

IMPACT OF ROAD DUST POLLUTED MULBERRY LEAVES ON THE FOOD INGESTION, ASSIMILATION AND CONVERSION EFFICIENCY OF SILKWORM, *BOMBYX MORI* L. IN KASHMIR VALLEY

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ABSTRACT : The present investigation was carried out to ascertain the possible effect of feeding the silkworm, *Bombyx mori* L. with road dust polluted mulberry leaves on the food consumption, assimilation and conversion efficiency using silkworm hybrids, SH6 × NB4D2, CSR2 × CSR4, DUN22 × DUN6 and CSR double hybrid in relation to the road dust from the National Highway 1A at Pampore, Kashmir, India. The roads generate vast amount of dust which got deposited on the mulberry leaves. As the distance from road to mulberry plots decreased the dust deposition on leaves increased. Impact on the silkworm nutritional indices - food consumption, assimilation and conversion efficiency was assessed by rearing the silkworm larvae on dust polluted mulberry leaves besides the amount of dust deposition on the mulberry leaves was also measured. The food consumption, assimilation and conversion efficiency of the silkworm larvae got significantly reduced in both the 4th as well as in the 5th instar, when they were fed with extreme dust polluted mulberry leaves as compared to those larvae which were fed with low dust polluted mulberry leaves. But the effect was more pronounced in the 5th instar as larvae utilize maximum amount of feed in the 5th instar. The reduced conversion efficiency culminated in the decreased average larval weight and average larval weight gain of the silkworm larvae. All the silkworm hybrids register a marked affect when they were fed with extreme dust polluted mulberry leaves, but CSR2 × CSR4 hybrid was affected most with the CSR double hybrid least affected.

Keywords: Assimilation, Hybrid, Larvae, Mulberry, Road Dust, Silkworm.

INTRODUCTION

Sericulture is the practice of rearing of silkworms for the production of raw silk. Sericulture is both an art as well as a science for the production of silk and has been practiced for thousands of years [1]. Sericulture is an agro based industry with a vast potential of employment generation for both rural as well as urban areas. Although there are several commercial species of silkworm, *Bombyx mori* is the most widely used and intensively studied, and practices and techniques for its rearing are the most developed [2]. This insect is the only living species in its family, Bombycidae, and has been domesticated for so long time that it probably no longer exists in the wild [3].

The mulberry silkworm, *Bombyx mori* L. is a monophagous insect which feeds exclusively on the mulberry (*Morus spp.*) leaves for its growth and development. Mulberry leaf quality and quantity has a direct impact on the overall performance of the silkworm such as larval and cocoon weight, amount of silk production, pupation, and reproductive traits. The efficiency of converting the ingesta and digesta into body, cocoon and cocoon shell varies with the silkworm breeds under the influence of environment, mulberry varieties and seasons [4]. Nutritional quality of mulberry leaves has a pivotal role to play on the quantum of ingesta, digesta and digestibility of food among silkworms [5]. The quality and quantity of ingesta and digesta ensures the proper growth and silk production in silkworms [6]. Environmental pollution is turning to be an another constraint which affects the quality and quantity of the mulberry leaves and feeding these polluted mulberry leaves affects the silkworm growth as well as cocoon and silk production [7].

Sericulture in Kashmir valley is being sustained by mulberry trees mainly existing on the road sides, river bunds, canals, farm/garden boundaries/fences, etc in an erratic form. Soils of Kashmir valley are predominantly alfisols which are susceptible to dust erosion during clear sunny and windy days which generates large amount of dust. Continuous and unending movement of vehicles adds to the problem with heavy amounts of dust pollution being generated along the roads of Kashmir valley. National highway 1A, which connects Srinagar, the summer capital of the state with rest of India via Jammu, witnesses a huge traffic load comprising of heavy commercial vehicles, light commercial vehicles, convoy and patrolling wagons of Indian army and Central Reserve Police Force besides motor vehicles of Kashmir inhabitants and tourists; counting to millions per annum and increasing. Mulberry leaf is known to have a tendency to attract the dust and particulate matter present in air and thus a preferred plant to assess the atmospheric air pollution especially particulate matter in urban environments [8]. Kashmir has been conferred with the salubrious climate which is highly conducive for sericulture. Besides facing various devastating factors of biotic and abiotic origin, the dust generated by vehicular movement on the roads of valley is emerging as one more limiting factor for quality mulberry leaf production in Kashmir valley. Feeding these dust polluted mulberry leaves to silkworm is certain in sericulture particularly in Kashmir valley where silkworm farmers fetch leaves from the mulberry trees, which mainly exist along roads. When these dust polluted mulberry leaves are finally consumed by *B. mori*, it may cause stressful condition for its growth and development, affects its performance and making it susceptible to various pathogens. The road dust polluted mulberry leaves may also affect the food consumption of the silkworms, hence can drastically affect its growth and development. Though a lot of studies have been carried out on the effect of road dust on agricultural and horticultural crops, the studies related to the mulberry and silkworm is very meagre [9]. In this regard an effort was made to assess the effect of the road dust polluted mulberry leaves on the food consumption, assimilation, conversion efficiency and growth of the silkworm, *Bombyx mori* L.

MATERIALS AND METHODS

The present investigation was carried out at Central Sericulture Research and Training Institute (CSR & TI), Central Silk Board, Pampore, Jammu and Kashmir, India located at an altitude of 1574 meters above mean sea level; 74.93° E longitude and 34.02° N latitude during spring 2013. Two plots of mulberry dwarf plantations with Goshoerami variety were selected for dust measurement and feeding the silkworms based on their proximity to the NH -1A and plots were named as site A located within 30m and site B (beyond 150m). Dust deposited on mulberry leaves in these plots was measured from 10 randomly selected mature leaves after washing off the dust and measuring the freshly deposited dust at 24 hours intervals for seven consecutive days [10].

Silkworm hybrids, SH6 × NB4D2, CSR2 × CSR4, DUN22 × DUN6 and CSR double hybrid were reared on the dust polluted mulberry leaves following the standard rearing procedure [11] to know the effect of road dust pollution on the food consumption, assimilation and conversion efficiency of silkworm. Two disease free layings (DFLs) of each race were kept in rearing trays; in which one set of trays contains the neonates from each race and were fed with the extreme dust polluted mulberry leaves and the another set of trays contains the neonates from each race were fed with the low dust polluted mulberry leaves, making the total number of rearing trays upto eight. After the resumption from the 3rd molt, the larvae in each tray were divided into three replicates by keeping hundred larvae in each tray, making the total number of rearing trays up to twenty four and the remaining larvae were maintained as reserved stock. One batch of twelve trays were fed with extreme dust polluted mulberry leaves and the other batch of twelve trays were fed with low dust polluted mulberry leaves. The young larvae (1st-3rd instars) were reared at 26-28 °C with 80-90% RH, and late age larvae (4th and 5th instars) were maintained at 24-26 °C with 70-80% RH. The feed utilization studies were carried out in the fourth and fifth instar as highest amount of food is consumed in these two instars by the silkworm larvae. The accurately weighted fresh mulberry leaves were fed three times a day to the experimental batches. Samples of mulberry leaves used for each feeding were taken as dummies for dry weight determination of ingested food. The healthy larvae were counted daily in each replication of every treatment, and unequal, unhealthy, and dead larvae were removed upon discovery and replaced from additional batches maintained separately. Observations on weight of leftover leaf and excreta were recorded daily on dry weight basis after oven drying at a constant temperature of 80 °C whereas the weight gain of the larvae was recorded on fresh weight basis [12]. Different nutritional parameters such as ingested food, digested food, reference ratio, growth rate, ingested food conversion efficiency, approximate digestibility (AD), consumption index (CI) and coefficient of metabolism (COM) were calculated by following the Gravimetric method [13]. Average larval weight and average larval weight gain was also calculated to assess the effect of the polluted mulberry leaves on the growth of the silkworm larvae. Following equations were used to calculate various nutritive parameters [13].

Ingested food (g). Total intake of the dry weight (g) of mulberry leaves by silkworm larvae during the 4th and 5th instars up to spinning or ripening stage.

Ingested food (IF) = Dry weight of leaf given – Dry weight of leftover leaf

Digested food (g). Total assimilated dry food from the intake or ingesta of dry weight of mulberry leaves by silkworm larva during the 4th and 5th stage until spinning or ripening

Digested food (DF) = Dry weight of food ingested – Dry weight of faeces

Consumption index. Consumption the rate of food intake to the mean weight of the larvae during the feeding period.

$$\text{Consumption index (CI)} = \frac{\text{Ingested food}}{\text{Mean fresh larval weight in the 4}^{\text{th}} \text{ or } 5^{\text{th}} \text{ instar} \times \text{larval duration (Days)}}$$

Reference ratio (g). An indirect expression of absorption and assimilation of food. Denotes the ingesta required per unit excreta produced

$$\text{Reference ratio (RR)} = \frac{\text{Dry weight of food ingested}}{\text{Dry weight of excreta}}$$

Growth rate. Growth rate refers to larval gain biomass and indicates the efficiency of conversion of nutrition into larval biomass.

$$\text{Growth rate (GR)} = \frac{\text{Fresh weight gained by worms}}{\text{Duration of feeding (days)} \times \text{Mean dry weight of the worms during feeding period}}$$

Approximate digestibility(%). Directly indicates the assimilation efficiency of mulberry leaves and depends on the passage rate of food through gut in silkworm.

$$\text{A.D.} = \frac{\text{Weight of food ingested} - \text{weight of faeces}}{\text{Weight of food ingested}} \times 100$$

Ingested food conversion efficiency (%). Associated with the efficiency conversion of ingested nutrition into biomass or body matter at different stages and expressed in percentage.

$$\text{IFCE} = \frac{\text{Fresh weight gained by larvae}}{\text{Dry weight of food ingested}} \times 100$$

Coefficient of metabolism (COM). Measure of total metabolic activity associated with the efficiency of silkworm larvae in converting digested food into larval biomass.

$$\text{COM} = \frac{\text{Weight of food digested} - \text{Increase in weight of larva}}{\text{Weight of food digested}} \times 100$$

$$\text{Average larval weight (ALWt)} = \frac{\text{Total larval weight}}{\text{Total number of the larvae}}$$

$$\text{Average larval weight gain (ALWtG)} = \frac{[\text{Total larval weight in the}] - [\text{Total larval weight in}]}{\text{Total number of the larvae}}$$

Previous instar the present instar

Statistical analysis

The resultant data on dust deposition, food consumption, assimilation, conversion efficiency and larval growth were analyzed deploying the factorial design after necessary transformations, using *MSTATC*[®] application.

RESULTS

The observations related to the deposition of the road dust on the mulberry leaves shows that varied amount of dust deposition takes place on the mulberry leaves in proportion to the distance from the national highway NH-1A (Table 1). The deposition of the dust on the mulberry leaves decreases proportionately as the distance from the road increases. Highest amount of road dust deposition on the mulberry leaves was observed at Site A located within 30 m proximity from the highway and were significantly highest than the Site B. The dust concentration on the leaves from both the plots increases as the period of exposure increases, but the dust accumulation on the leaves from the site A is far more than the leaves from site B. For our convenience in the further study, the leaves from the site A were regarded as the extreme dust polluted leaves (EDP) and the leaves from the site B were regarded as the low dust polluted leaves (LDP).

Table 1: Dust Deposition on Mulberry leaves at two test sites in CSR & TI, Pampore (During May-June, 2013)

Sites	Amount of Dust Deposited on the mulberry leaves (g/ 10 leaves)							
	Duration (in Days)							
	1	2	3	4	5	6	7	Average ± S.D
Site A	1.65	2.03	2.22	2.66	2.87	3.12	3.35	2.55 ± 0.61
Site B	0.79	0.99	1.07	1.26	1.39	1.58	1.72	1.25 ± 0.33

The effect of the road dust polluted mulberry leaves on the food ingestion, assimilation and the conversion efficiency of the silkworm, *Bombyx mori* L. in the 4th as well as in the 5th instar is shown in the table 2 and 3. The effect of the dust polluted mulberry leaves on the ingested food by the silkworm larvae was quite significant. The silkworm larvae in both the 4th as well as in the 5th instar which were fed with EDP leaves recorded a lowest ingested food as compared to the larvae which were fed with the LDP mulberry leaves. The lowest amount of ingested food in the 4th instar was recorded in the larvae of CSR double hybrid which were fed with EPD leaves and the highest amount of ingested food was recorded in the larvae of SH₆×NB₄D₂ which were fed with LDP leaves. In the 5th instar, the lowest amount of ingested food was recorded in the larvae of CSR₂ × 4 which were fed with EDP leaves and the highest amount of ingested food was observed in the larvae of CSR double hybrid larvae which were fed with LDP leaves. This significant difference in the ingested food may be due to the apathy of the silkworm towards the less nutritious dust polluted mulberry leaves. The reduced amount of ingested food paves the way for other nutritional parameters which are affected by the extreme dust polluted mulberry leaves.

The digested food directly depends on the ingested food by the silkworm larvae. The digested food in the 4th instar ranges from 14 - 30.67 g for the larvae which were fed with EDP mulberry leaves and in the range of 17.87 - 35.57 g for the larvae which were fed with LDP leaves. In the 4th instar, the highest digested food was observed in the larvae of SH₆ × NB₄D₂ and CSR₂ × 4 fed with LDP and the lowest amount of digested food was observed in the larvae of CSR double hybrid fed with EDP leaves. In the 5th instar, the digested ranges from 89.58 - 137.42 g for the larvae which were fed with EDP mulberry leaves and in the range of 116.55 - 173.38 g for the larvae which were fed with LDP leaves. In the 5th instar, the highest digested food was observed in the larvae of SH₆ × NB₄D₂ fed with LDP and the lowest amount of digested food was observed in the larvae of CSR double hybrid fed with EDP leaves. The amount of the digested food shows a decline when the larvae were fed with the extreme dust polluted mulberry leaves. The reference ratio observed was highest in the larvae fed with EDP mulberry leaves both in the 4th as well in the 5th instar as compared to the larvae fed with LDP mulberry leaves. In the 4th instar, the highest reference ratio value was observed in the larvae of DUN 22 × 6 (2.38 g) fed with EDP leaves and the lowest amount of reference ratio was observed in the larvae of CSR double hybrid (1.54 g) fed with LDP leaves. In the 5th instar, the highest reference ratio value was observed in the larvae of CSR₂ × 4 (2.45 g) fed with EDP leaves and the lowest amount of reference ratio was observed in the larvae of CSR double hybrid (1.47 g) fed with LDP leaves.

The highest growth rate was observed in the larval batches which were fed with LDP mulberry leaves as compared to the larval batches fed with EDP mulberry leaves. In the 4th instar, the highest growth rate was observed in the larvae of SH₆×NB₄D₂ (20.38) fed with LDP leaves and the lowest growth rate was observed in the larvae of CSR double hybrid (15.16) fed with EDP leaves. In the 5th instar, the highest growth rate was observed in the larvae of DUN 22 × 6 (13.87) fed with LDP leaves and the lowest growth rate was observed in the larvae of CSR double hybrid (10.04) fed with EDP leaves.

Table 2: Effect of Road Dust Polluted Mulberry Leaves on the Nutritional Parameters of Silkworm Larvae in the 4th Instar

4 th Instar Hybrids	IF			DF			RR			GR			IFCE																	
	EDP	LDP	Mean	EDP	LDP	Mean	EDP	LDP	Mean	EDP	LDP	Mean	EDP	LDP	Mean															
SH ₆ ×NB ₄ D ₂	53.27 ^c	63.67 ^a	58.47 ^a	30.20 ^c	35.57 ^a	32.88 ^a	2.37 ^e	2.20 ^{cd}	2.29 ^b	17.56 ^d	20.38 ^a	18.97 ^a	67.13 ^{cd}	83.71 ^{bc}	75.44 ^b															
CSR ₂ × 4	48.47 ^d	59.13 ^b	53.80 ^b	27.23 ^d	35.57 ^{bc}	29.90 ^b	2.29 ^{de}	2.15 ^c	2.23 ^b	16.92 ^d	19.45 ^{bc}	18.19 ^b	63.37 ^d	76.55 ^{cd}	69.96 ^c															
DUN 22 × 6	52.80 ^c	62.00 ^a	57.40 ^a	30.67 ^c	34.10 ^{ab}	32.38 ^a	2.38 ^e	2.19 ^c	2.29 ^b	16.99 ^d	20.12 ^{ab}	18.50 ^b	71.14 ^{ab}	87.64 ^b	79.39 ^b															
CSR Double Hybrid	35.43 ^e	46.37 ^d	40.90 ^e	14.00 ^f	17.87 ^e	15.93 ^e	1.73 ^b	1.54 ^a	1.64 ^a	15.16 ^e	18.73 ^c	16.95 ^e	87.16 ^b	116.53 ^a	101.84 ^a															
Mean	47.49	57.79		25.53	30.03		2.20	2.02		16.66	19.64		72.21	91.12																
LSD (p=0.05)	2.447			1.730			2.779			1.979			0.09481			0.06704			0.7643			0.5405			5.091			7.20		

4 th Instar Hybrids	CI			COM			ALWt			ALWtG														
	EDP	LDP	Mean	EDP	LDP	Mean	EDP	LDP	Mean	EDP	LDP	Mean	EDP	LDP	Mean									
SH ₆ ×NB ₄ D ₂	25.91 ^{cd}	24.24 ^d	25.08 ^c	0.18 ^e	0.50 ^{cd}	0.34 ^b	0.49 ^d	0.66 ^c	0.58 ^b	0.36 ^c	0.53 ^a	0.45 ^b												
CSR ₂ × 4	26.70 ^d	25.24 ^e	26.06 ^d	0.12 ^e	0.39 ^{cd}	0.26 ^b	0.43 ^e	0.59 ^d	0.51 ^c	0.31 ^d	0.45 ^b	0.38 ^d												
DUN 22 × 6	23.87 ^d	22.52 ^c	23.19 ^b	0.22 ^d	0.59 ^c	0.41 ^b	0.53 ^e	0.68 ^b	0.61 ^a	0.38 ^c	0.54 ^a	0.46 ^a												
CSR Double Hybrid	17.28 ^b	15.76 ^a	16.53 ^a	1.21 ^b	2.03 ^a	1.62 ^a	0.44 ^e	0.72 ^a	0.58 ^b	0.31 ^d	0.54 ^a	0.43 ^c												
Mean	23.44	21.99		0.44	0.88		0.47	0.66		0.34	0.52													
LSD (p=0.05)	1.151			0.8137			0.2896			0.2048			7.20			5.09			0.0173			0.0122		

Figures in a column superscripted by same alphabet(s) are not different from each other by least significant difference at p=0.05

Table 3: Effect of Dust Polluted Mulberry Leaves on the Nutritional Parameters of Silkworm Larvae in the 5th Instar

5 th Instar Hybrids	IF			DF			RR			GR			IFCE																	
	EDP	LDP	Mean	EDP	LDP	Mean	EDP	LDP	Mean	EDP	LDP	Mean	EDP	LDP	Mean															
SH ₆ ×NB ₄ D ₂	235.54 ^a	310.33 ^b	272.09 ^b	130.61 ^c	173.38 ^a	152.00 ^a	2.34 ^e	2.17 ^{de}	2.26 ^c	12.87 ^b	13.81 ^a	13.34 ^a	75.85 ^d	83.00 ^c	79.43 ^b															
CSR ₂ × 4	186.47 ^f	292.47 ^c	239.47 ^c	110.18 ^d	154.21 ^b	132.19 ^b	2.45 ^e	2.11 ^{cd}	2.28 ^c	12.03 ^c	13.55 ^a	12.79 ^c	68.21 ^e	81.41 ^c	74.81 ^c															
DUN 22 × 6	239.68 ^d	310.43 ^b	275.06 ^b	137.42 ^c	154.31 ^b	145.87 ^a	2.30 ^{de}	1.98 ^c	2.14 ^b	12.31 ^c	13.87 ^a	12.31 ^b	70.83 ^e	94.40 ^b	82.62 ^{ab}															
CSR Double Hybrid	226.68 ^e	361.23 ^a	293.92 ^a	89.58 ^e	116.55 ^d	103.06 ^c	1.68 ^b	1.47 ^a	1.58 ^a	10.04 ^d	11.20 ^d	10.62 ^d	79.35 ^{cd}	88.93 ^b	84.14 ^a															
Mean	222.07	318.62		116.95	149.61		2.19	1.94		11.81	13.12		73.56	86.94																
LSD (p=0.05)	9.156			6.475			11.50			8.130			0.1448			0.1024			0.3947			0.2791			4.672			3.304		

5 th Instar Hybrids	CI			COM			ALWt			ALWtG														
	EDP	LDP	Mean	EDP	LDP	Mean	EDP	LDP	Mean	EDP	LDP	Mean												
SH ₆ ×NB ₄ D ₂	16.40 ^{cd}	15.58 ^{bc}	15.99 ^b	0.48 ^c	0.64 ^c	0.562 ^b	2.47 ^e	3.13 ^c	2.78 ^b	1.94 ^d	2.47 ^c	2.21 ^c												
CSR ₂ × 4	17.66 ^d	16.63 ^{cd}	17.15 ^c	0.15 ^d	0.54 ^c	0.347 ^c	1.71 ^e	2.83 ^d	2.27 ^c	1.27 ^d	2.38 ^c	1.83 ^d												
DUN 22 × 6	17.41 ^{de}	14.70 ^b	16.06 ^b	0.23 ^d	0.90 ^b	0.565 ^b	2.23 ^d	3.41 ^b	2.82 ^b	1.69 ^e	2.93 ^b	2.31 ^b												
CSR Double Hybrid	12.66 ^a	12.30 ^a	12.48 ^a	1.07 ^b	1.76 ^a	1.385 ^a	2.24 ^f	3.75 ^a	2.99 ^a	1.80 ^e	3.21 ^a	2.51 ^a												
Mean	16.03	14.80		0.468	0.961		2.153	3.280		1.677	2.749													
LSD (p=0.05)	0.9943			0.7031			0.1974			0.1395			0.1341			0.09481			0.1224			0.8654		

Fig

ures in a column superscripted by same alphabet(s) are not different from each other by least significant difference at p=0.05

Ingested food conversion efficiency is an important physiological parameter which shows the conversion of the leaf matter into the body matter of the silkworm. The IFCE follows the same trend as the other parameters that is highest ingested food conversion efficiency was observed in the silkworm batches which were fed with low dust polluted mulberry leaves as compared with the larval batches which were fed with extreme dust polluted mulberry leaves. In the 4th instar, the highest IFCE was recorded in the larvae of CSR double hybrid (116.53 g) fed with LDP leaves and the lowest IFCE was recorded in the larvae of DUN 22 × 6 (63.37) fed with EDP leaves. In the 5th instar, the highest IFCE was observed in the larvae of DUN 22 × 6 (94.40) fed with LDP leaves and the lowest IFCE was observed in the larvae of CSR₂ × 4 (68.21) fed with EDP leaves.

Consumption index relates the rate of food intake with the mean weight gain of the larvae during the feeding period. The observed consumption index was highest in the silkworm batches which were fed with the extreme dust polluted mulberry leaves as compared with the larvae which were fed with the low dust polluted mulberry leaves. In the 4th instar, the highest consumption index was observed in the larvae of CSR₂ × 4 (26.70) fed with EDP leaves and the lowest consumption index was observed in the larvae of CSR Double Hybrid (15.76) fed with LDP leaves. In the 5th instar, the highest consumption index was observed in the larvae of CSR₂ × 4 (17.66) fed with EDP leaves and the lowest consumption index was observed in the larvae of CSR Double Hybrid (12.30) fed with LDP leaves.

Coefficient of metabolism denotes the metabolic activity associated with the conversion of mulberry leaf into the larval biomass. The highest coefficient of metabolism was observed in the silkworm larvae which were fed with the low dust polluted mulberry leaves and the lowest coefficient of metabolism was observed in the silkworm larvae which were fed with extreme dust polluted mulberry leaves. In the 4th as well in the 5th instar, the highest coefficient of metabolism was observed in the larvae of CSR Double Hybrid (2.03 and 1.76) fed with LDP leaves and the lowest coefficient of metabolism was observed in the larvae of CSR₂ × 4 (0.12 and 0.15) fed with EDP leaves.

The highest average larval weight was recorded in the larvae of CSR Double Hybrid followed by DUN 22 × 6, SH₆ × NB₄D₂ and CSR₂ × 4 fed with low dust polluted mulberry leaves both in the fourth as well as in the 4th instar. The lowest average larval weight was recorded in the larvae of CSR₂ × 4, followed by CSR Double Hybrid, SH₆ × NB₄D₂ and DUN 22 × 6 fed with extreme dust polluted mulberry leaves in the fourth instar. In the 5th instar, lowest average larval weight was recorded in the larvae of CSR₂ × 4, followed by DUN 22 × 6, CSR Double Hybrid and SH₆ × NB₄D₂ fed with extreme dust polluted mulberry leaves.

The highest average larval weight gain was recorded in the larvae of CSR Double Hybrid and DUN 22 × 6 followed by SH₆ × NB₄D₂ and CSR₂ × 4 fed with low dust polluted mulberry leaves in the fourth instar. And in the 5th instar, highest average larval weight gain was recorded in the larvae of CSR Double Hybrid followed by DUN 22 × 6, SH₆ × NB₄D₂ and CSR₂ × 4 fed with low dust polluted mulberry leaves. The lowest average larval weight gain was recorded in the larvae of CSR₂ × 4 and CSR Double Hybrid followed by SH₆ × NB₄D₂ and DUN 22 × 6 fed with extreme dust polluted mulberry leaves in the fourth instar. In the 5th instar, lowest average larval weight gain was recorded in the larvae of CSR₂ × 4, followed by DUN 22 × 6 fed, CSR Double Hybrid and SH₆ × NB₄D₂ fed with extreme dust polluted mulberry leaves.

DISCUSSIONS

Mulberry leaf quality (38.2%) along with climate (37.0%) is one of the important contributing factors in the production of good quality cocoons and silk [14]. So the cultivation of the mulberry plants used in the rearing of the silkworms should be healthy enough to ensure a good quality silk production. Solid matter comprised of soil, natural biogenic materials and anthropogenic metallic constituents is called as dust [15-16]. Automobiles introduce a number of pollutants and dust on both sides of the road [17]. Road dust is a natural sink of organic and inorganic contaminants viz., toxic pollutants, heavy metals and hydrocarbons [18]. Road dust also contains various toxic metals like cadmium, chromium, lead, copper, nickel and zinc [16]. With a blind rush in the traffic movement on the roads, the heavy metal levels in the plants along the roadsides have significantly increased [19]. It has been well established that, insects fed on road side plants have accumulated significantly higher lead owing to higher lead content in the host plants than the controls [20]. In Kashmir, the chance of getting the road dust polluted mulberry leaves are either or both during commercial chawki rearing centers under the government control as well in the late age rearing carried out at farmer's level. Findings of the present study enunciates the fact that mulberry plantations located along busy National Highway 1A are prone to air pollution of large dimensions in the form of road dust, particulate matter, automobile exhaust etc.

The studies showed that the feeding of dust polluted mulberry leaves significantly affects the nutritional parameters of the silkworm. The decline in the ingested food by the larvae in both the 4th as well in the 5th instar is probably due to the apathy of the silkworm for the dust polluted mulberry leaves. The reduced tendency of the silkworm larvae towards polluted mulberry leaves paves the way for other nutritional parameters which get affected by the dust polluted mulberry leaves. The reduced ingested food resulted in the reduction in the digested food.

The reference ratio in the silkworm batches was found to be very large which were fed with extreme dust polluted mulberry leaves depicting the reduced absorption and assimilation capacity of the silkworm larvae. The greater reference ratio means the larvae consumes more amounts of the mulberry leaves to derive the nutrition which could have otherwise obtained from a small amount of mulberry leaves if the leaves were free from the dust pollution. Moreover the polluted mulberry leaves were found to affect the metabolic activity of the silkworm reducing its efficiency to derive maximum gains from the leaf matter. The effect of the polluted mulberry leaves on the consumption index of the silkworm larvae further reveals the nutritional inadequacy of the mulberry leaves polluted with the dust. The large amount of the polluted leaves required by the larvae to synthesize larval biomass means that extra leaves are required to fed to the larvae which add to the cost of the production, which declines the profitability. These findings found the support from the studies that nutritional quality and the quantity affects nearly all biological processes including the rates of biochemical and physiological processes [21-22], and eventually can affect the larval quality or quantity of cocoon crops in the silkworm. In this regard, there is a scope for investigating the effect of road dust polluted mulberry leaves on the biochemical and the nutritive parameters of the mulberry leaves which forms the sole food for the silkworm.

The effect of the polluted mulberry leaves on these nutritional indices culminated into the reduced conversion efficiency of the silkworm larvae which consequently results in reduced average larval weight and average larval weight gain of the silkworm. The effect of the polluted mulberry leaves on the conversion efficiency of the silkworm is very important as it determines the performance and growth of the silkworm larvae which subsequently affects the productivity of the silkworm. This can be elucidated from the studies that efficiency of conversion of ingesta and digesta are the ultimate indices to evaluate nutritional efficiency of a silkworm breed or hybrid and to assess the nutritional quality of the feed used [23-27].

The observations related to the effect of the road dust on the quality of the mulberry leaf clearly challenge its suitability for the rearing of the silkworm. Food consumption, assimilation, conversion efficiency and growth of the silkworm show a marked decline due to the feeding of the silkworms with extreme dust polluted mulberry leaves. The reason for the decline in the food consumption, assimilation and the conversion efficiency of the silkworm may be due to less palatability of the mulberry leaves and stress conditions accrued to the larvae upon feeding. The effect of the extreme dust polluted mulberry leaves on the silkworms was more pronounced in the 5th instar as the silkworm consumes the highest amount of food in the 5th instar. The decline in the growth and conversion efficiency of the silkworm in the 5th instar can have derogatory effect on the productivity of the silkworm as this instar culminates in the production of the cocoons by the silkworms and determines the success and failure of the crop. These findings borrow the support from a study which reported that the dust polluted mulberry leaves causes silkworm mortality, increases microbial disease susceptibility of the silkworm and affects the larval, silk and cocoon characters of the silkworm [28].

When EDP leaves were fed to silkworms, the food consumption, conversion efficiency and growth of the larvae was reduced, clearly depicting the adverse effect of dust, which is proportional to the amount of dust consumed by the larvae along with leaf. The reduced growth of the larvae fed with dust polluted mulberry leaves may in turn affect the productivity of the crop thus affecting the economic gains of the produce.

A similar study related to the mulberry plots located near NH-34 in West Bengal reported the similar observations [9]. The study suggested identifying the mulberry varieties which will be suitable for cultivation in polluted areas and can promisingly address the problems faced by sericulture in these challenged environments. Although some information is available on the harmful effect of components of air pollution on mulberry and silkworm like Polynuclear Aromatic Hydrocarbons [29], fluorides [7, 30-33] and sulphur [30]; no knowledge is available about the impact of road dust and its toxic constituents such as lead, cadmium, etc on mulberry as well as silkworm. In that case, it is inevitable to cultivate mulberry plantations away from roads for sericulture to ensure better quality leaf. In conditions like Kashmir, where the mulberry plantations are grown along the roads in a scattered manner; which is the main leaf source for sericulture and automobile and tourism sectors are registering a hike day by day by leaps and bounds, it is required to alleviate the harmful effect of dust pollution on mulberry and sericulture to revert back the downslide of this industry for the last few decade in the state.

CONCLUSION

The present study conducted to assess the effect of the road dust polluted mulberry leaves on various nutritional indices of the silkworm hybrids SH6 × NB4D2, CSR2 × CSR4, DUN22 × DUN6 and CSR double hybrid during spring 2013 at CSR&TI, Pampore, Kashmir, India, reveals that the road dust polluted mulberry leaves leads to reduced food consumption, assimilation, conversion efficiency and growth of the silkworm larvae in both 4th as well as in the 5th instar, but the effects were more significant in the 5th instar. It also results in the reduced weight of the mature larvae which can in turn affect the productivity of the silkworm. The effect of the road dust polluted mulberry leaves on the nutritional indices and growth of the silkworm larvae is a serious cause of concern as the majority of the sericulture units are dependent on the mulberry plantations existing along the road sides. In that scenario, the possible harmful effects of the polluted mulberry leaves on the sericulture cannot be ruled out. So as to mitigate the effects of the road dust on the sericulture, various preventive and management strategies are required to formulate which can substantially alleviate the effect of the polluted mulberry leaves on the sericulture so as to ensure the sustainability of this vital industry and make it more promising.

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REFERENCES

- [1] Tamura, T., Thibert, C., Royer, C., Kanda, T., Abraham, E., Kamba, M., Komoto, N., Thomas, J. L., Mauchamp, B., Chavancy, G., Shirk, P., Fraser, M., Prudhomme, J. C., Couble, P. 2000. Germline transfor-mation of the silkworm using a piggyback transposon-derived vector. *Nat. Biotechnol.*, 18(1):81-84.
- [2] Barber, E. J. W., 1992. Prehistoric textiles: the development of cloth in the Neolithic and Bronze Ages with special reference to the Aegean . Princeton University Press. pp. 31.
- [3] Ron, C. 2008. History of Sericulture. *Bulletin of the Entomological Society of America*, 35: 83-84.
- [4] Anantha, R. K. V., Benchamin, K. V., Magadam, S. B., Remadevi, O. K. and Datta R. K. 1993. Studies on the Nutritional efficiency of hybrid, PM x NB4D2 of silkworm, *Bombyx mori* L. *Indian J. Seric.*, 32(1): 43-49.
- [5] Ito, T. 1972. An approach to nutritional control mechanisms in the silkworm *B. mori* L. *Israel J. Entomol.*, 7: 1-6.
- [6] Takeuchi, Y., Kosaka, T. 1962. On the amount of food ingested and digested during successive developmental periods of the first, second and third instars of silkworm, *Bombyx mori* L. *Tech. Bull. Exp. Stn.*, 79: 24-25.
- [7] Kuribayashi, S., Yatomi, K., Kadota, M. 1976. Effects of hydrogen fluoride and sulfur dioxide on mulberry trees and silkworms. *J. Jpn. Soc. Air Poll.*, 6: 155.
- [8] Daud, M., Khalid, N., Waheed, S., Wasim, M., Arif, M., Zaidi, J. H. 2011. *Morus nigra* plant leaves –Biomonitor for elemental air pollution monitoring. *Radochimica Acta*, 99: 243-252.
- [9] Ghosh, M., Mukhopadhyay, A., Mukhopadhyay, U. K. 2011. Studies on the effect of mulberry leaves of Lune variety on silkworm (*Bombyx Mori l*) rearing in polluted environment of Gangetic plains of West Bengal, India. *Research Journal of Chemical Sciences*, 1: 80-89.
- [10] Anthony, P. 2001. Dust from walking tracks, impact on rainforest leaves on epiphylls. *Cooperative Research Centre for Tropical Rainforest Ecology and Management*. Australia. www.rainforest-crc.jcu.edu.au.
- [11] Dandin, S. B., Giridhar, K. 2010. Hand Book of Sericulture Technologies. Central Silk Board, Bangalore, India. pp. 307-385.
- [12] Chinnaswamy, R., Hothur, L., Savarapu, S. K., Chebba, M. A., Chitta, S. K. 2011. Nutrigenetic screening strains of the mulberry silkworm, *Bombyx mori*, for nutritional efficiency. *J. Insect Sci.*, 12(3): 1-18.
- [13] Waldbauer, G. P. 1968. The consumption and utilization rate of food by insects. *Advanced Insect Physiology*, 5: 229-288.
- [14] Miyashata, Y. 1986. A report on mulberry cultivation and training methods suitable to bivoltine rearing in Karnataka. Central Silk Board, Bangalore, Karnataka, India.
- [15] Ferreira, B. L., De Miguel, E. 2005. Geochemistry and risk assessment of street dust in Luanda, Angola. *Tropical Urban Environment*, 39: 4501-4512.
- [16] Faiz, Y., Tufail, M., Tayyeb, J., Chaudhry, M., Naila-Siddique, M. M. 2009. Road dust pollution of Cd, Cu, Ni, Pb and Zn along Islamabad Expressway, Pakistan. *Microchemicals Journal*, 92: 186-192.
- [17] Honour, S. A., Bell, J. N. B., Ashenden, T. W., Cape, J. N., Power, S. A. 2009. Responses of herbaceous plants to urban air pollution: effects on growth, phenology and leaf surface characteristics. *Environmental Pollution*, 157: 1279-1286.
- [18] Quingsheng, L., Qingli, Z., Tao, Y., Ning, Q. and Lungsang, C. 2009. Magnetic properties of street dust from Chibi city, Hubei Province, China: its implications for urban environment. *Journal of Earth Sciences*, 20: 848-857.
- [19] Zhao, H., Cui, B., Zhang, K. 2010. The distribution of heavy metal in surface soils and their uptake by plants along roadside slopes in longitudinal range gorge region, China. *Environ. Earth Sci.*, 61: 1013-1023.
- [20] Beyer, W. N., Moore, J. 1980. Lead residues in eastern tent caterpillars (*Malacosoma americanum*) and their host plant (*Prunus serotina*) close to a major highway. *Environmental Entomology*, 9: 10-12.
- [21] Parra, J. R. P., Kogan, M. 1981. Comparative analysis of methods for measurements of food intake and utilization using the soyabean looper, *Pseudoplusia includens* and artificial media. *Entomological Experimental Application*, 30: 45-57.
- [22] Paul, D.C., subba, R. G., Deb, D.C. 1992. Impact of dietary moisture on nutritional indices and growth of *Bombyx mori* and concomitant larval duration. *J. Insect Physiol.*, 38 (3), 229-235.
- [23] Ding, N., Zhang, X. M., Jiang, M. Q., Xu, W. H., Wang, Z. E., Xu, M. K. 1992. Genetical studies on the dietary efficiency of the silkworm, *Bombyx mori* L. *Canye Kexue*, 18: 71-76.

- [24] Junliang, X., Xiaofeng, W. 1992. Research on improvement of efficiency of transferring leaf ingested into silk of the silkworm, *Bombyx mori* L. International Congress on Entomology. Abstract 169-003, pp. 623.
- [25] Maribashetty, V. G., Aftab, C. A., Chandrakala, M. V., Rajanna, G. S. 1999. Consumption and conversion efficiency of food and water in new multivoltine breeds of silkworm, *Bombyx mori* L. Indian J. Seric., 38: 140-144.
- [26] Prabhakar, M. K., Reddy, D. N. R., Narayanaswamy, K. C. 2000. Consumption and utilization of mulberry leaves by the silkworm, *Bombyx mori* L. Bulletin of Indian Academy Sericulture, 4: 52-60.
- [27] Ramesha, C., Anuradha, C. M., Lakshmi, H., Sugana, K. S., Seshagiri, S. V., Goel, A. K., Suresh, K. C. 2010. Nutrigenetic Traits Analysis for the Identification of Nutritionally Efficient Silkworm Germplasm Breeds. Biotechnology, 9(2): 131-140.
- [28] Khan, T. A., Ramegowda, G. K and Dar, M. Y. 2013. Effect of road dust pollution in mulberry on silkworm performance in Kashmir valley, India. Res. J. Agr. Sci., 4(4): 501-506.
- [29] Ghosh, M., Mukhopadhyay, A. and Mukhopadhyay, U. K. 2013. Effect of air pollution on physiology and yield of mulberry silkworm, *Bombyx mori* L. Research Journal of Chemistry and Environment, 1: 80-89.
- [30] Kuribayashi, S. 1971. Environmental pollution effects on sericulture and its countermeasures. Seric. Sci. and Tech., 10: 48-49.
- [31] Fuzii, M., Honda, S. 1972. Relative oral toxicity of some fluorine compounds for silkworm larvae. J. Seric. Sci. Jpn., 41: 104-110.
- [32] Imai, S., Sato, S. 1974) On the black spots observed in the integument of silkworms poisoned by fluorine compounds. J. Jpn. Soc. Air Poll., 9: 401.
- [33] Mochida, M., Yoshida, M. 1974. Symptoms of fluoride intoxication on silkworms, especially the abnormal arthroidal membrane. Japanese Society for Sericulture Annual Meet, 41: 24.