Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 392-395
Received: 06-09-2019
Accepted: 13-10-2019

DK Hadimani
Department of Sericulture, College of Agriculture Bheemarayanagudi, Karnataka, India

MC Devaiah
Department of Agril. Entomology (Zoology), UAS, GKVK, Bangalore, Department of Sericulture, UAS, GKVK, Bangalore, Karnataka, India

KC Narayanaswamy
Department of Agricultural Entomology, College of Agriculture, Raichur, Karnataka, India

RN Bhaskar
Department of Agril. Entomology (Zoology), UAS, GKVK, Bangalore, Department of Sericulture, UAS, GKVK, Bangalore, Karnataka, India

Radha D Kale
Department of Agril. Entomology (Zoology), UAS, GKVK, Bangalore, Department of Sericulture, UAS, GKVK, Bangalore, Karnataka, India

KR Shreeramulu
Department of Agril. Microbiology, UAS, GKVK, Bangalore, Department of Sericulture, UAS, GKVK, Bangalore, Karnataka, India

Corresponding Author:
DK Hadimani
Department of Sericulture, College of Agriculture Bheemarayanagudi, Karnataka, India

Studies on food consumption indices as influenced by *BM* NPV infection

DK Hadimani, MC Devaiah, KC Narayanaswamy, RN Bhaskar, Radha D Kale and KR Shreeramulu

Abstract
Silkworm *Bombyx mori* L. is highly susceptibility to grasserie disease caused by *Bombyx mori* nuclear polyhedrosis virus *(Bm*NPV). The amount of food ingested due to *Bm*NPV infection during fourth instar was significantly maximum in CSR_2 (225.56 g/100 larvae) followed by PM × CSR_2 (219.33 g/100 larvae, while significantly lowest food consumption was recorded in BL_{43} (191.58 g/100 larvae) followed by MH_1 (194.95 g/100 larvae). Among the PIBs concentrations, the amount of food ingested was significantly maximum in 10^{-6} PIBs (207.25 g/100 larvae) which was on par with 10^{-3} PIBs (206.74 g/100 larvae) compared to control (214.71 g/100 larvae).whereas, the amount of food ingested during fifth instar, the food ingested was significantly maximum in CSR_2 (1057.36 g/100 larvae), which was on par with that of PM × CSR_2 (1050.12 g/100 larvae). Among the PIBs concentrations, the food ingested was significantly maximum at 10^{-6} (1252.29 g/100 larvae) followed by 10^{-3} PIBs (1158.12 g/100 larvae) compared to the control (651.75 g/100 larvae). Similarly, The food digestion was significantly maximum in CSR_2 (180.03 g/100 larvae), which was on par with PM × CSR_2 (179.05 g/100 larvae) and MH_1 × CSR_2 (179.05 g/100 larvae). It was significantly minimum in BL_{43} (160.35 g/100 larvae).The food digestion was maximum in CSR_2 × CSR_4 (838.85 g/100 larvae) followed by PM × CSR_2 (834.08g / 100 larvae) and MH1 × CSR_2 (834.44 g/100 larvae). It was minimum in MH_1 (797.25 g / 100 larvae) followed by BL_{43} (798.33 g/100 larvae).

Keywords: Food, consumption indices, by *Bm* NPV, infection

Introduction
Sericulture is a sustainable, eco-friendly and agro-based rural industry comprising cultivation of mulberry plant varieties, rearing of silkworms, and production of silk. It is one of the most labor-intensive sectors and has played a critical role in rural development and economic growth. Most of the marketable silk around the world is being produced from the mulberry silkworm, *Bombyx mori* L. Mulberry silkworm is an essentially monophagous and host plant-specific insect that feeds solely on mulberry leaves (*Morus alba*, Family: Moracea). Two kinds of silk proteins have been distinguished as major components of silk cocoons, the first being fibroin – a fibrous protein secreted in the lumen of the posterior silk gland of *B. mori* and the second being sericin, a natural macromolecular protein that serves as an adhesive to unite fibroin for making silk cocoons of silkworm. India has rich resources of mulberry varieties that are traditionally cultivated, and a few exotic varieties have been introduced from time to time. Besides the influence of environmental factors, the silk productivity is related to the quantity and quality of mulberry leaves. Nutritional physiology has a vital role in influencing the performances of different stages of silkworm. To better understand the chemical ecology of the insect-plant relationship, studying the quantitative aspect of nutrition in the insect is important. Development of silkworm is greatly influenced by the nutrient composition of the mulberry host leaves, which is also the determining factor of the quality of silk. The performance of silkworm is evident by the digestion and assimilation of the nutritional materials present in mulberry leaves. The life cycle routine of the silkworm from identical genetic stock varies significantly based on nutritional quality of mulberry leaves. As such, the amount of food consumed and the quantity digested by the silkworms have a direct effect on its physiological performance and silk production. Pioneering research has proved that deficiency of certain nutrients or imbalance of nutrient on the diet affects the digestibility and metabolic activity of larvae (Waldbauer, 1968) [5].

The amount of food consumed and the quantity digested by the silkworms will have direct effects on its performance, mating success and reproduction. Deficiency of certain nutrients or a nutritionally imbalanced diet affects the digestibility and metabolic activity of larvae (Waldbauer, 1968) [5]. In the present study, an attempt has been made to assess on the food consumption indices as influenced by *Bm*NPV infection

Material and Methods

The four improved breeds (CSR_2, CSR_4, MH_1, BL_{43}) and five hybrids of mulberry silkworm ($CSR_2 \times CSR_4$, $CSR_4 \times CSR_2$, $MH_1 \times CSR_2$, $BL_{43} \times CSR_2$ and PM × CSR_2) with two PIBs of 10^{-3} and 10^{-6} *Bm*NPV was tested under laboratory conditions. The standard rearing techniques were fallowed as recommended by Krishnaswami (1978) [3] and three replications by using 100 worms of each breed and their hybrid.

Grasserie diseased fifth instar larvae were collected and haemolymph was taken into sterilized glass tubes from infected worms shortly before death by puncturing the front pair of prolegs with the help of sterilized pin followed by a gentle pressing. The turbid milky haemolymph was stored and then subjected to refrigeration for several days till the polyhedra settled at the bottom. Later, the haemolymph was filtered through four layers of muslin cloth. The filtrate was subjected to centrifugation at 15,000 rpm for 15 minutes using Remi C-24 refrigerated centrifuge. The pellet was resuspended in sterile distilled water to half of the original volume and centrifuged at 5,000 rpm for 15 minutes. Another cycle of centrifugation at 15,000 rpm followed by 5,000 rpm was done for 15 minutes each. The process of the differential centrifugation was repeated till a milky whitish amorphous sediment of highly purified nuclear polyhedra was confirmed from microscopic examination. The polyhedra were suspended in distilled water and stored at 5 °C in the refrigerator.

The polyhedral concentration was determined using the Neubauer's haemocytometer. From the original stock suspension of the polyhedra, two polyhedral concentrations *viz*., 10^{-3} (7.73 x 10^{-7}) and 10^{-6} (4.12 x10^{-10}) were prepared and used for the study.

The infection was carried out orally by feeding the silkworms with virus suspension smeared mulberry leaves, soon after third moult. The leaf bits of 10 × 12 cm size were prepared using suitable aged leaf and washed in running water and sterilized by using 70 per cent alcohol (by cotton swab). The sterilized leaves were shade dried and such leaves were smeared evenly on both the sides with virus suspension (0.5 ml / replication) using non-absorbant cotton. The leaves were shade dried and fed to the silkworms. Control batches were fed with surface sterilized mulberry leaves for the first feed. For subsequent feedings, inoculum free leaves suitable for the age were provided for both treated and untreated batches. Two concentrations (10^{-3} and 10^{-6}) of polyhedral bodies were smeared uniformly to the surface of sterilized mulberry leaves and fed to improved breeds and hybrids of silkworm which were replicated thrice.

The consumption indices were computed by gravimetric method (Waldbauer, 1968) [5]

Results and Discussion

Food ingested (g/100 larvae) during fourth instar

The amount of food ingested was significantly maximum in CSR_2 (225.56 g/100 larvae) followed by PM × CSR_2 (219.33 g/100 larvae) and $MH_4 \times CSR_2$ (215.99 g/100 larvae), while significantly lowest food consumption was recorded in BL_{43} (191.58 g/100 larvae) followed by MH_1 (194.95 g/100 larvae). Similarly, the amount of food ingested ranged between 195.85 g/100 larvae (BL_{43}) to 234.60 g/100 larvae (CSR_2) in non-inoculated batches of silkworm. Among the PIBs concentrations, the amount of food ingested was significantly maximum in 10^{-6} PIBs (207.25 g/100 larvae) which was on par with 10^{-3} PIBs (206.74 g/100 larvae) compared to control (214.71 g/100 larvae). The interaction effect between the silkworm breeds / hybrids and PIBs concentrations was significant with regard to food ingested. The food ingested was significantly maximum in CSR_2 at 10^{-3} PIBs (221.07 g/100 larvae), which was on par with 10^{-6} PIBs (221.02 g/100 larvae). It was significantly minimum in BL_{43} (179.86 g/100 larvae) followed by MH_1 (198.05 g/100 larvae) at 10^{-6} PIBs concentration. This variation in food consumption among the improved breeds/hybrids might be due to the genotypic variation. As the PIBs concentration increased, the food consumption decreased due to reduced metabolic activities. This occurs due to the initiation of virus multiplication in the nuclei of infected tissues. The present findings are in conformity with Gururaj *et al*. (2001) [1] who have also reported that the food consumption decreased in the *Bm*NPV (4.282 g/larva) infected silkworm compared to the control (4.839 g/larva).

Harper (1973) [2] reported significantly decreased food consumption by cabbage loopers infected with nuclear polyhedrosis virus with increase in the virus dosage. Ramakrishnan and Chaudhari (1974) [4] reported the effect of nuclear polyhedrosis disease on food consumption in tobacco caterpillar, *Spodoptera litura*. The total food intake on 4th day was 0.065 mg/larva in control. While, virus fed larvae showed decreasing amounts of food consumption with increasing dose of virus, the intake being 0.0449 and 0.0489 mg/larva respectively, for the virus stock suspension (7.78 × 10^8 PIBs/ml) and viral dilution (1.55 × 10^8 PIBs) concentration. Drastic reduction in the food intake was observed on the 5th day in the virus fed larvae.

Food ingested (g/100 larvae) during fifth instar

The food ingested was significantly maximum in CSR_2 (1057.36 g/100 larvae), which was on par with that of PM × CSR_2 (1050.12 g/100 larvae). The food ingested was significantly maximum in BL_{43} (973.83 g/100 larvae), which was on par with that of MH_1 (981.46 g/100 larvae). Similarly, the food ingested ranged between 596.11 g/100 larvae in BL_{43} to 719.79 g/100 larvae in $CSR_2 \times CSR_4$ in non-inoculated batches of silkworm. Among the PIBs concentrations, the food ingested was significantly maximum at 10^{-6} (1252.29 g/100 larvae) followed by 10^{-3} PIBs (1158.12 g/100 larvae) compared to the control (651.75 g/100 larvae). The interaction effects between silkworm breeds/hybrids and PIBs concentrations was found to be significant with respect to food ingested during Vth instar. The food ingested was significantly maximum in CSR_2 (1301.47 g/100 larvae), which was on par with that of CSR_4 (1290.89 g/100 larvae), PM × CSR_2 (1270.71 g/100 larvae) and $MH_1 \times CSR_2$ (1259.16 g/100 larvae) at 10^{-6} PIBs concentration. It was significantly minimum in BL_{43} (1106.57 g/100 larvae), which was on par with MH_1 (1118.72 g/100 larvae) at 10^{-3} PIBs concentration. This might be due to the genotypic variation in improved silkworm breeds and hybrids. Significantly higher food ingested during fifth instar in the *Bm*NPV infected larvae may be attributed to the effect of gut physiology and hormonal system of silkworm. Even though the *Bm*NPV infected larvae behave normally during early stage of the infection with respect to food ingested. The food consumption increased in the *Bm*NPV infected larvae due to prolonged larval duration compared to non-inoculated batches of silkworm during fifth instar. As the PIBs concentration increased, the food consumption decreased in the *Bm* NPV infected larvae, which may be attributed to the accelerated metabolic activities as result of the infection/disease. These observations are in close conformity with findings of Gururaj *et al*. (2001) [1] who reported that food consumption has reduced in the *Bm*NPV (4.282 g/larva) infected silkworms compared to non-inoculated (4.839 g/larva) silkworms.

Harper (1973) [2] studied the food consumption in cabbage looper infected with NPV, which showed with significant reduction in food consumption increased viral dosages. Effect of NPV on food consumption by tobacco caterpillar, *S. litura* showed decreased in ingested with increasing dose of virus (Ramakrishnan and Chaudhari, 1974) [4].

Food digestion (g) during fourth instar

The food digestion was significantly maximum in CSR_2 (180.03 g/100 larvae), which was on par with PM × CSR_2 (179.05 g/100 larvae) and MH_1 × CSR_2 (179.05 g/100 larvae). It was significantly minimum in BL_{43} (160.35 g/100 larvae) followed by MH_1 (162.95 g/100 larvae). Similarly, the food digestion ranged from 165.28 g/100 larvae (BL_{43}) to 185.39 g/100 larvae (CSR_2) in non-inoculated batches of silkworm. Among the viral dilutions, higher food digestion was recorded at viral dilution of 10^{-6} PIBs (171.43 g/100 larvae) followed by 10^{-3} PIBs (169.56 g/100 larvae) when compared to control (175.57 g/100 larvae). The interaction effect between silkworm breeds/ hybrids and PIBs concentrations was significantly different with respect to food digestion during fourth instar. The food digestion was significantly maximum in CSR_2 (178.89 g/100 larvae), which was statistically on par with that of PM × CSR_2 (179.09 g/100 larvae) and MH_1 × CSR_2 (178.88 g/100 larvae) at viral dilution of 10^{-3} PIBs. It was significantly minimum in BL_{43} (151.95 g/100 larvae) followed by MH_1 (156.55 g/100 larvae) at viral dilution of 10^{-3} PIBs. The decrease in digestion rate in *Bm* NPV infected silkworms during IVth instar may be attributed to reduced physiological activities. As the PIBs concentration increased, the food digestion decreased due to the initiation of virus multiplication in the nuclei of infected tissue. The present findings are in conformity with the work of Gururaj *et al*. (2001) [1] who reported that food assimilated in the *Bm*NPV (1.844 g/larva) infected larva was significantly minimum compared to the control (2.036 g/larva).

Ramakrishna and Chaudhari (1974) [4] studied the effect of NPV disease on excretion by the tobacco caterpillar, *S. litura* which showed maximum in control batches (0.118 mg/larva) of larvae compared to infected larvae in virus stock (7.78 × 108 PIBs/ml) (0.080 mg/larva) and viral dilution (1.55 × 108 PIBs/ml) (0.0852 mg/larva).

Food digestion (g) during V instar

The food digestion was maximum in CSR_2 × CSR_4 (838.85 g/100 larvae) followed by PM × CSR_2 (834.08g / 100 larvae) and MH1 × CSR_2 (834.44 g/100 larvae). It was minimum in MH_1 (797.25 g / 100 larvae) followed by BL_{43} (798.33 g/100 larvae).

The food digestion ranged between 446.25 g/100 larvae (BL_{43}) to 544.59 g/100 larvae (CSR_2 × CSR_4) among non-inoculated batches. The food digestion was significantly maximum at viral dilution of 10^{-3} PIBs (987.31g / 100 larvae) which was on par with that of 10^{-6} PIBs (974.90 g/100 larvae) compared to control (474.29 g/100 larvae). The interaction for food digestion was non-significant. The food digestion was maximum in PM × CSR_2 (1038.35 g/100 larvae) followed by MH_1 × CSR_2 (1026.10 g/100 larvae) and CSR_2 (1020.40 g / 100 larvae) at viral dilution of 10^{-3} PIBs. It was minimum in MH_1 (957.48 g/100 larvae) followed by CSR_4 × CSR_2 (958.34 g/100 larvae) at viral dilution of 10^{-6} PIBs. This might be due to the genotypic variation among improved breeds and hybrids of silkworm. The maximum rate of food digestion in the *Bm*NPV infected larvae may be attributed to the metabolic activities in the silkworm. The higher quantity of food digestion in the *Bm*NPV infected larvae may be attributed to the prolonged larval duration compared to the control batches of silkworm during fifth instar, eventhough the *Bm*NPV infected larvae behave normally during early stage of infection. Among the PIBs concentration, food digestion was similar at different viral dilution in *Bm*NPV infected larvae compared to control. The current findings are in contrast to those of Gururaj *et al*. (2001) [1], who have reported maximum food assimilation (20.36 g/larva) in non-inoculated batches of silkworm compared to the *Bm*NPV (1.844 g/larva) infected silkworm. Ramakrishnan and Chaudhari (1974) [4] reported the effect of NPV disease on excretion by the tobacco caterpillar, *Spodoptera litura* which showed maximum in control batches of larvae (0.118 mg/larva) compared to infected larvae in virus stock (7.78 × 10^8 PIBs/ml) (0.080 mg/larva) and viral dilution (1.55 × 10^8 PIBs / ml) (0.0852 mg/larva).

Table 1: Food ingestion (g) in improved breeds and hybrids of *B. mori* as influenced by *Bm*NPV infection

Stage / Breeds /hybrids	IVth instar				Vth instar			
	PIBs concentrations		Control	Mean	PIBs concentrations		Control	Mean
	10^{-3}	10^{-6}			10^{-3}	10^{-6}		
CSR_2	221.07	221.02	234.60	225.56	1197.81	1301.47	672.81	1057.36
CSR_4	205.23	217.62	220.99	214.61	1185.99	1290.89	669.66	1048.85
MH_1	185.00	198.05	201.80	194.95	1118.72	1223.49	602.18	981.46
BL_{43}	119.08	179.86	195.85	191.58	1106.57	1218.81	596.11	973.83
CSR_2 × CSR_4	219.39	205.00	206.00	210.13	1160.62	1244.91	719.79	1041.77
CSR_4 × CSR_2	197.20	199.09	204.19	200.16	1131.81	1228.55	615.72	992.03
MH_1 × CSR_2	209.09	211.88	227.00	215.99	1180.11	1259.16	683.33	1040.87
BL_{43} × CSR_2	210.65	213.70	217.03	213.79	1142.48	1232.59	625.46	1000.18
PM × CSR_2	213.98	218.99	225.02	219.33	1198.93	1270.71	680.73	1050.12
Mean	206.74	207.25	214.71	209.57	1158.12	1252.29	651.75	1020.72

Food ingestion (g/100 larvae)

Test of significance	IVth instar			Vth instar		
	F-test	SEm±	CD at 5%	F-test	SEm±	CD at 5%
Silkworm breeds and hybrids (A)	*	0.627	1.74	*	13.66	38.71
PIBs concentrations (B)	*	0.362	1.00	*	7.88	22.35
Interaction (A × B)	*	1.09	3.01	*	23.65	67.05

*significant at 5%

Table 2: Food digestion (g) in improved breeds and hybrids of *B. mori* as influenced by *Bm*NPV infection

Food digestion (g/100 larvae)								
Stage / Breeds / hybrids	IVth instar				Vth instar			
	PIBs concentrations		Control	*Mean*	PIBs concentrations		Control	Mean
	10^{-3}	10^{-6}			10^{-3}	10^{-6}		
CSR_2	178.89	175.82	185.39	180.03	1020.40	995.61	457.59	824.53
CSR_4	164.98	174.52	174.18	171.23	1016.33	990.75	468.52	825.20
MH_1	156.55	164.63	166.68	162.95	987.71	957.48	446.57	797.25
BL_{43}	151.95	163.82	165.28	160.35	980.15	968.59	446.25	798.33
$CSR_2 \times CSR_4$	175.85	170.09	179.10	175.01	1010.00	961.95	544.59	838.85
$CSR_4 \times CSR_2$	167.06	163.90	166.29	165.75	991.57	958.34	544.74	501.88
$MH_1 \times CSR_2$	178.88	173.88	184.39	179.05	1026.10	970.02	501.19	832.44
$BL_{43} \times CSR_2$	172.82	176.45	178.91	176.06	815.15	996.38	459.32	756.95
$PM \times CSR_2$	179.09	178.74	179.92	179.25	1038.35	975.02	488.87	834.08
Mean	169.56	171.43	175.57	172.19	987.31	974.90	474.29	812.17

Test of significance	IVth instar			Vth instar		
	F-test	SEm±	CD at 5%	F-test	SEm±	CD at 5%
Silkworm breeds and hybrids (A)	*	0.461	1.28	NS	22.64	64.19
PIBs concentrations (B)	*	0.266	0.738	*	13.07	37.06
Interaction (A × B)	*	0.799	2.21	NS	39.21	111.18

* : significant at 5% NS : Non-significant

References

1. Gururaj CS, Sekharappa BM, Sarangi SK. Chronological variation in the food consumption and utilization in *Bm* NPV infected silkworm, Bombyx mori L. Indian J. Seric. 2001; 40:115-118.
2. Harper JD. Food consumption by cabbage loopers infected with nuclear polyhedrosis virus. J Invertebr. Pathol. 1973; 21:191-197.
3. Krishnaswami S. New Technology of Silkworm Rearing. Bulletin No. 2, CSR&TI, Mysore, 1978, 19.
4. Ramakrishnan N, Chaudhari S. Effect of nuclear polyhedrosis disease on consumption, digestion and utilization of food by the tobacco caterpillar, *Spodptera litura* (Fabricius). Indian J. Ent. 1974; 36(2):93-97.
5. Waldbauer GP. The consumption and utilization of food by insects. Adv. Insect. Physiol. 1968; 5:229-288.