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RESEARCH ARTICLE

Manganese from Soil to Silk in Tasar Silkworm, *Antheraea mylitta* Drury and Its Effect in Cocoon Yield

Shantakar Giri[1], Sanjoy Misra[2]

[1]P_4 Tasar Breeding Station, Central Tasar Research and Training Institute, Central Silk Board, Dumka, Jharkhand, India, [2]Department of Chemistry, Ranchi University, Ranchi, Jharkhand, India

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ABSTRACT

An investigation on the status of available manganese in soil and its content in a leaf of the host plant, larva, pupa, excreta, and cocoon shell of Tasar silkworm, *Antheraea mylitta* Drury was undertaken to study the dynamics and impact of consumption of manganese in cocoon production. Availabilities of Mn, Fe, Cu, and Zn in the soil of Tasar rearing plot at Nagri, Ranchi was found as 82.79 ± 9.78 mg/kg, 23.32 ± 2.96 mg/kg, 0.83 ± 0.202 mg/kg, and 0.68 ± 0.097 mg/kg, respectively. Manganese is adequately available in the soil, but its contents in leaf, larva, litter, pupa, and cocoon shell vary remarkably. The content of manganese in the leaf of *Terminalia tomentosa* was found in the range of 50–100 ppm which was optimum for the success of the crop. Excess consumption of Mn by the larva of Tasar silkworm resulted in the failure of the crop yielding 2 cocoons/dfl when reared on *Shorea robusta* which has Mn content in the range of 353 ppm–2543 ppm. Daily requirement of manganese in optimum level for healthy development of Tasar silkworm larva of Daba bivoltine Eco Race was calculated to be in the range of 0.007–0.035, 0.018–0.36, 0.045–0.090, 0.099–0.198, and 0.354–0.708 mg for 1st, 2nd, 3rd, 4th, and 5th instars, respectively. Manganese content in a leaf of *Shorea robusta* (Sal) indicated that its translocation capacity compared to *Terminalia arjuna* (Arjun) and *T. tomentosa* (Asan) is very high which is detrimental for the health of Tasar silkworm of Daba bivoltine Eco Race.

Key words: *Antheraea mylitta* D, concentration manganese, *shorea robusta, terminalia arjuna, terminalia tomentosa*

INTRODUCTION

The presence of about 40 different elements has been established in living bodies,[4] out of which, manganese is one of the important essential metal elements present in trace quantity. The plant takes manganese from the soil as essential micronutrient, which is one of the 17 essential plant nutrients. Thus, manganese is an essential metal element for both plant and animal and has been categorized as essential biometal which acts as a catalyst in many biochemical reactions. However, the excess of manganese concentration in the diet is detrimental to health and becomes toxic after a certain limit. A wide variety of metal-dependent enzymes are found in nature which acts in fundamental biological processes, including photosynthesis, respiration, and nitrogen fixation.

Mn cycle in soil

The equilibrium among solution, exchangeable, organic, and mineral forms determines Mn availability to plants [Figure 1]. The major processes are Mn oxidation-reduction and complexing solution Mn with natural organic chelates. Like Fe, the continuous cycling of organic matter (OM) significantly contributes to soluble Mn. Factors influencing the solubility of soil Mn include pH, redox, and organic complexation.[9] Soil moisture, aeration, and microbial activity influence redox, while complexation is affected by OM and microbial activity.

Address for correspondence:
Shantakar Giri,
E-mail: shantakar69@gmail.com

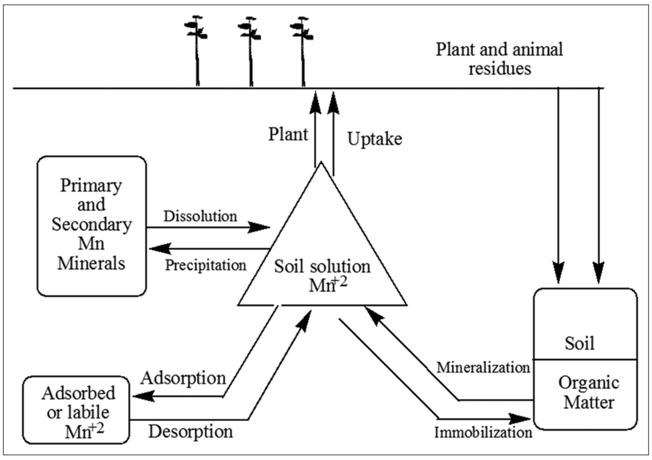

Figure 1: Mn cycle in soils

Plants absorb Mn^{+2} and low molecular weight organically complexed Mn. Mn concentration in plants typically ranges from 20 to 500 ppm, while Mn deficient plants contain <15–20 ppm Mn. Mn must be reduced to Mn^{+2} for absorption by roots by:

$$MnO_2 + 4H^+ + 2e^- \xrightarrow{\text{microbes}} Mn^{+2} + 2H_2O$$

Low molecular weight organic compounds are exuded by roots into the rhizosphere. Microbial degradation of these exudates establishes reducing conditions and provides electrons to reduce Mn to Mn^{+2} for absorption.

Mn^{+2} enters root cells through the plasmalemma by a specific transporter protein that establishes an electrical gradient where the cell wall is more (+) than the cell interior. Few other cations compete with Mn^{+2} for transport across membranes, which are unique since other cations do compete with each other (e.g. Cu^{+2} and Zn^{+2}). However, high concentrations of Ca^{+2} and Mg^{+2} adsorbed to apoplasmic (root) cell walls, especially in high pH soils, can reduce Mn^{+2} adsorption to cell walls and eventual transport into the cell.

Mn is essential to photosynthesis reactions, enzyme activation, and root growth.

O_2 and Photosynthesis

Most O_2 in the atmosphere originates from Mn-facilitated electron transport in photosynthesis. Photosynthetic reduction of CO_2 to carbohydrates $[(CH_2O)_n]$ given by:

$$CO_2 + 2H_2O \xrightarrow{\text{Light}} (CH_2O)n + O_2 + H_2O)$$

Involves several electron transfer steps. When chlorophyll absorbs light energy, it is oxidized (loses an electron) and provides the energy to reduce CO_2. The oxidized chlorophyll accepts electrons from an Mn-containing protein. When Mn donates electrons to chlorophyll, the oxidized Mn protein will oxidize H_2O to produce O_2.

$$2H_2O + 4Mn^{+3} \longrightarrow 4Mn^{+2} + O_2 + 4H^+)$$

The reduced Mn protein again donates electrons to another photo-oxidized chlorophyll. Therefore, Mn is essential to electron transfer through chlorophyll to reduce CO_2 to carbohydrate and produce O_2 from H_2O.

Reducing agents formed in cellular reactions can donate an electron to O_2, forming the superoxide free radical O_2^-. Free radicals are highly reactive and toxic to cellular metabolic reactions (e.g. chlorophyll degradation). Superoxide dismutase (SOD) enzymes are produced to readily convert O_2^- to O_2. Fe-SOD and CuZn-SOD occur in chloroplasts, while Mn-SOD occurs in mitochondria. This protection mechanism is especially important in plants grown under high light intensity where potential free radical production and photo-oxidation damage are the greatest.

Mn and lignin synthesis

Like Cu, Mn activates several enzymes that synthesize several amino acids and phenols important to lignin production. In addition to lignin, these compounds are used to synthesize phenolic acids and alcohols that provide resistance to infection by pathogens.

Mn deficiency and toxicity

Due to its essential role in photosynthesis, root and shoot growth rates are substantially reduced in Mn-deficient plants. As a result, N and P accumulate, which increases the potential for root and leaf diseases. Mn deficiency also restricts the formation of lignin and phenolic acids that also help to reduce the incidence of diseases. Soil fungi that generally do not infect plant roots can cause disease in Mn-deficient plants. Grasses low in Mn are often more susceptible to root rot diseases.

Mn is immobile in the plant, so younger leaves initially exhibit deficiency symptoms. Mn deficiency produces interveinal chlorosis most crops. In some crops, low-Mn-related chlorosis of younger leaves can be mistaken for Fe deficiency. Mn deficiency of several crops has been described by such terms as a gray speck of oat, marsh spot of pea, and speckled yellows of sugar beet. Mn toxicity occurs in sensitive crops grown on acid soils.

Mn sources

Organic Mn

Mn concentration in most animal wastes is similar to Zn, ranging from 0.01% to 0.05% Mn. Average application rates of most manure will provide sufficient plant-available Mn. As the Fe, Zn, and Cu, the primary benefit of organic waste application is increased OM and associated natural chelation properties are increasing Mn availability. As with the other micronutrients, Mn content in municipal waste varies greatly depending on the source. On the average, Mn content is about half the Cu content (0.05%).

The tasar silkworm, *Antheraea mylitta* drury is polyphagus in nature having a number of primary and secondary host plants. Extensively, it thrives on the leaves of primary tasar food plants - *Terminalia tomentosa* (Asan), *Terminalia arjuna* (Arjun), and *Shorea rubusta* (Sal**)** which are widely available in the tropical belt in India. Out of many ecological and biological factors which influence the crop production, the quality of Tasar food plants used for rearing is one of the most contributing factors. The growth and development of the silkworms and commercial characters of cocoons produced by them are greatly influenced by the nutrient contents of leaves.[21] Further, the quality of leaves used for tasar silkworm rearing depends on the nutritional status of the soil. It has also been reported that improvement of both mulberry and Tasar food plants' leaves can be made by micronutrients such as Fe, Cu, Zn, Mn, B, and Mo. However, the requirement of micronutrients level in soil and leaves of tasar host plants has least been studied. In insect, the growth rate is related to the nutrients absorbed by the body from different host plants.[5,6,15] Evaluation of nutritional quality in spiraling whitefly infested in mulberry was studied by Mahadeva and Nagaveni[11] and enzymatic effect of manganese on mulberry silkworm was studied by Yamamoto *et al.*[27] It has also been reported[12] that the variation in metal ion concentrations in the hemolymph of silkworm, *Bobmyx mori* occurs during developmental stages. Several studies have been carried out on foliar constituents of Tasar food plants.[1,22-25] In mulberry; mineral nutrition deficiency and requirement were studied by several workers.[8,18-20] Soil micronutrient status of mulberry gardens was studied by some workers.[3,14] For human being, the most important source of Mn for the general population is diet, and the average intake of Mn from food ranges from 2 to 9 mg/day.[16] However, no study has so far been carried out to know the transfer dynamics of a nutrient element from soil to silk in Tasar culture. Moreover, the optimal requirements of insects are largely unknown.[12] Hence, a study was undertaken to know the optimum requirement of manganese concentration in Tasar host plant leaves for the healthy development of Tasar silkworm. The requirement of manganese content in Tasar silkworm feed was estimated on the basis of consumption of a quantity of leaf by the larva and crop performance of Daba bivoltine Eco Race.

MATERIALS AND METHODS

Ten composite soil samples for an area of around one hectare under Tasar host plants of *T. tomentosa* at field laboratory CTR and TI, Ranchi (Jharkhand) were collected from one feet depth of the land during the month of June. Ranchi is located in the southern part of the Chhota Nagpur plateau at 23.35° N Latitude and 85.33°E Longitude and the average elevation of the city is 629 m above sea level. It has a humid subtropical climate with hilly topography and dense tropical forests. Temperature ranges from maximum 42 to 20 C during summer and from 25 to 0°C during winter. The annual rainfall is about 1430 mm.

All collected soil samples were air dried ground and passed through 2 mm sieve before analysis. Important soil characteristics, namely pH and organic carbon percentage were determined by the potentiometric method, 1:2.5 soil-water suspension (Jackson, 1973) and organic carbon content by Walkley and Black method, respectively, as described in the book of soil testing and recommendation by Basak, 2006.[2] As primary nutrients, available Nitrogen was estimated by Alkaline $KMnO_4$ method (Subhiah and Asija, 1956), Phosphorous by Bray and Kruz (1945), and Potassium by Ammonium acetate extracts (using flame photometer). The available transition metals as micronutrients were estimated by DTPA extractable,[10] method using Atomic Absorption Spectrophotometer as described in the book of Tandon.[26]

Composite leaf samples from *T. tomentosa* under block plantation were collected during rearing period in 13 replications. The samples were thoroughly washed with tap water followed by 0.1 N HCl solution and double distilled water. The washed samples were air dried and then kept in a hot air oven at 70°Ċ. The dried leaves were powdered and stored in plastic bottles for chemical analysis. 1.00 g of each leaf sample was digested in 10 ml 9:4 mixture of HNO_3:$HClO_4$ at 200°C until the liquid becomes colorless. Manganese content in leaf was estimated by the methods as described in the book of Tandon (2001).[26] Leaf samples of Arjun and Sal were also collected in the same way and analyzed following the same procedure.

Litters and cocoons of Daba bivoltine race of *A. mylitta D.* were collected from the rearing field from where leaf and soil samples collected for the study. Litters collected and Pupae taken out from the collected cocoon samples were dried by keeping these in a hot air oven at 70°Ċ. The dried litter and pupa samples were ground to powder and stored in plastic bottles for chemical analysis. For estimation of manganese in litters, pupae and cocoon shell, and 1.00 g each of these samples were digested in 10 ml 9:4 mixture of HNO_3:$HClO_4$ at 200°C until the liquid becomes colorless. Manganese was estimated by the method as described in the book of Tandon (2001).[26] Daily requirement of manganese content in milligram was calculated on the basis of average leaf consumption by the larvae. The calculation of leaf consumption was adopted as suggested by Jolly *et al.*[7]

RESULTS

Table 1 indicates that the soil of block plantation of *T. tomentosa* at CTR and TI, Ranchi is strongly acidic having a low level of organic carbon content. The availability of nitrogen and phosphorous in the soil is in low level whereas, the available potassium in the soil is in the medium range. Availability of micronutrients such as Cu, Fe, Mn, and Zn is in the adequate level. Mn content in a leaf of Asan and Arjun was found to be in the range of 59.29 ± 8.88 ppm–49.56 ± 11.52 ppm whereas it was found in the range of 353 ppm–2543 ppm in case of Sal [Figure 2]. Daily requirement of manganese was calculated on the basis of leaf consumption and considering 70% leaf moisture during larval feeding. The optimum range was calculated to be of 0.007–0.035, 0.018–0.36, 0.045–0.090, 0.099–0.198, and 0.354–0.708 mg for 1st, 2nd, 3rd, 4th, and 5th instars, respectively, for healthy development of Tasar silkworm larva of Daba bivoltine Eco Race. Further, it was found that manganese content in tender leaf of natural Sal tree is in less quantity compared to mature leaves of the same plant.

DISCUSSION

Availability of manganese in the soil of Tasar host plant gardens is in adequate to excess level as per the critical limits of Mn, Fe, Cu, and Zn rated by Follet and Lindsay. The limits for Mn, Fe, Cu,

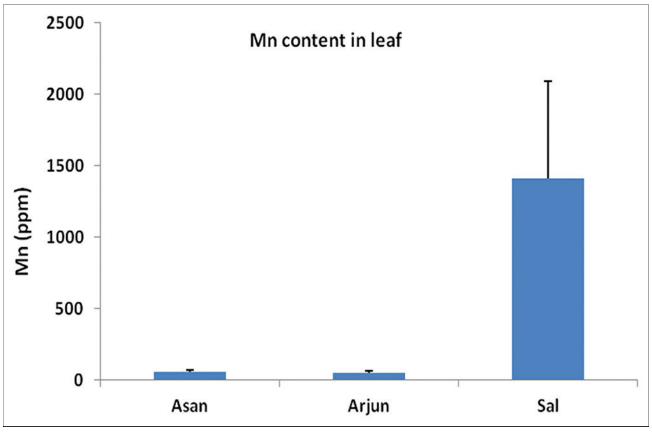

Figure 2: Content of Mn(ppm) in leaves of tasar host plants

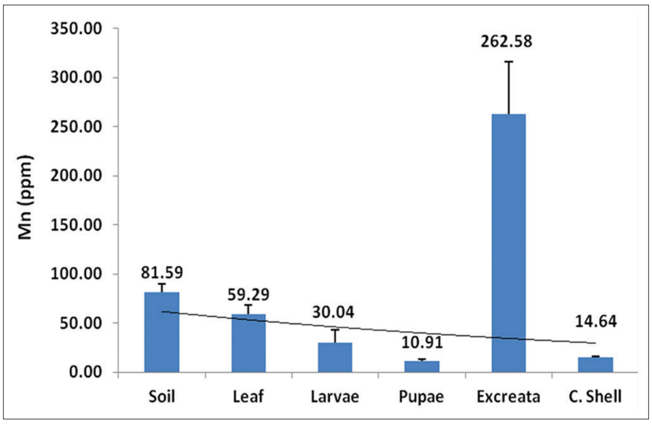

Figure 3: Mn content at variable status from soil to silk in tasar silkworm of Daba bivoltine eco-raceplants

Table 1: Nutrient status of soil at field laboratory of CTR and TI, Ranchi

Name of the place and type of plantation	Soil property		Nutrient status of soil (in available form)						
	pH	O.C %	N (kg/ha)	P (kg/ha)	K (kg/ha)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Nagri, Ranchi	5.15±0.36	0.48±0.012	261.92±9.03	9.26±0.55	179.68±9.12	0.83±0.202	23.32±2.96	82.79±9.78	0.68±0.097

and Zn are 2.00 mg/kg, 4.5 mg/kg, 0.20 mg/kg, and 0.60 mg/kg, respectively. Some plants can tolerate extremely high manganese level without detrimental effects whereas some are sensitive to excess manganese. Manganese content in *Shorea robusta* (Sal) indicates that its translocation capacity compared to *T. arjuna* (Arjun) and *T. tomentosa* (Asan) is very high which may be attributed to its genic effect adapting to all the growing area of *Shorea robusta*. Therefore, quantum of manganese was recorded higher. Moreover, a high Mn level in plants is a sign of low soil pH.[17]

Transfer cycle of Mn from soil to silk is presented in Figure 3. When Tasar silkworm was fed with Asan leaf, it was found that larger quantity of manganese got disposed of as excreta in the form of litters. Some 59.29/mg/kgofMn present in the leaf (Asan) was consumed by the larva and retained only 30.24 mg/kg in larva and 10.91 mg/kg in pupa stage, respectively. This indicated that manganese is required for the health of Tasar silkworm in very less quantity. Thus, manganese concentration in the range of 50–100 ppm in the leaf of Tasar host plant is sufficient for the healthy development of the Tasar insect *A. mylitta* D.

The optimum range of per day consumption was calculated to be 0.007–0.035, 0.018–0.36, 0.045–0.090, 0.099–0.198, and 0.354–0.708 mg for 1st, 2nd, 3rd, 4th, and 5th instars, respectively, for the healthy development of Tasar silkworm larva of Daba bivoltine Eco Race. When larva consumes leaf of *Shorea robusta*, an excess quantity of manganese intake takes place to its body which results in a toxic effect. Further, it was found that manganese content in a tender leaf of natural *Shorea robusta* (Sal) tree is less than mature leaves of the same plant. Analytical data also reveal that tender leaves of coppice Sal plant contain a higher content of Mn compared to tender leaves of tree type Sal plant. Since manganese is mostly an immobile element in the plant, transportation of its ions to the tip of the twigs and ultimately to the tender leaves of the much taller tree takes place in fewer quantity. Varied range of manganese content in a leaf of Shorea *robusta* (Sal) exists due to the same fact. Since rearing performance of Tasar silkworm of Daba bivoltine Eco Race is not good on Sal trees; it is inferred that higher content of manganese must be interfering with the digestion and could not be assimilated by the Tasar silkworm properly leading to an imbalance in the physiological activities causing heavy mortality of the larvae. Further, rearing on leaves of coppice Sal tree for young age larva is also not advisable because of higher content of manganese exists in tender leaves of coppice plant. This is further evident from Tables 2 and 3 that rearing was highly affected when reared on Sal plants whereas it was a success crop

Table 2: Instar-wise consumption of leaf of *T. tomentosa* and requirement of Manganese by Tasar silkworm larva, *Antheraea mylitta* D. for Daba bivoltine Eco Race

Stage of larva	Percentage out of total consumption of leaf during larval period	Consumption of leaf per larva (in g)	Larval period (in days)	Per day consumption of fresh leaf/larva (in g)	Dry weight of per day consumption of leaf/larva (in g)	Consumption of content of manganese per day through leaf (in mg) when leaf contains 50 ppm Mn.	Consumption of content of manganese per day through leaf (in mg) when leaf contains 100 ppm Mn.
1st instar	0.30	0.898	4	0.225	0.07	0.0035	0.007
2nd instar	1.60	4.792	4	1.198	0.36	0.018	0.036
3rd instar	6.00	17.972	6	2.995	0.90	0.045	0.09
4th instar	13.20	39.537	6	6.589	1.98	0.099	0.198
5th instar	78.90	236.325	10	23.632	7.09	0.354	0.708
Total/ Average	100.00	299.524	30	9.984	2.99	0.149	0.298

A. mylitta: Antheraea mylitta, T. tomentosa: Terminalia tomentosa

Table 3: Rearing performance of Tasar silkworm on different host plants

Number of DFLs utilized	Hatching %	Fec.	Number of larvae brushed	Food plants used	Leaf consumption/ larva (in gram)*	Manganese content consumed by larva per day (in mg)	Cocoon yield/dfl
8	72	200	1152	*T. tomentosa*	299.58	0.16-0.31	53
8	83	200	1328	*T. arjuna*	299.58	0.12-0.31	67
8	72	200	1152	*S. robusta*	299.58	1.05-7.61	02

*As per[7,13] reported that Sal is not preferred by Daba Eco Race. *T. arjuna: Terminalia arjuna, Shorea robusta: S. robusta, T. tomentosa: Terminalia tomentosa*

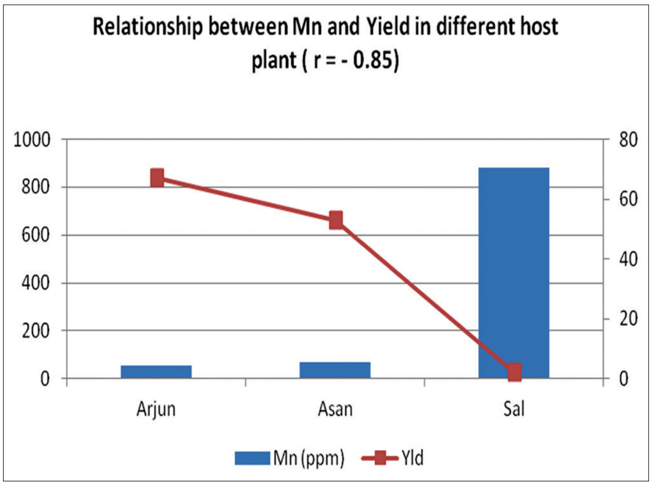

Figure 4: Relationship of Mn content in leaf with cocoon yield

when reared on *T. tomentosa* and *T. arjuna*. The relationship between manganese content in leaf of different primary Tasar host plants and yield of cocoons [Figure 4] validated the findings as these are negatively correlated (r = −0.85).

CONCLUSION

Higher intake of manganese by the larva of Tasar silkworm, *Antheraea mylitta* D occurs when feeds on leaf of Sal plant whereas it is within optimum range when feeds on leaf of *T. tomentosa* and *T. arjuna* plants. Manganese content more than 500 ppm in leaf of Tasar host plant is toxic to Tasar silkworm of Daba bivoltine Eco Race. There is zero percentage survival of Tasar silkworm larva when per day consumption of manganese exceeds 1 mg.

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