 at Raichur, which is situated in the North Eastern Dry Zone (Zone-II) of Karnataka between 16º 15' N latitude and 77º 20' E longitude with an altitude of 389 m above the mean sea level.

Preparation of probiotic concentrations solution used for the study

Azolla was collected freshly, brought to the laboratory, washed thoroughly with clean tap water and subsequently with distilled water. Azolla was weighed to 1000 grams for extraction using 1000 ml distilled water. Initially azolla was grinded with 100 ml of distilled water (out of 1000 ml) and sieved, the residue was mixed with 100 ml distilled water and grinded and sieved, and procedure was repeated 4-5 times and finally the filtrate volume was made up with the remaining distilled water and treated as stock solution. From this stock solution different concentrations were prepared *viz*., one, two, three, four and five per cent and the same was used for feed supplementation as done by Vijaykumar *et al*., 2016 [34]. *Spirulina* tablets were purchased from Raichur pharmaceutical shop and prepared 500 ml *spirulina* solution of different concentrations *viz*., one, two, three, four and five per cent. Soy milk was purchased from the super market of Raichur and from that stock, different concentration of the study was prepared and Baker's yeast (powder form) was purchased from the super market of Raichur and prepared the required concentrations of yeast solution appropriately used for feed supplementation.

Method of supplementation

Different concentrations of probiotic supplements of half litre solution was prepared and taken in separate containers every alternate day treatment wise. The harvested mulberry leaves were dipped in probiotic solutions separately treatment and concentration wise and then fed to silkworms once daily (morning feed) treatment and concentration wise from fourth instar first day onwards till they reached spinning stage. Spinning larvae were separately mounted on netrikas for cocoon construction treatment and replication wise. Observations were made on larval weight a day before spinning, fifth instar and total larval duration; effective rate of rearing and fresh weight of silk glands on the day of 50 per cent spinning.

Results and Discussion

Larval weight (g/10 larvae)

The silkworms reared on mulberry leaves supplemented with probiotics had better final fifth instar larval weight in comparison with control (unsupplementation) in both rearings. The final larval weight was highest in azolla (32.79 g for June-July rearing) and (30.32 g for March-April rearing) followed by soy milk, yeast and *spirulina* (28.40 to 30.37 g for June-July rearing) and (25.56 to 27.13 g for March-April rearing). Among the concentration tested, two per cent gave higher fifth instar larval weight (29.60 g for June-July rearing) and (26.82 g for March-April rearing). The interaction of feed additives along with concentration indicated significantly highest fifth instar larval weight in azolla at two per cent concentration during June-July rearing (34.12 g) and March-April rearing (31.72 g) (Table 1). Kamaraj *et al.* (2017) [16] observed higher larval weight when tasar silkworm host plant *Terminalia arjuna* was sprayed with soya aqueous solution at five per cent. Rahul *et al.* (2017) [25] reported that yeast at one per cent concentration fed to silkworm (Hybrid - MCon4 × BCon4) had no significant increase in larval weight and in the present study yeast was next best to azolla and soy milk. Vijaykumar *et al.*, 2016 [34] who reported the silkworm hybrid PM × CSR-2 reared on mulberry leaves supplemented with 50 per cent azolla concentrations gave higher larval weight (33.15 g) followed by 75 per cent concentration (32.14 g) when compared to control (28.31 g). He further, reported that higher larval weight in azolla treatment might be due to the fact that, azolla is a rich source of protein (25-30%), essential amino acids (7-10%) and minerals (10-15%). Sampath *et al.* (2013) [29] reported improved larval weight of 7.23 g as compared to control (4.52 g) when eri silkworms were fed with *Anabaena variabilis* at 500 ppm concentration. Sarker *et al*. (1995) [30] opined larval weight was maximum when mulberry leaves supplemented with mixture of soy milk + sugar + vitamins + potassium iodide salt. Mathavan *et al.* (1984) [22] also reported that final instar larvae of *Bombyx mori* L. fed on mulberry leaves supplemented with *Spirulina fusiformis* (Worohichin), a single cell protein (SCP) registered higher larval weight (2.09 g) over control (1.47 g) is in conformity with the findings of the present study. Furthermore, the quality of food has direct relevance on weight of larva and supplementation with probiotics perhaps increased the haemolymph protein and amino acid content in the silkworm larvae resulting in better silkworm growth as observed by Horie and Watanabe (1983) [13].

Table 1: Effect of probiotic feed supplementation to mulberry silkworm, PM × CSR-2 on fifth instar larval weight a day before spinning

Treatment	Fifth instar larval weight a day before spinning (g/10 larvae)													
	March - April rearing							June - July rearing						
	Concentration							Concentration						
	1%	2%	3%	4%	5%	Treatment mean	% ↑ over Ab. control	1%	2%	3%	4%	5%	Treatment mean	% ↑ over Ab. control
Spirulina	27.68	26.63	25.59	24.55	23.54	25.56	9.56	30.46	29.40	28.38	27.37	26.37	28.40	8.81
Azolla	30.48	31.72	30.86	29.78	28.78	30.32	29.96	33.05	34.12	33.33	32.25	31.22	32.79	25.63
Yeast	24.62	25.61	26.62	27.63	28.63	26.62	14.10	27.27	28.27	29.35	30.36	31.36	29.32	12.34
Soy milk	29.16	28.16	27.15	26.13	25.04	27.13	16.29	32.38	31.38	30.40	29.34	28.34	30.37	16.36
Water control	25.42	25.42	25.42	25.42	25.42	25.42	8.96	28.32	28.32	28.32	28.32	28.32	28.32	8.51
Absolute control	23.33	23.33	23.33	23.33	23.33	23.33	-	26.10	26.10	26.10	26.10	26.10	26.10	-
Concentration mean	26.78	26.82	26.50	26.14	25.78	26.40	-	29.59	29.60	29.32	28.95	28.61	29.21	-
	S.Em ±		CD @ 1%					S.Em ±		CD @ 1%				
Treatment (A)	0.17		0.46					0.16		0.42				
Concentration (B)	0.16		0.42					0.14		0.38				
Interaction (A×B)	0.38		1.02					0.35		0.93				

Note: % ↑ over Ab. control denotes per cent increase over absolute control.

Larval duration (h)

The results of the present investigation showed shorter larval duration in case of March-April rearing as compared to June-July rearing. Since silkworms are poikilothermic in nature and the development of silkworm stages are highly influenced by temperature. As surrounding temperature rises to higher levels, the physiological and biochemical changes are accelerated within the silkworms. Several investigations (Bursell, 1964: Landien, 1973) [5, 19] reviewed the relationship between temperature and duration of different stages in the life history of silkworm. The larval duration will intensifies with the increase of temperature, so temperature influences the rate of metabolism in insects and hence the reduced larval duration in March-April rearing. Significant shorter fifth instar larval duration was observed when mulberry supplemented with azolla (193.59 h for June-July rearing) and (170.27 h for March-April rearing) followed by soy milk (197.28 h for June-July rearing) and during March-April rearing soy milk, yeast and *spirulina* gave shorter fifth instars larval duration of 173.09 to 175.28 h. Among the concentrations tried, one and two per cent gave significantly shorter larval duration (200.04 and 200.08 h for June-July rearing) and during March-April rearing two per cent gave significantly shorter larval duration (177.66 h). The interaction of feed additives along with concentration indicated that significant shorter larval duration in azolla at two per cent concentration (192.03 h) during June-July rearing andFurther, the interaction effect indicated that azolla at two per cent concentration gave significant shorter larval duration (168.33 h) and was on par with azolla at three per cent concentration (169.00 h) (Table 2). The total larval duration was significantly shorter in mulberry supplemented with azolla (675.03 h for June-July rearing) and (608.11 h for March-April rearing) which was followed by mulberry leaves supplemented with soy milk, yeast and *spirulina* (681.42 to 686.91 h for June-July rearing) and (614.17 to 618.60 h for March-April rearing). Among the concentrations tested, two per cent gave significantly shorter larval duration (687.50 h for June-July rearing) and (621.92 h for March-April rearing). The interaction of feed additives along with concentration indicated that significant shorter total larval duration in azolla at two per cent concentration (672.03 h) during June-July and (604.33 h) during March-April rearing (Table 3). The silkworms reared on mulberry leaves supplemented with probiotics had reduced larval duration in comparison with control (unsupplementation) in both the rearings. The present results are in agreement with the findings of Vijaykumar *et al*. (2016) [34] according to whom the fifth instar duration up to spinning (173.61 h) was shorter in PM × CSR-2 reared on mulberry leaves dipped with 50 per cent of azolla. Further, the results are in line with Roychoudhary *et al.* (1994) [28] who reported that fifth instar bivoltine hybrid (Jd6 × $SF_1$9) reared on artificial diet containing soybean and defatted soybean as protein supplements had a short larval duration as compared to larvae reared on mulberry leaf alone. Furthermore, the increase in larval period was directly related to concentrations of probiotics used and attributed to the assumption that, the worm's nutritive requirement might have been fulfilled within lesser duration because of feed supplementation of probiotics *viz*., azolla, soy milk, yeast and *spirulina* as compared with unsupplemented mulberry leaves. Though there was variation in the concentrations tried by different workers, yet the results are positive with regard to use of probiotics. Mathavan *et al.* (1984) [22] reported that final instar of *Bombyx mori* L. fed on mulberry leaves supplemented with *Spirulina fusiformis* (Worohichin), a single cell protein (SCP) required only six days to attain spinning stage as against the control (nine days).

Table 2: Effect of probiotic feed supplementation to mulberry silkworm, PM × CSR-2 on fifth instar larval duration

Treatment	Fifth instar larval duration (h)													
	March - April rearing							June - July rearing						
	Concentration							Concentration						
	1%	2%	3%	4%	5%	Treatment mean	% ↓ over Ab. control	1%	2%	3%	4%	5%	Treatment mean	% ↓ over Ab. control
Spirulina	173.67	174.17	175.10	176.13	177.33	175.28	-11.82	197.27	198.00	199.20	200.43	202.00	199.39	-8.06
Azolla	169.40	168.33	169.00	171.77	173.00	170.27	-14.34	193.40	192.03	193.07	194.17	195.27	193.59	-10.73
Yeast	176.10	175.83	174.77	174.00	173.27	174.79	-12.06	199.17	198.27	197.50	196.93	196.00	197.57	-8.90
Soy milk	171.27	172.00	173.10	174.10	175.00	173.09	-12.92	195.13	196.40	197.60	198.10	199.17	197.28	-9.03
Water control	176.83	176.83	176.83	176.83	176.83	176.83	-11.04	198.67	198.67	198.67	198.67	198.67	198.67	-8.39
Absolute control	198.77	198.77	198.77	198.77	198.77	198.77	-	216.87	216.87	216.87	216.87	216.87	216.87	-
Concentration mean	177.67	177.66	177.93	178.60	179.01	178.17	-	200.08	200.04	200.49	200.86	201.33	200.56	-

	S.Em ±	CD @ 1%	S.Em ±	CD @ 1%
Treatment (A)	0.16	0.42	0.14	0.38
Concentration (B)	0.14	0.38	0.13	0.34
Interaction (A×B)	0.35	0.94	0.31	0.84

Note: % ↓ over Ab. control denotes per cent decrease over absolute control.

Table 3: Effect of probiotic feed supplementation to mulberry silk worm, PM × CSR-2 on total larval duration

Treatment	Total larval duration (h)													
	March - April rearing							June - July rearing						
	Concentration							Concentration						
	1%	2%	3%	4%	5%	Treatment mean	% ↓ over Ab. control	1%	2%	3%	4%	5%	Treatment mean	% ↓ over Ab. control
Spirulina	615.53	616.40	618.50	620.23	622.33	618.60	-6.14	681.70	682.60	685.53	690.53	694.20	686.91	-4.72
Azolla	606.93	604.33	606.00	610.43	612.83	608.11	-7.74	674.73	672.03	673.73	676.27	678.37	675.03	-6.37
Yeast	620.10	618.93	616.60	615.00	613.37	616.80	-6.42	687.30	684.60	682.60	681.03	679.43	682.99	-5.27
Soy milk	610.27	612.27	614.20	616.10	618.00	614.17	-6.82	677.13	679.47	681.70	683.20	685.60	681.42	-5.49
Water control	620.50	620.50	620.50	620.50	620.50	620.50	-5.86	685.33	685.33	685.33	685.33	685.33	685.33	-4.94
Absolute control	659.10	659.10	659.10	659.10	659.10	659.10	-	720.97	720.97	720.97	720.97	720.97	720.97	-
Concentration mean	622.07	621.92	622.48	623.56	624.36	622.88	-	687.86	687.50	688.31	689.56	690.65	688.78	-
	S.Em ±			CD @ 1%				S.Em ±			CD @ 1%			
Treatment (A)	0.21			0.56				0.22			0.59			
Concentration (B)	0.19			0.51				0.20			0.53			
Interaction (A×B)	0.47			1.24				0.50			1.32			

Note: % ↓ over Ab. control denotes per cent decrease over absolute control.

Silk gland weight (g)

The silk gland weight was highest in azolla (0.973 g for June-July rearing) and (0.949 g for March-April rearing) which was followed by mulberry leaves supplemented with soy milk, yeast and *spirulina* (0.889 to 0.950 g for June-July rearing) and (0.820 to 0.928 g for March-April rearing). Among the concentrations tested, two per cent gave significantly higher silk gland weight (0.908 g for June-July rearing) and (0.869 g for March-April rearing) (Table 4). The interaction of feed additives along with concentration indicated significantly highest silk gland weight was observed in azolla at two per cent concentration (0.991 g) and was on par with three per cent concentration (0.987 g) during June-July rearing. Further, the interaction effect indicated that azolla at two per cent concentration gave significantly highest silk gland weight (0.970 g) during March-April rearing. The nutrients supplemented through mulberry leaves in terms of probiotics might have contributed to the additional growth of silkworm, thereby increasing the body weight and silk gland weight and this might be due to stimulatory effect on protein synthesizing machinery of silkworm and silk gland cells. The variation in the response of probiotics and their concentration might be attributed to supplementation of nutrients which in turn might have played role on the metabolic process in silkworm. The protein is effective only up to an optimal level beyond which the larval growth is impeded considerably and accordingly at higher concentration of probiotics the response was marginal and this may be attributed to the nature of chemicals including phagostimulants and nutrients which act as deterrents when applied in excess (Narayanaswamy and Ananthanarayana (2006) [23]. Contrary to present findings with regard to *spirulina*, Kumar and Balasubramanian (2014) [17] observed *spirulina* supplementation at higher concentration at five per cent had better response (617.43 mg) when compared to one per cent (485.47 mg).

Table 4: Effect of probiotic feed supplementation to mulberry silk worm, PM × CSR-2 on silk gland weight

Treatment	Silk gland weight (g)													
	March - April rearing							June - July rearing						
	Concentration							Concentration						
	1%	2%	3%	4%	5%	Treatment mean	% ↑ over Ab. control	1%	2%	3%	4%	5%	Treatment mean	% ↑ over Ab. control
Spirulina	0.862	0.853	0.831	0.809	0.788	0.820	11.87	0.923	0.901	0.882	0.861	0.842	0.889	6.72
Azolla	0.942	0.970	0.952	0.932	0.910	0.949	29.47	0.979	0.991	0.987	0.973	0.967	0.973	16.81
Yeast	0.837	0.857	0.877	0.897	0.917	0.877	19.65	0.874	0.893	0.912	0.933	0.951	0.912	9.48
Soy milk	0.957	0.943	0.929	0.912	0.893	0.928	26.60	0.981	0.971	0.963	0.942	0.937	0.950	14.05
Water control	0.830	0.830	0.830	0.830	0.830	0.826	12.69	0.868	0.868	0.868	0.868	0.868	0.863	3.06
Absolute control	0.731	0.731	0.731	0.731	0.731	0.733	-	0.832	0.832	0.832	0.832	0.832	0.833	-
Concentration mean	0.857	0.869	0.853	0.852	0.848	0.856	-	0.906	0.908	0.902	0.901	0.896	0.903	-
	S.Em ±			CD @ 1%				S.Em ±			CD @ 1%			
Treatment (A)	0.002			0.004				0.001			0.002			
Concentration (B)	0.003			0.009				0.003			0.009			
Interaction (A×B)	0.006			0.015				0.002			0.005			

Note: % ↑ over Ab. control denotes per cent increase over absolute control.

Table 5: Effect of probiotic feed supplementation to mulberry silk worm, PM × CSR-2 on effective rate of rearing

Treatment	Effective rate of rearing (%)													
	March - April rearing							June - July rearing						
	Concentration							Concentration						
	1%	2%	3%	4%	5%	Treatment mean	% ↑↓ over Ab. control	1%	2%	3%	4%	5%	Treatment mean	% ↑↓ over Ab. control
Spirulina	90.67 (72.23)*	90.00 (71.57)	89.33 (70.94)	88.00 (69.73)	87.33 (69.15)	89.07 (70.72)	-2.84	92.33 (73.93)	91.33 (72.88)	90.67 (72.23)	89.33 (70.94)	88.00 (69.73)	90.33 (71.94)	-4.24
Azolla	93.00 (74.66)	94.67 (76.66)	93.67 (75.43)	92.00 (73.59)	91.00 (72.56)	92.87 (74.59)	1.31	95.33 (77.54)	97.33 (80.64)	96.33 (78.98)	95.00 (77.08)	94.67 (76.66)	95.73 (78.18)	1.48
Yeast	87.67 (69.44)	88.33 (70.03)	89.67 (71.25)	91.67 (73.23)	92.00 (73.57)	89.87 (71.50)	-1.96	90.67 (72.23)	91.00 (72.56)	92.33 (73.93)	93.67 (75.43)	94.00 (75.85)	92.33 (74.00)	-2.12
Soy milk	92.67 (74.30)	91.33 (72.88)	90.67 (72.22)	89.00 (70.63)	88.67 (70.33)	90.47 (72.07)	-1.31	95.00 (77.08)	94.00 (75.82)	93.33 (75.05)	92.67 (74.30)	91.67 (73.23)	93.33 (75.10)	-1.06
Water control	87.67 (69.46)	87.67 (69.46)	87.67 (69.46)	87.67 (69.46)	87.67 (69.46)	87.67 (69.46)	-4.36	91.00 (72.56)	91.00 (72.56)	91.00 (72.56)	91.00 (72.56)	91.00 (72.56)	91.00 (72.56)	-3.53
Absolute control	91.67 (73.23)	91.67 (73.23)	91.67 (73.23)	91.67 (73.23)	91.67 (73.23)	91.67 (73.23)	-	94.33 (76.24)	94.33 (76.24)	94.33 (76.24)	94.33 (76.24)	94.33 (76.24)	94.33 (76.24)	-
Concentration mean	90.56 (72.22)	90.61 (72.31)	90.44 (72.09)	90.00 (71.64)	89.72 (71.38)	90.27 (71.93)	-	93.11 (74.93)	93.17 (75.12)	93.00 (74.84)	92.67 (74.42)	92.28 (74.05)	92.84 (74.67)	-
	S.Em ±			CD @ 1%				S.Em ±			CD @ 1%			
Treatment (A)	0.28			0.73				0.29			0.78			
Concentration (B)	0.25			0.67				0.26			0.70			
Interaction (A×B)	0.62			1.64				0.65			1.73			

Note: % ↑↓ over Ab. control denotes per cent increase or decrease over absolute control.
*Figures in the parentheses are arc sin transformed values.

Effective rate of rearing (ERR %)

Maximum ERR of (95.73% during June-July rearing) and (92.87% during March-April rearing) was recorded in case of silkworms fed with azolla supplemented leaves. The next best ones were absolute control and soy milk (94.33 and 93.33% for June-July rearing) and (91.67 and 90.47% for March-April rearing) respectively. Among the five concentrations tested, one to four per cent concentrations gave significantly higher ERR (92.67 to 93.17% in June-July rearing) and (90.00 to 90.61% for March-April rearing). Further, the interaction effect of indicated that azolla at two and three per cent concentration gave significantly higher ERR (97.33 and 96.33%) during June-July rearing and (94.67 and 93.67%) in March-April rearing (Table 5). The present results are in line with the results of Vijaykumar *et al.* (2016) [34] who reported that the use of aqueous solution of azolla at 50 per cent and 25 per cent concentrations had the maximum ERR of 97.16 per cent and 96.33 per cent, respectively. The lowest ERR was found in absolute control (85.27%); Anitha *et al.* (2015) [2] found Dorlac at two per cent had higher ERR in eri silkworms (95.00%) when compared to control (85.00%); Sampath *et al.* (2013) [29] reported that eri silkworms fed with *Anabaena variabilis* at 500 ppm concentration showed significantly maximum ERR (71.95%) as compared to control (39.78%) and Rekha and Neelu (2010) observed maximum ERR of 90.02 per cent when supplemented with soybean and in absolute control the same was minimum (82.55%). The increased ERR observed in the present study may be due to lower incidence of diseases in the treated silkworms and this might be due to influence of feed additives containing protein, amino acids, carbohydrates and minerals which act as building blocks of tissues making them robust and thus imparted healthiness to larvae. Further, due to better growth and development on account of nutritious feed received during silkworm rearing. The lowest ERR in *spirulina* might be due to increased concentration of *spirulina* supplementation to mulberry leaves resulted in poor feeding and reduced survival rate.

Azolla supplementation increased fifth instar larval weight to the tune of 25.63 and 29.96 per cent over absolute control during both the rearings. Azolla supplementation gave shorter fifth instar larval and total larval duration to the tune of -10.73 & -14.34 and -6.37 & -7.74 per cent over absolute control during both the rearings respectively. Silk gland weight was increased to the tune of 16.81 and 29.47 per cent due to azolla supplementation over absolute control during both the rearings. During June-July rearing and March-April rearing azolla supplementation enhanced ERR to the tune of 1.48 and 1.31 per cent over absolute control.

It is concluded that the mulberry silkworm hybrid, PM × CSR-2 reared on mulberry leaves supplemented with two per cent azolla once every day in the morning from fourth instar till spinning stage exhibited superiority for larval traits in both the rearings followed by soy milk, yeast and *spirulina*. Hence, azolla as a feed supplement at two per cent concentration may be conveniently exploited after large scale trials to enhance the larval growth and development parameters.

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