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Investigation of BmNPV infection on physiological and biochemical parameters of *Bombyx mori* administrated with botanicals

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ABSTRACT

The present study is based on the impact of BmNPV infection on physiological and biochemical parameters of Bombyx mori administrated with botanicals (Plectranthus amboinicus and Psoralea corylifolia). The study result revealed that activities of alkaline phosphatase and amylase enzymes in multivoltine silkworm breeds in response to botanical, higher enzyme activity was recorded for untreated control in all the breeds, Nistari (7.10 µg/ 100 µl, 1471.52), C. Nichi (6.60µg/ 100µl, 1400.29), Tamil Nadu White (6.20µg/ 100µl,1190.20) followed by P. corylifolia for Nistari (5.00 µg/ 100 µl, 1190.28), C. Nichi (4.65µg/ 100µl,825.35), Tamil Nadu (5.25µg/ 100µl, 900.12) and P. amboinicus for Nistari (5.23µg/ 100µl, 1201.99), C. Nichi (4.95 µg, 735.20), Tamil Nadu White (4.90 µg/100 µl,885.11). Studies on biochemical parameters of multivoltine breeds to botanical treatment revealed that untreated control recorded the highest content of carbohydrates, protein, lipid, and uric acid in Nistari (9.90mg/ ml, 97.45mg/ ml, 5.12mg/ g, 4.70mg/ g of fresh excreta), C. Nichi (8.92, 90.00, 4.90, 4.90) and Tamil Nadu white (9.80, 93.11, 5.35, 4.60) followed by P. amboinicus and P. corylifolia and both were found on par.

***Keywords*:** Biochemical, botanicals, NPV, physiological, multivoltine, silkworm breeds, silkworm

Bombyx mori is a monophogus silkworm which feeds only on mulberry leaves. Silkworm larva is infected by Nucleopolyhedrovirus (NPV) in both tropical and temperate areas. Generally, a significant yield loss is recorded on every stage of silkworm rearing. *Bombyx mori* NPV typically causes milky or Grasserie diseases. It causes great economic loss in sericulture industry due to malformation of cocoon, least cocoon shell ratio and weight etc. Yield loss up to 40 to 80 per cent has been reported by BmNPV infection in south India (Mudasir *et al.,* 2017). Silkworm larva is very delicate and highly sensitive to temperature and humidity. The disease is caused by improper bed cleaning, feeding of older leaves, and unhygienic condition in rearing hall during the rearing period. Various categories of microbes, virus, bacteria, fungai and protozoa cause different diseases in silkworm (Babu *et al.,* 2009). At this moment, viruses that tremendously infect silkworm larva include Nucelopolyhedrovirus (NPV), Cytoplasmic polyhedrovirus (CPV), Infection flacherie virus (IFV) and Denso virus (DNV). However, this virus is connected with the infection of one or other viruses and bacteria. Bacterial infection of silkworm is known as flacherie. *Streptococcus faecalis*, *S. faeciu, Staphylococci* sp and *Serratia marcescens* cause bacterial flacherie singly or associated with viruses such as IFV and DNV (Nataraju *et al.,* 1999). Among the viral diseases, NPV causes massive infestation and is contagious. It can affect various stages of silkworm which causes serious damage and significant financial losses to sericulture farmers posing serious threat to world level (Sengupta *et al.,* 1990; Brancalhao, 2002). In India the direct silkworm cocoon crop loss due to diseases is reported at 20 per cent (Balavenkatasubbaiah *et al.,* 2015). Losses due to viral disease are estimated at almost 70 per cent of the total loss due to diseases (Babu *et al.,* 2009). The chemical control is not desirable against silkworm diseases because of the soft and very sensitive body. Hence, botanicals are used to minimize Grasserie diseases in silkworm larva and to improve the economic parameters of cocoon and silk. The present study was conducted on BmNPV infection on physiological and biochemical parameters of *B. mori* administrated with botanicals.

MATERIALS AND METHODS

The experiments were conducted during 2019-2020 in the Department of Agricultural Entomology, Adhiyamaan College of Agriculture and Research, Krishnagiri, Tamil Nadu. Mulberry silkworm rearing was carried out in analytical studies in a properly disinfected rearing house without pebrine diseases in 25^{0}C temperature and 80 % relative humidity Disease-free laying (Dfls) were fetched from various central sericulture institutions and the state department of sericulture, Tamil Nadu, and Karnataka. Grasserie infected fifth instar silkworms were collected, washed and macerated with

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pestle and mortar. Virus suspension thus obtained was filtered using a muslin cloth. The filtrate was then centrifuged at 500 rpm for 30 seconds and the supernatant was collected and again centrifuged at 3000 rpm for three minutes. The supernatant was then discarded, and the pellets obtained were suspended in distilled water and stored in the refrigerator at 4^0C for bioassay studies (Sugumori *et al.,* 1990). Polyhedral occlusion bodies in the suspension were counted with the help of an improved Neubauerhaemo cytometer using a phase-contrast microscope.

Preparation of solvent seed extract of Psoralea corylifolia: Seeds of *P.corylifolia* were dried in the shade and powdered. The powered seed sample was weighed and extracted with hexane in a Soxhlet apparatus for six hours (Khatune, 2000). It was then kept at room temperature for evaporation of solvents. The residue were weighed and dissolved in an equivalent volume of acetone (w/v) to fetch a working stock solution. The necessary concentration of 1000 ppm was prepared using distilled water from the working standard.

Preparation of solvent leaf extracts of Plectranthus amboinicus: Leaf material of *P. amboinicus* was fully cleaned in running tap water followed by two times washing with distilled water. The leaves were dried in the shade and making powered. The powdered leaf sample were weighed and extracted with hexane in Soxhlet equipments for six hours (Khatune, 2000). It was kept at room temperature for evaporation of solvents. Then the residue was weighed and dissolved in a same volume of acetone (w/v) to fetch a working standard.

Method of administration of botanical: Soft mulberry leaf was drenched in a viral suspension of 10^6 POB/ml, then shade dried and to the silkworm larva after the second instar. The drenched leaves were provided during the first feed on the first day. Furthermore, after that, the silkworm larva was fed with common mulberry leaf. Another day, the leaves treated with 1000 ppm extracts of *Psoralea corylifolia* and *Plectranthus amboinicus* were fed to the worms. Soft leaf was dipped in the needed concentration of extracts and shade dried before feeding it to silkworms. The botanical was administrated twice, once on the 2^{nd}day of the 3^{rd} instar and the other on the 1^{st} day of the 4^{th} instar. The silkworms fed with BmNPV alone served as treated control. An untreated control was also maintained.

The physiological parameters (alkaline phosphatase and amylase activity) are estimated by following the proteolytic activity method (Eguchi *et al.,* 1976). Biochemical parameters of *B. mori* and estimation of total sugar in haemolymph were estimated by anthrone method (Dubois *et al.,* 1956). Lowry method (Lowryet *et al*., 1951) was used for the total protein calculation. Quantitative analysis of uric acid was carried out as per the method described by Tojo and Hirano (1966) and estimation of total lipids was extracted with the chloroform-methanol mixture (2:1). 20 ml of solvent was used per gram of tissue.

The experiment was conducted using three breeds of multivoltine silkworm (Nistari, C.Nichi, and TN White), four treatments, seven replications (each replication of about 50 larvae), 10^6 POBs/ml concentration of virus, and 1000ppm concentration of plant extract in complete randomized design (CRD). Statistical analysis of data from factorial completely randomized design was done using methods suggested by Panse and Sukhatme (1957), and means were compared with Duncuns multiple range test (Duncun, 1995).

RESULTS AND DISCUSSION

Physiological parameters

Alkaline phosphatase activity (Nistari, C. Nichi, TN White): Results on performance of breeds in response to alkaline phosphatase activity revealed that on the administration of *P. corylifolia*, TN White recorded the highest activity (5.25 µg/100 µl) followed by Nistari (5.00 µg / 100 µl and C. Nichi (4.65 µg/100 µl). For *P.amboinicus*, C. Nichi and TN White were on par, recording an activity of 4.90 to 4.95µg/100ml followed by Nistari (5.23 µg/ 100µl). No significant difference in enzyme activity was observed between breeds for untreated control, for treated control highest activity was recorded in TN White (5.00 µg/100 µl) followed by C. Nichi (4.50 µg/100 µl) and Nistari (3.90µg/100µl) (Table 1). In the present study, the breeds showed variation in their response to treatments. With regard to response of breeds to BmNPV infection upon administration of botanicals, TN White, C.Nichi and Nistari recorded higher activity. The results also revealed that TN White among multivoltine breeds showed excessive activity of alkaline phosphatase. The present result on the top activity of alkaline phosphatase in Nistari falls in line with Kasmaei *et al.* (2012). Matindoost (2006) revealed that BmNPV had caused an abundant decrease in activity of alkaline phosphatase enzyme in silkworm after infection of a cell line established from silkworm embryo.

Amylase activity (Nistari, C. Nichi, TN White): Results on performance of breeds in response to amylase activity revealed that on the administration of *P. corylifolia*, Nistari recorded the highest activity (1190.28 µg/100 µl) followed by TN White (900.12 µg/100 µl) and C. Nichi (825.35 µg/100 µl). A similar trend was observed for *P. amboinicus* (1201.99, 885.11, 735.20 µg/100 µl). In the case of both treated and untreated

Table 1: Effect of botanicals on alkaline phosphatase and amylase activity (µg/100µl) of multivoltine breeds of *B. mori* exposed to BmNPV (KRI 1 strain)

Sl. No.	Treatments	Alkaline phosphatase (µg/100µl) multivoltine breeds			Amylase activity (µg/100µl) multivoltine breeds		
		Nistari	C. Nichi	TN White	Nistari	C. Nichi	TN White
1.	*P. corylifolia*	5.00^{b}_{C}	4.65^{b}_{B}	5.25^{b}_{A}	1190.28^{b}_{A}	825.35^{b}_{C}	900.12^{b}_{B}
2.	*P. amboinicus*	5.23^{b}_{B}	4.95^{b}_{A}	4.90^{b}_{A}	1201.99^{b}_{A}	735.20^{b}_{C}	885.11^{b}_{B}
3.	Treated	3.90^{c}_{C}	4.50^{c}_{B}	5.00^{b}_{A}	1096.85^{c}_{A}	733.11^{c}_{B}	490.50^{c}_{C}
4.	Untreated	7.10^{a}_{A}	6.60^{a}_{A}	6.20^{a}_{A}	1471.52^{a}_{A}	1400.29^{a}_{B}	1190.20^{a}_{C}
	Mean	4.77	4.90	5.20	1250.00	894.39	880.88
	SEm(±)	**0.0574**	**0.1240**	**0.1110**	**23.2504**	**22.6484**	**12.5251**
	LSD (0.05)	**0.1402**	**0.3026**	**0.2708**	**55.1781**	**53.9456**	**29.2022**

Means followed by a common small letter in a column and capital letter (lower case) in a row are not statistically different by DMRT (P = 0.05).

Table 2: Effect of botanicals on total carbohydrate and protein content (mg/ ml) of multivoltine breeds of *B. mori* exposed to BmNPV (KRI 1 strain)

Sl. No.	Treatments	Total Carbohydrates (mg/ml) multivoltine breeds			Protein content (mg/ml) multivoltine breeds		
		Nistari	C. Nichi	TN White	Nistari	C. Nichi	TN White
1.	*P. corylifolia*	9.21^{b}_{A}	7.90^{b}_{B}	7.25^{b}_{B}	92.10^{b}_{A}	82.10^{b}_{C}	79.10^{b}_{B}
2.	*P. amboinicus*	8.90^{b}_{A}	7.80^{b}_{B}	7.11^{b}_{B}	90.00^{b}_{A}	83.22^{b}_{C}	82.23^{b}_{B}
3.	Treated	5.35^{c}_{A}	3.91^{c}_{B}	5.10^{c}_{A}	66.20^{c}_{A}	52.55^{c}_{C}	61.55^{c}_{B}
4.	Untreated	9.90^{a}_{A}	8.92^{a}_{B}	9.80^{a}_{A}	97.47^{a}_{A}	90.00^{a}_{C}	93.11^{a}_{B}
	Mean	7.80	6.70	7.23	85.85	78.99	78.93
	SEm(±)	**0.1575**	**0.0626**	**0.1338**	**0.8280**	**1.5226**	**1.1470**
	LSD (0.05)	**0.3810**	**0.1388**	**0.2585**	**2.0018**	**3.7112**	**2.78020**

Means followed by a common small letter in a column and capital letter (lower case) in a row are not statistically different by DMRT (P = 0.05).

Table 3: Effect of botanicals on lipid content (mg/ g) in *B. mori* multivoltine breeds exposed to BmNPV (DMI strain)

Sl. No.	Treatments	Lipids (mg/ g) multivoltine breeds			Uric acid (mg/ g of fresh weight of excreta) multivoltine breeds		
		Nistari	C.Nichi	TN White	Nistari	C.Nichi	TN White
1.	*P. corylifolia*	4.70^{b}_{B}	4.00^{b}_{C}	4.40^{b}_{A}	3.40^{b}_{A}	2.90^{b}_{C}	3.10^{b}_{B}
2.	*P. amboinicus*	4.10^{b}_{B}	4.00^{b}_{C}	4.10^{b}_{A}	3.20^{b}_{A}	2.40^{b}_{C}	2.90^{b}_{B}
3.	Treated	3.20^{c}_{B}	3.10^{c}_{C}	2.90^{c}_{A}	2.00^{c}_{A}	2.10^{c}_{C}	2.00^{c}_{B}
4.	Untreated	5.12^{a}_{B}	4.90^{a}_{C}	5.35^{a}_{A}	4.70^{a}_{A}	4.90^{a}_{A}	4.60^{a}_{A}
	Mean	4.2625	3.8850	4.7025	3.1450	2.3550	2.9575
	SEm(±)	**0.0745**	**0.0563**	**0.0998**	**0.0654**	**0.0426**	**0.0914**
	LSD (0.05)	**0.1825**	**0.1375**	**0.2440**	**0.1210**	**0.1318**	**0.1502**

Means followed by a common small letter in a column and capital letter (lower case) in a row are not statistically different by DMRT (P = 0.05)

control, Nistari recorded the highest activity (1096.85µg/100µl and 1471.52µg/100µl)) followed by C. Nichi (733.11 µg/100 µl, 1400.29 µg/100µl) and TN White (490.00 µg/100µl, 1190.20 µg/100µl) (Table 1). It was also observed in the present study that the activity of amylase were significantly higher in multivoltine breeds. This indicates the tolerance of multivoltine breeds to BmNPV by improving the digestion by an increase in enzyme secretion. Similar study has been reported, where increased tolerance of Indian multivoltine races to NPV had already been documented (Funakoshi and Aizawa, 1989; Watanabe *et al.* 1990).

Biochemical parameters (Nistari, C. Nichi, TN White)

Total carbohydrates: Results on performance of breeds in response to total carbohydrate content revealed that on the administration of *P. corylifolia*, Nistari recorded the highest content (9.21 mg/ ml) followed by both C. Nichi (7.90 mg/ml) and TN White (7.28 mg/ml). Similar trend was observed for *P.amboinicus* (8.90 mg/ml, 7.80 mg/ml, and 7.11 mg/ml). In case of both treated and untreated control both Nistari (5.35 mg/ml, 9.90 mg/ml) and TN White (5.10 mg/ml, 9.80 mg/ml) recorded highest content of carbohydrates (Table 2). In the present investigation, total carbohydrate content was significantly higher in untreated control in the multivoltine followed by botanical treatment. Treated control recorded the lowest content. Among the multivoltine breeds in botanical treatment, higher activity was observed in Nistari followed by C. Nichi and TN White and both were on par. Jacob (1972) has earlier reported abundant decrease in concentration of glycogen in NPV infected larva. Significant reduction in carbohydrate content was noticed in silkworm larva upon infection by virus by Rajitha *et al*. (2013).

***Total protein*:** Results on the performance of breeds to different treatments revealed that for all the treatments *viz*., untreated control, *P. corylifolia, P.amboinicus*, and treated control, Nistari recorded the highest protein content (mg/ml) (97.47, 92.10, 90.00, 66.20, respectively) followed by TN White (93.11,79.10,82.23,61.55) and C.Nichi (90.00,82.10, 83.22, 52.33) (Table 2). In the present study, total protein content was significantly higher in untreated control in the multivoltine breeds. Treated control recorded the lowest content. The present result on decrease in protein content in treated control falls in line with findings of Sarma *et al*. (1994) and Etebari *et al*. (2007) who reported significant decrease in total protein content in BmNPV infected silkworm larva. Gururaj (1996) reported a decline in level of glycogen, reducing sugar and protein in the mid gut of BmNPV infected larva.

***Total lipids*:** The result showed that, on assessment of breeds on their response to different treatments revealed that TN White recorded the highest lipid content (mg/g) for untreated control (5.35 mg/g), *P. corylifolia* (4.40 mg/g), *P.amboinicus* 4.10 mg/g) and treated control (2.90 mg/g) followed by Nistari (mg/g) (5.12,4.70,4.10,3.20) and C.Nichi (mg/g) (4.90,4.00,4.00,3.10) (Table 3). Significant reduction in total lipid content was reported in *B. mori* infected with *Serratia marcescens* by Sam Devdas *et al.* (1994). Considerable decline in lipid content was showed in course of infection by pathogen. This might be due to utilization of lipids in metabolic activity of host to combat against the infection by pathogen. Secondly, raised lipase activity in infected haemolymph may be the reason for declined content of lipid in haemolymph. Rajasekhar (1993) reported reduced lipid content in infected silkworm larva and attributed this may be due to utilization of lipid components by pathogen.

***Uric acid*:** Comparison of breeds on their response to different treatments revealed that Nistari recorded the highest excretion of uric acid content (mg/g of fresh excreta) for untreated control (4.50 to 4.70, *P.amboinicus* (3.20), *P. corylifolia* (3.40), and treated control (2.00 mg/g) followed by TN White (mg/g of fresh excreta) (4.60, 2.90, 3.10, 2.00) and C.Nichi (4.90, 2.40, 2.90, 2.00) (Table 3). Reduction in uric acid content in larvae infected with virus may be due to the choices for reduction of carbohydrate because virus rapidly minimize on all the nutritional content in insect body or may be due to the fact that metabolic reactions affected virus. Urea changes are directly related to nitrogen metabolism and amino acids (Hirayarna *et al.* 1996).

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