CHAPTER III

COMBINED EFFECT OF LEAF QUALITY AND NUCLEAR POLYHEDROSIS ON THE HAEMODYNAMICS AND ECONOMIC PARAMETERS
INTRODUCTION

Unfavoured changes in the natural environment prevent insects from attaining their physiological potential for best performances (Slansky and Scriber, 1985). Nutrition is one such major factor that determines the potential fitness of an insect towards quality, quantity and digestibility of nutritional substances. The consumption and utilization of food constitutes a *sine qua non* for growth, development and reproduction.

Nutritional composition of mulberry leaf plays an important role in cocoon production and ultimately the silk productivity. Nutritional composition of mulberry leaves changes with maturity (Krishnaswamiet al., 1973; Pillai and Jolly 1985; Hanif and Islam, 1987; Venkataramu, 1987; Sreedhara et al., 1988; Rupa et al., 1993; Sinha et al., 1993 and Jadhav, 1994). Therefore, changes in quality of leaf may reflect on changes in silkworm haemolymph (Bhatt and Krishna, 1984).

3.1.1 Silkworm haemolymph serves as a repository for stored nutrients. Many primary and secondary metabolites derived from the digestion of ingested food, are continuously poured into it. Improper and unbalanced composition of nutrient's constituents may cause physiological disturbances
(Shvetsova, 1950; Shimizu, 1982; Bhatt and Krishna, 1984; Udpudi, 1989 and Jeyakumar et al., 1995) leading to the enhanced susceptibility to diseases (Shvetsova, 1950 and Haseena and Muralikumaran, 1992 and Sivaprakasam et al., 1996). Therefore the nutritional quality of the host plant tends to influence the incidence of a disease through sudden changes in the haemolymph composition of the silkworm (Ambika et al., 1992 and Jeyakumar et al., 1995).

Several researchers have reported changes in haemocyte count (Arnold and Hinks, 1976; Wago and Ishikawa, 1979; Pathak, 1986; Pathak and Soni, 1990) as influenced by metabolites (Aizawa, 1959; Benz, 1963; Bergold, 1963; Martignoni, 1964; Komano et al., 1966; Bhosale and Kallapur, 1990 and Dinesh and Rao, 1992), pH (Downer, 1981; VenkataRami Reddy et al., 1990 and Ambika et al., 1992) and cations (Sharma and Pathak, 1992). However, there is no report on the combined effect of leaf quality and nuclear polyhedrosis on the haemodynamics of B.mori. Therefore the present investigation was carried out.

3.1.2 Available information suggests that silkworms fed on different mulberry varieties show variable performances (Das and Sikdar, 1970; Krishnaswami et al., 1970; Kushwaha and Verma, 1978; Opender et al., 1979; Venugopal et al., 1980 Tayade and Tamale, 1984; Bari et al., 1985; Das and Vijayaraghavan, 1990 and
Haque et al., 1990). The variation in the quality of food can influence body weight, fuel reserves, adult performance, fecundity and reproduction (Edelman, 1963; Rodrerequez, 1972; Thompson et al., 1972; Reese, 1979; Slansky and Scriber, 1985 and Udapudi, 1989). About 70% of the silk proteins produced by silkworm are directly derived from mulberry leaves (Narayanan et al., 1967; Krishnaswami et al., 1970 and Petkov and Dona, 1979). Therefore, the quality of mulberry leaf influences the growth, development and productivity of the silkworm and thereby greatly affects the economics of sericulture industry (Yokayama, 1963 and Das et al., 1983). Feeding on nutritionally rich leaves showed better growth and development of silkworm larvae as well as gain in economic characters of cocoons (Krishnaswami et al., 1978). However, very scanty information is available on the effect of feeding variable quality leaves and nuclear polyhedrosis on larval and cocoon parameters of silkworm B. mori.

B. mori prefers to feed on mulberry leaves of specific age. In field conditions, the farmers may feed the worms with variable quality leaves owing to the scarcity of leaves, environmental changes and negligence etc. Such variations may have crucial bearing on nutritional status of the silkworm as well as on economic parameters of cocoons. Moreover, when a stock of silkworm is contaminated with the
disease like nuclear polyhedrosis, the damage may be quite heavy because of the synergistic nature of the two factors. Therefore, it was planned to investigate the combined effect of leaf quality and nuclear polyhedrosis on the haemodynamics and economically important characters of the silkworm B. mori.
MATERIALS AND METHODS

Most of the materials and methods used and employed for these set of experiments are common to those already described in the previous chapter.

Laboratory bred NB\textsubscript{18} larvae, maintained by conventional methods, were used in these experiments. Chemicals and reagents were of analytical grade.

The tender leaves of M\textsubscript{5} mulberry variety were dipped thoroughly in LC\textsubscript{50} strong suspension of the virus and the excess fluid was drained out by gravity. The leaves were dried in shade for sometime. The silkworms, just coming out of third moult were starved for 4 hours before being allowed to feed on treated leaves for one complete feed. Beds were cleaned and complete trays were changed soon after the treatment was over. These groups were then fed with varying quality leaf namely a) medium (50 to 70 days old) b) mature (more than 70 days old) c) tender (25 to 45 days old) and d) mixed (all the above types alternately) every day. While both the control and treated larvae were maintained on leaves as prescribed by Krishnaswami (1979), the changes to the experimental group of leaves was brought into effect from fourth instar. And the change was continued up to spinning for both the test groups. Respective control
groups were also fed with similar type of leaves. And, each control and experimental group contained similar number of larvae (100 x 3).

3.2.1 Haematological changes were determined as in the previous chapter with respect to that haemocyte count (as per Nittono, 1960), trehalose (as per Roe, 1955), protein (as per Lowry et al., 1955), pH (as per VenkataRami Reddy et al., 1990) and cation concentrations (as per Willis, 1960 a,b and c).

3.2.2 The observations were recorded as follows:
1. Per cent larval mortality - This was calculated from the larval mortality due to nuclear polyhedrosis in each groups by using the following formula:
   \[
   \% = \frac{\text{Number of larvae dead}}{\text{Total number of larvae under observation}} \times 100
   \]

2. Larval duration - This was calculated in hours from the first day of fourth instar till the spinning day in fifth instar.

3. Mature worm weight - The larval weight (mg) was recorded after the sixth day of fifth instar by using digital balance (Anamed, Bangalore).
4. Per cent cocoon formation - This was calculated from the cocoons formed in each group by using the following formula:

\[ \% = \frac{\text{Number of cocoons formed}}{\text{Total numbers of larvae under observation}} \times 100 \]

5. Per cent moth emergence - Per cent moth emergence was calculated after the completion of moth emergence by using the formula:

\[ \% = \frac{\text{Number of moth emerged}}{\text{Total numbers of larvae under consideration}} \times 100 \]

6. Cocoon and shell weight - Total cocoon weight and shell weights were recorded on fifth day after spinning of cocoons using digital balance.

7. Shell ratio - Shell ratio was calculated by using the following formula:

\[ \text{Ratio} = \frac{\text{Shell weight}}{\text{Total cocoon weight}} \times 100 \]

Results were compared by employing Student's 't' test and ANOVA. The effect of combinations was assessed by calculating the statistical significance.
RESULTS

3.3.1 The data pertaining to the combined effect of leaf quality and nuclear polyhedrosis on haemodynamics of B. mori are presented in table 3.1, 3.2 and 3.4. The statistical analysis of the data is presented in table 3.3 and 3.5.

Total haemocyte count:

In the control group, maximum total haemocyte count (THC) was recorded in tender leaf fed group (6450 cell/mm$^3$). It was followed by that fed by medium (6379 cell/mm$^3$) and mixed leaf fed group (6170 cell/mm$^3$). THC was least in mature leaf fed group (5830 cell/mm$^3$) (Table 3.1 and Fig.3.1).

In BmNPV treated groups, least THC was recorded in tender leaf fed group (1237 cell/mm$^3$) followed by that in mature (1356 cell/mm$^3$), and mixed leaf fed groups (1434 cell/mm$^3$). Among the treated ones the medium leaf fed group (1734 cell/mm$^3$) demonstrated the higher THC. The THC indicated significantly less count in treated groups as compared to that in the respective untreated groups (Table 3.1 and 3.3).
Table 3.1: Combined effect of leaf quality and nuclear polyhedrosis on haematological parameters

<table>
<thead>
<tr>
<th>Test group</th>
<th>THC (cell/mm³)</th>
<th>Trehalose (µg/µl)</th>
<th>Protein (µg/µl)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Treated</td>
<td>Control Treated</td>
<td>Control Treated</td>
<td>Control Treated</td>
</tr>
<tr>
<td>Medium</td>
<td>6379 ± 124 1734 ± 143**</td>
<td>6.25 ± 0.13 3.83 ± 0.10**</td>
<td>8.62 ± 0.90 11.00 ± 0.11</td>
<td>6.27 ± 0.25 6.76 ± 0.03</td>
</tr>
<tr>
<td>Mature</td>
<td>5830 ± 300 1356 ± 121**</td>
<td>5.46 ± 0.91 4.32 ± 0.07**</td>
<td>7.20 ± 0.13 10.67 ± 0.22**</td>
<td>6.34 ± 0.04 6.58 ± 0.01**</td>
</tr>
<tr>
<td>Mixed</td>
<td>6170 ± 101 1434 ± 131**</td>
<td>7.25 ± 0.12 3.43 ± 0.13**</td>
<td>8.15 ± 0.10 15.27 ± 0.18**</td>
<td>6.41 ± 0.02* 7.01 ± 0.09**</td>
</tr>
<tr>
<td>Tender</td>
<td>6450 ± 131 1237 ± 192**</td>
<td>8.42 ± 0.17 3.20 ± 0.10**</td>
<td>8.65 ± 0.08 13.77 ± 0.10**</td>
<td>6.43 ± 0.03 7.24 ± 0.04**</td>
</tr>
</tbody>
</table>

a - Mean ± S.E of 5 observations
b - Mean ± S.E of 3 observations
+ - Indicate larvae treated with LC₅₀ concentration,
* - Indicate significant difference at 5% level as compared with that of the respective control,
*₁ - Indicate significant difference at 1% level as compared with that of the respective control,
(Student's 't' test by Fisher, 1956)
Table 3.2: Combined effect of leaf quality and nuclear polyhedrosis on cation concentration

<table>
<thead>
<tr>
<th>Test group</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca⁺⁺</th>
<th>Mg⁺⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>Medium</td>
<td>15 ± 1.00</td>
<td>20 ± 2.52*</td>
<td>40 ± 2.52</td>
<td>49 ± 2.00**</td>
</tr>
<tr>
<td>Mature</td>
<td>12 ± 0.57</td>
<td>17 ± 1.16**</td>
<td>32.67 ± 2.52</td>
<td>46 ± 1.73**</td>
</tr>
<tr>
<td>Mixed</td>
<td>20 ± 2.00</td>
<td>26 ± 2.08*</td>
<td>46 ± 1.53</td>
<td>51 ± 1.53**</td>
</tr>
<tr>
<td>Tender</td>
<td>23 ± 2.52</td>
<td>28 ± 1.53*</td>
<td>48 ± 1.53</td>
<td>56 ± 2.00**</td>
</tr>
</tbody>
</table>

Mean ± S.E of 3 observation, NB₈₁ silkworm larvae treated during fourth instar and assessed after sixth day of fifth instar,
+ Indicate larvae treated with LC₅₀ concentration during fourth instar,
* Indicate significant difference at 5% level as compared with that of the respective control,
** Indicate significant difference at 1% level as compared with that of the respective control. (Student's 't' test by Fisher, 1956)
Table 3.3: Statistical analysis of the data from table 3.1 and 3.2

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>'F' values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
</tr>
<tr>
<td>Between groups (L)</td>
<td>1</td>
</tr>
<tr>
<td>Between the control and test group (T)</td>
<td>6</td>
</tr>
<tr>
<td>Interaction (L x T)</td>
<td>28</td>
</tr>
</tbody>
</table>

* Values significant at 5% level,
** Values significant at 1% level.

(Statistical analysis by ANOVA as described by Fisher, 1956).

NS Non-significant.
Trehalose and protein concentration:

In BmNPV treated groups, trehalose concentration decreased significantly as compared with that in the respective untreated groups. Among the treated groups, least trehalose concentration was observed in tender leaf fed group ($3.20 \pm 0.10 \mu g/\mu l$). It was followed by that in mixed ($3.34 \pm 1.3 \mu g/\mu l$) and medium leaf fed groups ($3.83 \pm 0.1 \mu g/\mu l$). Maximum trehalose concentration ($4.32 \pm 0.07 \mu g/\mu l$) was found in medium leaf fed group (Table 3.1 and Fig.3.2).

The protein concentration of all the treated groups increased significantly (Table 3.1 and Fig. 3.3) over that of the respective control groups. Maximum protein concentration was recorded in mixed leaf fed group (15.27 $\mu g/\mu l$) followed that fed by tender (13.77 $\mu g/\mu l$), medium leaf fed groups (11.00 $\mu g/\mu l$). And the least protein concentration was observed in mature leaf fed group (10.67 $\mu g/\mu l$).

Interestingly, in untreated groups maximum trehalose and protein contents were observed in tender leaf fed groups (8.42 and 8.65 $\mu g/\mu l$ respectively). For trehalose it was followed by that in mixed (7.25 $\mu g/\mu l$) and medium leaf fed groups (6.25 $\mu g/\mu l$). For protein, however, it was followed by medium (8.62 $\mu g/\mu l$), mixed leaf (8.15 $\mu g/\mu l$) fed groups.
pH:

In the untreated control, maximum pH was recorded in tender leaf fed group (6.43 pH), followed by that fed by mixed (6.41 pH), and mature leaf fed groups (6.34 pH). Least pH was observed in medium leaf (6.27 pH) fed group (Table 3.1 and Fig. 3.4).

In BmNPV treated groups, least pH was recorded in mature leaf fed group (6.58 pH). Maximum pH (7.24 pH) was observed in tender leaf fed group, followed by that in mixed (7.01 pH), and medium (6.76 pH) leaf fed groups. pH concentration of treated groups was higher as compared with that of respective untreated control groups (Table 3.1, Fig. 3.4).

Cations:

During nuclear polyhedrosis, concentration of all cations increased significantly as compared with that of the respective untreated groups. Maximum cation concentration was recorded in tender leaf fed group, while least was recorded in mature leaf fed ones. Among both the treated and untreated groups, the magnesium demonstrated maximum, and sodium indicated the minimum concentration among the groups which were kept on different quality leaves (Table 3.2, Fig. 3.5, 3.6, 3.7 and 3.8).
Figure 3.1: Combined effect of leaf quality and nuclear polyhedrosis on total haemocyte count

Figure 3.2: Combined effect of leaf quality and nuclear polyhedrosis on trehalose concentration
Figure 3.3: Combined effect of leaf quality and nuclear polyhedrosis on protein concentration

Figure 3.4: Combined effect of leaf quality and nuclear polyhedrosis on pH concentration
Figure 3.5: Combined effect of leaf quality and nuclear polyhedrosis on Na concentration

Figure 3.6: Combined effect of leaf quality and nuclear polyhedrosis on K concentration
Figure 3.7: Combined effect of leaf quality and nuclear polyhedrosis on Ca concentration

Figure 3.8: Combined effect of leaf quality and nuclear polyhedrosis on Mg concentration
The statistical analysis of the data indicated that there was significant variation between treated and untreated groups with respect to THC, trehalose, protein, pH and cations concentration (Table 3.1). Analysis of variance also showed significant differences when the comparisons were made between the different leaf fed groups, as well as within the respective groups (Table 3.3).

3.3.2 The results pertaining to the combined effect of leaf quality and nuclear polyhedrosis on larval and cocoon characters are presented in table 3.4, and the statistical analysis in table 3.5.

Per cent larval mortality:

In the treated groups the maximum incidence of the larval mortality due to NPV was recorded in tender leaf fed groups (76%). Whereas the mixed (64%) and the mature leaf fed (55%) groups showed slightly higher mortality than the medium leaf fed (48%) group.

In the untreated groups, the groups fed with tender leaves indicated the maximum (31%) mortality. It was followed by that with the mixed (20%) and mature (16%) leaves. However, the group fed with medium leaves demonstrated no mortality. Statistical analysis revealed the fact that there was significant difference between the
Table 3.4: Combined effect of leaf quality and nuclear polyhedrosis on larval and cocoon characters

<table>
<thead>
<tr>
<th>Quality leaf</th>
<th>Per cent larval mortality</th>
<th>Larval duration after third moult onward (hrs)</th>
<th>Mature worm wt (mg)</th>
<th>Per cent cocoon formation</th>
<th>Per cent moth emergence</th>
<th>Cocoon wt (mg)</th>
<th>Shell wt (mg)</th>
<th>Shell ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>A 0.00</td>
<td>324.00±4.00</td>
<td>2477.00±84.00</td>
<td>96.00±3.00</td>
<td>94.00±2.00</td>
<td>1555.00±120.00</td>
<td>282.33±3.00</td>
<td>18.41±1.68</td>
</tr>
<tr>
<td></td>
<td>B 48.00±2.33</td>
<td>343.00±5.00</td>
<td>2433.00±128.00</td>
<td>52.00±2.33</td>
<td>48.00±1.00</td>
<td>1417.00±130.00</td>
<td>265.00±5.29</td>
<td>18.94±1.34</td>
</tr>
<tr>
<td>Mature</td>
<td>A 16.00±2.96</td>
<td>371.00±5.00</td>
<td>2319.00±21.00</td>
<td>84.00±2.96</td>
<td>68.00±6.00</td>
<td>1301.93±23.00</td>
<td>203.87±3.00</td>
<td>15.66±0.40</td>
</tr>
<tr>
<td></td>
<td>B 55.00±3.76</td>
<td>395.00±3.00</td>
<td>2135.00±91.00</td>
<td>45.00±4.00</td>
<td>38.00±9.00</td>
<td>1336.00±95.00</td>
<td>185.00±1.73</td>
<td>13.98±0.95</td>
</tr>
<tr>
<td>Mixed</td>
<td>A 20.00±4.63</td>
<td>342.00±5.00</td>
<td>2878.00±56.00</td>
<td>80.00±4.63</td>
<td>65.00±3.00</td>
<td>1727.00±123.00</td>
<td>295.33±2.00</td>
<td>17.36±1.93</td>
</tr>
<tr>
<td></td>
<td>B 64.00±6.06</td>
<td>381.00±4.00</td>
<td>2870.00±143.00</td>
<td>36.66±6.06</td>
<td>28.00±6.00</td>
<td>1343.00±34.00</td>
<td>241.00±6.11</td>
<td>17.96±0.57</td>
</tr>
<tr>
<td>Tender</td>
<td>A 31.00±4.36</td>
<td>317.00±4.00</td>
<td>3465.00±105.00</td>
<td>69.00±4.35</td>
<td>45.00±3.00</td>
<td>1640.67±13.00</td>
<td>346.67±2.00</td>
<td>21.13±0.67</td>
</tr>
<tr>
<td></td>
<td>B 76.00±2.00</td>
<td>331.00±3.00</td>
<td>3324.00±74.65</td>
<td>24.00±2.00</td>
<td>19.00±3.00</td>
<td>1556.33±36.41</td>
<td>300.00±0.58</td>
<td>19.28±0.06</td>
</tr>
</tbody>
</table>

Mean ± S.E. of 3 observations, NB. silkworm larvae treated during fourth instar and assessed after sixth day of fifth instar.
A represent untreated group,
B represent larvae treated with LC50 concentration during fourth instar,
* indicate values significant at 5% level among respective untreated group,
** indicate values significant at 1% level among respective treated group,
(Statistical analysis by 'F' value using one way ANOVA - Fisher, 1956).
Corrected by, Abbott's formula.
Table 3.5: Statistical analysis of the data from table 3.4

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Per cent larval mortality</th>
<th>Larval duration</th>
<th>Mature worms wts.</th>
<th>Per cent cocoon formation</th>
<th>Per cent moth emergence</th>
<th>Cocoon wt</th>
<th>Shell wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between the different leaf fed group (L)</td>
<td>3</td>
<td>38.81*</td>
<td>240.56*</td>
<td>52.43*</td>
<td>38.81*</td>
<td>48.81*</td>
<td>8.74*</td>
<td>4.23**</td>
</tr>
<tr>
<td>Between the test groups (T)</td>
<td>1</td>
<td>507.52*</td>
<td>168.39*</td>
<td>1.76 NS</td>
<td>507.52*</td>
<td>408.52*</td>
<td>1.29*</td>
<td>0.44 NS</td>
</tr>
<tr>
<td>Between the replication</td>
<td>2</td>
<td>7.20 NS</td>
<td>6.42 NS</td>
<td>0.22 NS</td>
<td>7.20 NS</td>
<td>5.20 NS</td>
<td>1.19 NS</td>
<td>1.48 NS</td>
</tr>
<tr>
<td>Interaction (L x T)</td>
<td>3</td>
<td>0.97*</td>
<td>10.23*</td>
<td>0.83*</td>
<td>0.97*</td>
<td>0.84*</td>
<td>4.55*</td>
<td>4.17*</td>
</tr>
</tbody>
</table>

* Values significant at 5% level,
** Values significant at 1% level,
NS Values non-significant.

(Statistical analysis by ANOVA as described by Fisher, 1956).
various leaf fed groups as well as in the untreated and treated groups (Table 3.5 and Fig.3.9).

Larval duration:

Among the untreated groups, the group fed with mature leaves had the maximum larval duration (371 ± 5 hrs). It was followed by that in mixed (342 ± 5 hrs) and medium leaf fed (324 ± 4.00 hrs) groups. The tender leaf fed group indicated the least larval duration (317 ± 4.00 hrs). The statistical comparison showed significant variation among the groups which were maintained on different type of leaves (Table 3.4, Fig.3.10).

In the treated groups, as well, the same trend was observed i.e. the group fed with mature leaves showed maximum larval duration (395 ± 3.00), followed by that with mixed (381 ± 4.00) and medium leaf fed groups (343 ± 5.00 hrs). The group fed with tender leaves demonstrated the least larval duration (331 ± 3.00 hrs). The statistical analysis of the data indicated that there was a significant variation among the groups, and there was a significant prolongation of larval duration as compared with that without the disease (Table 3.4, Fig.3.10).

Mature worm weight:

In the control group, the maximum larval weight was recorded in tender leaf fed group (3465 mg) followed by that
Figure 3.9: Combined effect of leaf quality and nuclear polyhedrosis on per cent larval mortality

Figure 3.10: Combined effect of leaf quality and nuclear polyhedrosis on larval duration
fed by mixed (2878 mg) and medium leaf fed group (2477 mg). The least larval weight was observed in mature leaf fed group (2319 mg) (Table 3.4, Fig.3.11).

In BmNPV treated groups, least larval weight was recorded in mature leaf fed group (2135 mg), followed by that in medium (2433 mg) and mixed leaf fed groups (2870 mg). Among the treated ones the tender fed group (3324 mg) demonstrated the higher larval weight. The larval weight marginally decreased in treated groups as compared with that in the respective untreated groups (Table 3.4 and Fig.3.11).

Per cent cocoon formation and moth emergence:

In BmNPV treated group, the per cent cocoon formation and moth emergence decreased significantly as compared with that in the respective untreated groups. Among the treated groups, least cocoon formation and moth emergence was observed in tender leaf fed group (24 and 19% respectively), followed by that in mixed (36 and 28% respectively), and mature leaf fed groups (45 and 38% respectively). Maximum cocoon formation and moth emergence (52 and 48% respectively) was found in medium leaf fed group (Table 3.4).

However, similar trend was observed in the untreated controls. The group fed with medium leaf recorded maximum cocoon formation and moth emergence (96 and 94% respectively).
Figure 3.11: Combined effect of leaf quality and nuclear polyhedrosis on larval weight

![Bar chart showing larval weight by leaf quality and treatment]

Figure 3.12: Combined effect of leaf quality and nuclear polyhedrosis on per cent cocoon formation

![Bar chart showing per cent cocoon formation by leaf quality and treatment]
respectively). It was followed by that fed with mature (84 and 68% respectively) and mixed leaves (80 and 65% respectively). Least cocoon formation and moth emergence was recorded in tender leaf fed group (69 and 45% respectively) (Table 3.4, Fig. 3.12 and 3.13).

Cocoon characters:

The cocoon weight, shell weight and shell ratio of all the treated groups decreased significantly (Table 3.4, Fig.3.14, 3.15 and 3.16) compared to those of the respective untreated control groups. The maximum cocoon and shell weight and shell ratio was recorded in tender leaf fed group (1556 and 300 mg and 19.28% respectively). It was followed by that fed with medium (1417 and 265 mg and 18.94% respectively) and mixed (1343 and 241 mg and 17.96% respectively) leaf fed groups. Cocoon weight, shell weight and shell ratio of mature leaf fed group was the lowest (1336 and 185 mg and 13.98% respectively).

Similar trend was observed in respective untreated control groups also. Minimum cocoon and shell weights and shell ratios were observed in mature leaf fed group (1302 and 204 mg, 15.66% respectively). Slightly higher values were observed in mixed (1727 and 295 mg, 17.36% respectively) and medium (1555 and 282 and 18.41% respectively). Maximum cocoon and shell weight and shell ratios were observed in tender leaf fed group (164 and 547 mg, and 21.13% respectively) (Table 3.4, Fig.3.14, 3.15 and 3.16).
**Figure 3.13:** Combined effect of leaf quality and nuclear polyhedrosis on per cent moth emergence

**Figure 3.14:** Combined effect of leaf quality and nuclear polyhedrosis on cocoon weight
Figure 3.15: Combined effect of leaf quality and nuclear polyhedrosis on shell weight

![Bar graph showing single shell weight (mg) for different leaf qualities and treatments.]

Figure 3.16: Combined effect of leaf quality and nuclear polyhedrosis on shell ratio

![Bar graph showing shell ratio (%) for different leaf qualities and treatments.]
Statistical analysis of ANOVA (Table 3.5) revealed significant variation with respect to per cent mortality, larval duration, mature worm wt., per cent cocoon formation, moth emergence and cocoon shell wt. of *B. mori*. However, the different quality leaf fed groups showed significant variation at 1% in respect of shell wt.

Significant variation was also observed in per cent larval mortality, duration, per cent cocoon formation, moth emergence and cocoon wt. among treated groups. But mature worm wt. and shell wt. did not show any significant variation between untreated and treated group. Replications of test groups did not show any variation. Interaction between the different leaf fed groups, and treated groups demonstrated significant variation in respect of larval and cocoon characters.
DISCUSSION

3.4.1 Effect on haemodynamics

Total haemocyte count (THC):

In the present investigations maximum number of haemocyte was observed in tender leaf fed group, followed by that in the medium and mixed leaf fed groups. Least number of haemocytes (cell/mm$^3$) was observed in mature leaf fed group. High nutrient content of tender leaves might have increased the growth rate of the silkworms (Jadhav, 1994), which in turn enhanced release of haemocytes from the haemopoietic tissues in terms of the increased physiological need of the insect (Beaulaton and Monpeyssin, 1976 and Jeyakumar et al., 1995). Similar observations have been reported by Jeyakumar et al. (1995), who observed increased THC of Pericallia riciniae larvae when fed on nutrient rich green stemmed castor leaves in comparison to red stemmed castor leaves. Minimum THC was recorded in mature leaf fed group. This could be attributed to the low nutrient quality of leaf (Jadhav, 1994 and Jeyakumar et al., 1995), and its subsequent effect on the growth and development of haemopoietic organs (Shapiro, 1968 and Akai and Sato, 1971).

In NPV treated groups, the THC decreased in general as compared with that of their respective controls. Minimum
THC was recorded in tender leaf fed group followed by that in mixed and mature leaf fed groups. This may be due to lysis of haemocytes and the effect on haemopoietic organs. Different groups varied in their degree of susceptibility owing to feeding on different quality of leaf. Tender leaf fed group possibly suffered more lysed haemocytes, resulting in lowest THC in contradiction to the expectation. Perhaps the multiplication of the virus was more in tender leaf fed group due to some other physiological change.

**Trehalose and protein concentration**

Maximum trehalose and protein concentration was observed in tender leaf fed group. This was followed by that in mixed and medium leaf fed groups. This may be due to enriched primary metabolites (carbohydrate, protein and amino acid etc. Narayanan *et al.*, 1967; Rupa *et al.*, 1993 and Jadhav, 1994). Minimum trehalose and protein concentration was observed in the group fed with mature leaf. Mature leaves are difficult to digest because of their texture and fibrous material (Anonymous, 1980). This low intake of food might have resulted in poor growth and hence low deposition of protein content in the blood.

During nuclear polyhedrosis, minimum trehalose and maximum protein concentrations were observed in tender leaf fed group. It was followed by that with mixed, medium and
mature leaf fed groups. The low trehalose concentration in all NPV treated groups may be due to its utilization by the host under stress and starvation (Bhosale and Kallapur, 1990) and destruction of fat body (Ingalhalli, 1993), the main site for the trehalose synthesis. Concentration of protein in haemolymph varied in different leaf fed groups depending upon the susceptibility of each group. The tender leaf fed group was the most susceptible followed by mixed, mature and medium leaf fed groups. High protein concentration of treated groups may be due to destruction/lysis and drainage of infected tissues (Takei and Tamashiro, 1975 and Bhosale and Kallapur, 1990).

pH:

The pH of tender leaf fed group was maximum, followed by mixed and mature leaf fed groups under untreated condition. However, in BmNPV treated groups, pH increased in all the leaf fed groups with maximum in tender followed by mixed and medium leaf fed group, respectively. Significantly in mature leaf fed group, variation in pH value was minimum under untreated and treated condition. These changes in pH of haemolymph may be attributed to varied quality leaf fed to silkworm B.mori and nuclear polyhedrosis.
Cations concentration:

The dietary conditions are known to affect the cation concentration (Shimizu, 1982), and this may alter some of the characteristics of susceptibility, voltinism and moltinism in the silkworm (Takamiya, 1975 and Sumimoto, 1974).

There was considerable increase in cation concentration during nuclear polyhedrosis among all groups. Maximum cation concentration was observed in tender leaf fed group. It was followed by that fed medium and mixed leaves. pH was more toward acid side in mature leaf fed groups. The cation concentration in untreated group could be attributed to the quality of leaf. Their subsequent utilization or accumulation during the active feeding stage might also affect the concentration. Higher cation concentration in treated group may be due to accumulation of the same from lysed tissues into the haemolymph (Ramakrishna, 1968) depending upon the susceptibility of the group fed with varied quality leaf.

3.4.2 Larval and cocoon characters

Incidence of nuclear polyhedrosis:

The treated tender leaf fed group demonstrated maximum susceptibility to nuclear polyhedrosis. This susceptibility may be due to the increased water content and decreased carbohydrate and cation content of the tender leaf
(Anonymous, 1980 and Jadhav, 1994). Least mortality was recorded in the treated group fed with the medium leaf. Perhaps the balanced nutrient content of the leaf (Jadhav, 1994) and the minimum susceptibility of the silkworms may be the reason. Therefore NPV treated groups showed higher susceptibility depending upon the nutrient content of the leaf. Similar results have been reported by Sivaprakasam et al. (1996). The per cent cocoon formation and moth emergence possibly depended on the per cent mortality respectively at larval and pupal stages.

Larval duration

During nuclear polyhedrosis, prolongation of larval period was observed in all groups fed with different quality leaves. The maximum life span was observed in mature leaf fed group, and least in tender leaf fed group. The varied prolongation of larval period in different treated groups, may be due to nutritional state of worms and alteration of rate of synthesis of the insect hormones.

Mature worm weight

Feeding activity is an important factor in the accumulation of reserve materials. The amount of food consumed by a larva influences its growth rate, development final body weight and probability of survival (Slansky and Scriber, 1985). Food intake is also regulated by the
physical nature of food and also presence of phagostimulants in the food (Dadd, 1970).

The increase in larval weight of tender leaf fed group, may be due to its higher palatability and nutrient quality of these leaves (Anonymous, 1980). The difference in physical nature (hardness) and nutrient quality of mature leaf could be accounted for the lower intake of food leading to the poor growth in mature leaf fed group (Narayanan et al., 1967 and Anonymous, 1980).

Significant decrease in larval weights of treated worms depended on the quality of leaf fed to each group. This may be due to the starvation induced in different groups of silkworms proportionate to the stress caused by different quality of food (Harper, 1973). Similar results have been reported by Narayanan et al. (1967), and Basavarajappa (1996) during nuclear polyhedrosis.

Cocoon characters

The cocoon weight, shell weight and per cent shell ratio are important as they reflect on the productivity of the silkworm. Increase in cocoon wt., shell wt. and shell ratio in tender, mixed and medium leaf fed groups may be according to the availability of protein content in such leaves (Table 3.1 and Fig. 3.3). Low protein content of
mature leaf (Jadhav, 1994) might have accounted for reduction in cocoon and shell wts. and shell ratio (Anonymous, 1980 and Basavarajappa, 1996). It is evident from the results that in untreated controls these parameters were at a significantly higher level as compared to those in the respective treated groups.

However, all the NPV treated groups showed decreased cocoon parameters. This may be due to the induced stress or starvation during nuclear polyhedrosis (Harper, 1973). Moreover, in addition to the differential lack of availability of proteins from leaves, the virus might have also blocked the protein synthesis and utilized the proteins for its own maturation.

Leaf quality have differential impact on haemodynamics of B. mori. Combined effect of leaf quality and nuclear polyhedrosis is more pronounced due to the possible stress. Changes in the total haemocyte count, trehalose, protein and ionic concentration affect the silkworm physiology depending upon the host nutritional stress. These physiological changes reflect upon the larval and cocoon characters of B. mori.