CONSUMPTION OF TRANSITIONAL METAL CONTENTS BY TASAR SILKWORM, ANtheraea mylitta D FOR ITS HEALTHY DEVELOPMENT

Shantakar Giri*, Susmita Das, Jitendra Singh and K. Sathyanarayana

Introduction

Tasar silkworm is polyphagus in nature and feeds on several host plants. However, it has food preference. The host plants, which silkworm normally prefers are known as primary host plants. Other host plants, where the silkworm can sustain its life, but normally do not prefer, are called secondary host plants. The tasar silk worm, Antheraea mylitta D thrives mostly on the leaves of three primary host plants namely Terminalia tomentosa (Asan), Terminalia arjuna (Arjun) Shorea robusta (Sal). It derives nutrients required for its growth from the leaves of these host plants. The growth and development of the silkworm and economic characters of cocoons produced by them are greatly influenced by the nutrient content of the leaves. Further, the quality of tasar host plant and leaves depend on nutrient status of the soil. The host plants take their food elements such as transition metals (Mn, Fe, Cu and Zn) and also other elements in their available form from the soil which helps the plants to grow resulting better quality of leaves.

The requirement of trace elements as micronutrients are generally comes under first row transition metals in the periodic table. The presence of about forty different elements has been established in living bodies (Das, 2007), out of which, manganese, iron, copper and zinc are the important essential metal elements present in trace quantities. The micronutrients (Zn, Cu, Fe, Mn) play a pivotal role both in maintaining and reinforcing immune and antioxidant performance and in affecting the complex system of genes implicated in encoding necessary proteins for a correct inflammatory immune response (Prasad, 2008). Plant takes these elements from soil in the form of ions and then these get transferred to different parts of plant and finally to the animal body which consumes it. This essential trace element which comes under micronutrient, there are two ranges of intake associated with adverse health effects: intakes that are too low and can lead to nutritional deficits and intakes that are too high and can lead to toxicity. Between these two ranges, there is a range of safe and adequate intakes that is compatible with good health. Tasar silk worm is polyphagus having a number of primary and secondary food plants. Terminalia tomentosa is one of the primary food plants of tropical tasar silk worm, Antheraea mylitta Drury (Jolly et al., 1968). Several studies have been carried out on foliar constituents of tasar food plants (Sinha & Jolly, 1971;...
Antheraea mylitta consumption of transition metal contents by tasar silkworm, voltinism. Hence, a study was undertaken to know the synergistic effect of metal contents of Cu, Fe, Mn and Zn on pupa stage of different eco-races of tasar silkworm on their critical limit are defined as inadequate level in the soil elements i.e. Mn, Fe, Cu and Zn in the soil of investigated area were found as per the graphical representation shown in Figure-1. Fifty composite leaf samples of T. tomentosa under block plantation and forest were collected during the month of June (45 days after sprouting of leaves). The samples were thoroughly washed with tap water followed by 0.1N HCl solution and double distilled water. The washed samples were air dried and then kept in hot air oven at 70°C. The dried leaves were powdered and stored in plastic bottles for chemical analysis. 1.00 g each leaf sample was digested in 10 ml 9:4 mixture of HNO₃: HClO₄ at 200°C until the liquid becomes colorless. Mn, Fe, Cu and Zn contents were estimated by the methods as described in the book of H.L.S. Tandon (2001) using Atomic Absorption Spectrophotometer (Varian make, 280FS).

Cocoon sampling and pupa analysis

A total of 60 cocoons, 10 cocoons each of six different eco-race viz. Daba, Sukinda, Modal, Raily, Bogai and Laria were collected from the study area. Collected cocoon samples of these eco-races were cut and pupae were taken out from their shells. Then, pupa samples were dried by keeping all those in hot air oven at 70°C. The dried pupa samples were ground to powder for chemical analysis. For estimation of metals in pupae, 1.00 g each of these samples was digested in 10 ml 9:4 mixture of HNO₃: HClO₄ at 200°C until the liquid becomes colorless. Mn, Fe, Cu and Zn contents in pupa were estimated by using Atomic Absorption Spectrophotometer (Varian make, 280FS) with diluting the digested samples in 100 to 500 times as per the range of metals contents present. Before aspirating the sample solution in AAS, blank solution and standard solutions were aspirated and run the equipment as per guidelines.

Materials and Methods

Soil, leaf and cocoon samples were collected from different tropical tasar producing areas in India, from important locations of six districts namely West Singhbhum, and Dumka districts of Jharkhand, Mayurbhanj and Keonjhar districts of Odisha and Raigarh and Bastar districts of Chhattisgarh respectively as shown in Figure-1.

![Map of Study area](image)

**Fig. 1 : Map of Study area**

Soil and Leaf sampling and analysis

The composite soil samples of 1’ depth were collected from farmers’ fields of fifty-eight different locations. The soil samples were air-dried, pulverized and passed through a 2 mm sieve before analysis. Available micronutrients of transition metal elements manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) were estimated by using DTPA extractant as outlined by Lindsay and Norvell (1978) and atomic absorption spectrophotometer.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Statistical analysis for co-relation of nutrient content of transitional metals with cocoon weights, shell weight and silk ratio % were determined using MS-excel tool. Co-relation factor were considered statistically significant when p<0.05(*), p<0.01(**) and p<0.001(***)

Results and Discussion

Status of available micronutrient of transition metals in tasar growing areas

Available micronutrient status of first row transition elements i.e. Mn, Fe, Cu and Zn in the soil of investigated area were found as per the graphical representation shown in Figure-2 to Figure-5. Critical limits of Mn, Fe, Cu and Zn were rated as per Folllet and Lindsay. As per the same, the critical limits of available Mn, Fe, Cu and Zn in the soil are 2.00 mg/kg, 4.5mg/kg, 0.20 mg/kg and 0.60 mg/kg respectively. Available quantity of micronutrients less than their critical limit are defined as inadequate level in the soil

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for healthy growth of the plant whereas available quantity of micronutrients more than their critical limit are defined as adequate level in the soil for healthy growth of the plant. Study revealed that the availability of micronutrients such as Cu, Fe and Mn in soil of tasar growing areas is much more than their critical limits whereas the availability of Zn in soil of these areas is slightly higher than its critical limit except Raigarh district of Chhattisgarh. Availability of Zinc in the soil of tasar growing areas of Raigarh district of Chhattisgarh is in low level, i.e. less than its critical limit.

The mean value and standard deviation for the availabilities of Mn, Fe, Cu and Zn in the soil of tasar producing areas of Jharkhand, Odisha and Chhattisgarh were found in the range of 42.03±20.44 mgkg⁻¹, 28.37±18.61 mgkg⁻¹, 1.45±0.50 mgkg⁻¹ and 1.70±0.88 mgkg⁻¹ respectively as per graphical representation shown in Figure-6. Data reveals that Mn, Fe and Cu are abundantly available in the soil whereas availability of Zn is adequate or just above its critical limit in the soil.

Fig. 2: Availability of Mn in the soil of tasar growing areas

Fig. 3: Availability of Fe in the soil of tasar growing areas

Fig. 4: Availability of Cu in the soil of tasar growing areas

Fig. 5: Availability of Zn in the soil of tasar growing areas

Fig. 6: Status of available micronutrients of transition metals in the soil of tasar producing area

Content of micronutrient of transition metals in tasar host plant of *Terminalia tomentosa*

Contents of four trace elements of transition metals viz. Mn, Fe, Cu and Zn as micronutrients in leaf of *T. tomentosa*, a primary tasar host plants to which silkworm consumes as food were found in average as 46.53 ppm, 213.82 ppm, 24.91 ppm and 37.98 ppm respectively which has been shown in Figure-7. Data reveals that micronutrient contents in leaf of, *T. Tomentosa* (Asan) exist in the following order: Cu < Zn < Mn < Fe and is essentially required for healthy development of tasar silkworm. As there was no adverse effect observed during the developmental stage of tasar silkworm larva and during its cocoon formation, consumption of these trace elements was within safe range.

Fig. 7: Micronutrient content of transition metals in *T. tomentosa* leaf

Instar-wise leaf consumption and per day intake of transition metal content by tasar silkworm larva of Daba bi-volntine eco-race

Intake quantity of transition metal contents by the tasar silkworm larva of Daba bi-volntine eco-race was estimated on the basis of leaf consumption during average larval period of 32 days. The data of the same has been shown in Table-1. Results revealed that manganese and iron intake were more than copper and zinc.
Table 1: Instar-wise consumption of leaf of *T. tomentosa* and intakes of Mn, Fe, Cu & Zn by tasar silkworm larva. *Antheraea mylitta* D. for Daba bivoltine ecorace.

<table>
<thead>
<tr>
<th>Stage of larva</th>
<th>Per day consumption of leaf/larva (in g)</th>
<th>Dry wt. of per day consumption of leaf/larva (in g)</th>
<th>Per day intake of Mn (in mg)/larva</th>
<th>Per day intake of Fe (in mg)/larva</th>
<th>Per day intake of Cu (in mg)/larva</th>
<th>Per day intake of Zn (in mg)/larva</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>0.225</td>
<td>0.07</td>
<td>0.003</td>
<td>0.015</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>2nd</td>
<td>1.198</td>
<td>0.36</td>
<td>0.017</td>
<td>0.077</td>
<td>0.010</td>
<td>0.014</td>
</tr>
<tr>
<td>3rd</td>
<td>2.995</td>
<td>0.90</td>
<td>0.042</td>
<td>0.192</td>
<td>0.022</td>
<td>0.034</td>
</tr>
<tr>
<td>4th</td>
<td>6.589</td>
<td>1.98</td>
<td>0.092</td>
<td>0.423</td>
<td>0.049</td>
<td>0.075</td>
</tr>
<tr>
<td>5th</td>
<td>19.693</td>
<td>7.09</td>
<td>0.329</td>
<td>1.52</td>
<td>0.176</td>
<td>0.269</td>
</tr>
<tr>
<td>Av.</td>
<td>6.14</td>
<td>2.08</td>
<td>0.097</td>
<td>0.445</td>
<td>0.052</td>
<td>0.079</td>
</tr>
</tbody>
</table>

Table 2: Content of transition metals in pupa and cocoon character of some important eco-races of tasar

<table>
<thead>
<tr>
<th>Eco-Race</th>
<th>Mn (ppm)</th>
<th>Fe (ppm)</th>
<th>Cu (ppm)</th>
<th>Zn (ppm)</th>
<th>C.W. (g)</th>
<th>S.W. (g)</th>
<th>S.R.%</th>
<th>F.L. (metre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daba BV</td>
<td>10.91</td>
<td>178.71</td>
<td>16.10</td>
<td>294.58</td>
<td>13.18</td>
<td>1.87</td>
<td>14.19</td>
<td>1040</td>
</tr>
<tr>
<td>Modal</td>
<td>3.71</td>
<td>163.40</td>
<td>14.15</td>
<td>346.51</td>
<td>18.48</td>
<td>3.84</td>
<td>20.78</td>
<td>1625</td>
</tr>
<tr>
<td>Raily</td>
<td>10.94</td>
<td>149.33</td>
<td>34.07</td>
<td>257.47</td>
<td>17.24</td>
<td>3.14</td>
<td>18.21</td>
<td>1410</td>
</tr>
<tr>
<td>Bogai</td>
<td>10.25</td>
<td>461.81</td>
<td>14.09</td>
<td>175.47</td>
<td>9.26</td>
<td>1.33</td>
<td>14.23</td>
<td>715</td>
</tr>
<tr>
<td>Laria</td>
<td>12.72</td>
<td>186.42</td>
<td>133.43</td>
<td>216.57</td>
<td>8.92</td>
<td>1.21</td>
<td>13.62</td>
<td>690</td>
</tr>
<tr>
<td>STV</td>
<td>7.91</td>
<td>176.32</td>
<td>16.58</td>
<td>224.69</td>
<td>12.15</td>
<td>1.48</td>
<td>12.17</td>
<td>816</td>
</tr>
</tbody>
</table>

**Effected of transition metals on health of silkworm and commercial traits of tasar cocoons:**

Nutritional ecology is a useful way to synthesize research on both basic and applied aspects of insect behavior of tasar towards oozing out the silk content from the silk gland. The metal elements of first transition series, Manganese (Mn), Iron (Fe), Copper (Cu) and Zinc (Zn) have a major role in the life process which come under essential gland. The natural catalysts in biological systems are enzymes. About 30% of the enzymes are metalloenzymes or metal activated enzymes. Evaluation of nutritional quality in spiraling whitefly infested in mulberry was studied by Mahadeva et al., 2012 and enzymatic effect of manganese on mulberry silkworm was studied by Yamamoto et al., 2005. It has also been reported (Malik et al., 2009) that the variation in metal ion concentrations in the haemolymph of silkworm, *Bombyx mori* occurs during developmental stages. The content of transition metals such as manganese, iron, copper and zinc which play a vital role in governing the physiology of both plant and the silkworm were estimated and their relationship with the cocoon characteristics were studied. Comparative data on content of transition metals in different eco-races of tasar silkworm pupa and their cocoon characters have been shown below in Table-2.

Table 3: Correlation table of micronutrient content in pupa and cocoon characters with value of correlation coefficient (r).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cu (ppm)</th>
<th>Fe (ppm)</th>
<th>Mn (ppm)</th>
<th>Zn (ppm)</th>
<th>C.W. (g)</th>
<th>S.W. (g)</th>
<th>S.R.%</th>
<th>F.L. (metre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (ppm)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe (ppm)</td>
<td>-0.017</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn (ppm)</td>
<td>0.387</td>
<td>0.474</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn (ppm)</td>
<td>-0.122</td>
<td>-0.387</td>
<td>-0.222</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.W. (g)</td>
<td>-0.391</td>
<td>-0.651</td>
<td>-0.480</td>
<td>0.476</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.W. (g)</td>
<td>-0.297</td>
<td>-0.607</td>
<td>-0.464</td>
<td>0.430</td>
<td>0.943</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.R.%</td>
<td>-0.166</td>
<td>-0.462</td>
<td>-0.352</td>
<td>0.300</td>
<td>0.762</td>
<td>0.927</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>F.L. (metre)</td>
<td>-0.319</td>
<td>-0.636</td>
<td>-0.449</td>
<td>0.432</td>
<td>0.966</td>
<td>0.981</td>
<td>0.872</td>
<td>1</td>
</tr>
</tbody>
</table>

It was found that filament length, cocoon weight and shell weight are positively correlated with the zinc content of pupa with highly significant value of correlation coefficient(r) which is significant at p<0.001. Shell ratio% is also positively correlated with zinc content of pupa which is significant at p<0.05. On the contrary, copper, iron and
manganese are negatively correlated with these cocoon characters. The inter relationship established by correlation analysis has been shown in Table-3. Metal content in pupa of *T. tomentosa* fed Daba BV ecorace has been shown in Figure-8 which clearly indicates that Zinc is required in larger quantity for tasar silkworm.

![Figure 8](image)

**Fig. 8:** Content of transition metals in pupa of tasar silkworm

**Conclusion**

It was inferred that tasar silkworm *Antheraea mylitta D.* has content of micronutrient of transition metal elements in its pupa stage in the ascending order as Mn < Cu < Fe < Zn whereas it was found in the order of Cu < Zn<Mn< Fe in tasar host plant leaf of *T. tomentosa*. Available Zn in the soil of tasar producing areas were also found relatively less than compared to Mn and Fe. Hence, it is evident that zinc supplementation to tasar silkworm larva in appropriate dose will be beneficial for the silk industry as zinc is required in larger quantity than other essential trace element as micronutrient for healthy development of tasar silkworm and silk production.

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**References**


