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CONSUMPTION OF TRANSITIONAL METAL CONTENTS BY TASAR SILKWORM, *ANTHERAEA MYLITTA* D FOR ITS HEALTHY DEVELOPMENT

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ABSTRACT

The important bio-metals involved in metalloproteins and metalloenzymes appear mainly from the first transition series. Manganese (Mn), Iron (Fe) Copper (Cu) and Zinc (Zn) are essential transition metals which come under this series and enters to tasar silkworm body for its survival from leaves of its host plants to which it consumes. Metal contents of these four essential trace elements were estimated for soil, leaf and pupa with Atomic Absorption Spectrometer. 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 whereas the contents of these elements 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. On the basis of instar-wise consumption of host plant leaves during larval stage of tasar silkworm *Antheraea mylitta* D, daily intake of these metal contents were estimated for healthy tasar silkworms of Daba bi-voltine(DBV) eco-race. Contents of these metal elements in pupa stage of tasar silkworm of different eco-races viz. Daba, Sukinda, Modal, Raily, Bogai and Laria were also determined through chemical analysis and found the average with standard deviation as 8.37±3.41 ppm of Mn, 130.18±77.05 ppm of Fe, 20.23±9.29 ppm of Cu and 280.81±52.28 ppm of Zn. It was ascertained that iron and zinc required in larger quantities than compared to manganese and copper for physiological activity of the tasar silkworm. Chemical analysis of pupa of eco-races revealed 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*. In the study it was inferred that zinc is required in larger quantity than other essential trace element as micronutrient for healthy development of tasar silkworm and silk production.

Keywords : *Antheraea mylitta* D, Eco-race, *T. tomentosa,* Transition metals and Tasar.

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. *Teminalia 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;

Agrawal *et al*., 1980; Sinha *et al.,* 1989; Sinha *et al.,* 1992 & Sinha *et al.,* 2005). In mulberry; mineral nutrition deficiency and requirement were studied by several workers (Krishnaswami *et al*., 1971; Singhal *et al*., 1999; Singhvi *et al*., 2002; Singhal *et al*., 2004). Nutrient contents in leaf and efficacy of tasar silkworm rearing on distinctly different host plant was studied by Giri *et al*, 2015. A wide variety of metal-dependent enzymes are found in nature which acts in fundamental biological processes, including photosynthesis, respiration and nitrogen fixation. These metal atoms also play vital role in governing the different physiological phenomenon in insect body of tasar silkworm *Antheraea mylitta* D. 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 been reported (Malik *et al*., 2009) that the variation in metal ion concentrations in the hymolymph of silkworm, *Bobmyx mori* occurs during developmental stages. It has also been reported that the voltinism (uni /bi /tri) in *A. mylitta* is regulated by environmental factors like temperature, relative humidity, day length and rainfall. Some have reported that voltinism pattern is found to be stable for a particular zone can change in different environmental conditions (Kar *et al*., 2000). However, no study has so far been carried out to know the synergistic effect of metal contents of Cu, Fe, Mn and Zn in pupa stage of different eco-races of tasar silkworm on their voltinism. Hence, a study was undertaken to know the consumption of transition metal contents by tasar silkworm, *Antheraea mylitta* D for its healthy development along with its influence in racial character.

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.

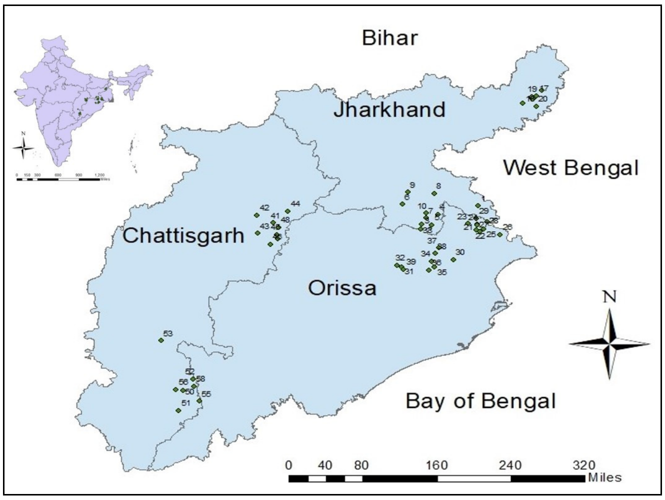

Fig. 1 : Map of Study area

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.

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 70ºĊ. 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 HNO_3: $HClO_4$ at 200º 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 70ºĊ. 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 HNO_3: $HClO_4$ at 200º 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.

Instars wise per day intake of nutrient content of Mn, Fe, Cu and Zn were calculated on the basis of per day leaf consumption by single larva and assuming 70% leaf moisture during larval feeding. Jolly *et al.* (1974) and Ojha *et al.* (1998) reported the quantum of leaf consumption of tasar silkworm in outdoor and indoor condition respectively.

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 Follet 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 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.

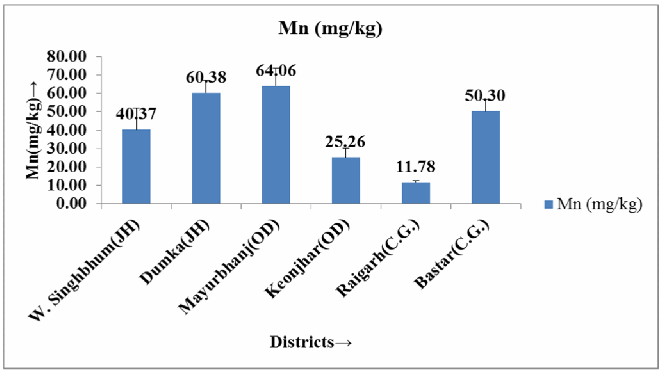

Fig. 2 : Availability of Mn in the soil of tasar growing areas

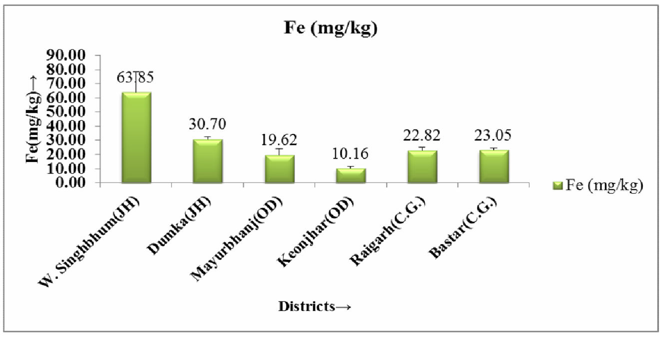

Fig. 3 : Availability of Fe in the soil of tasar growing areas

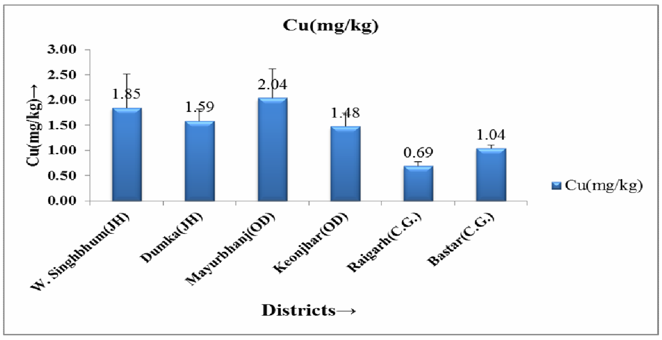

Fig. 4 : Availability of Cu in the soil of tasar growing areas

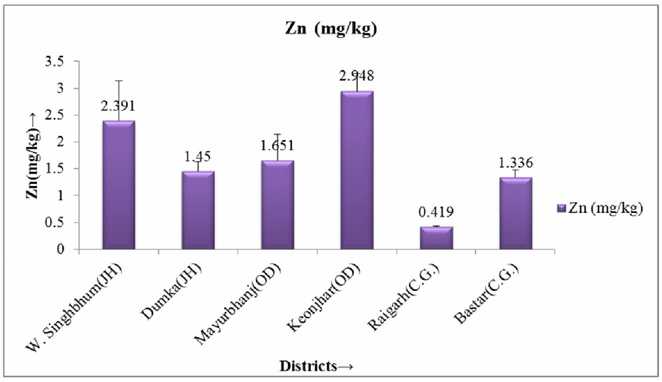

Fig. 5 : Availability of Zn in the soil of tasar growing areas

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.

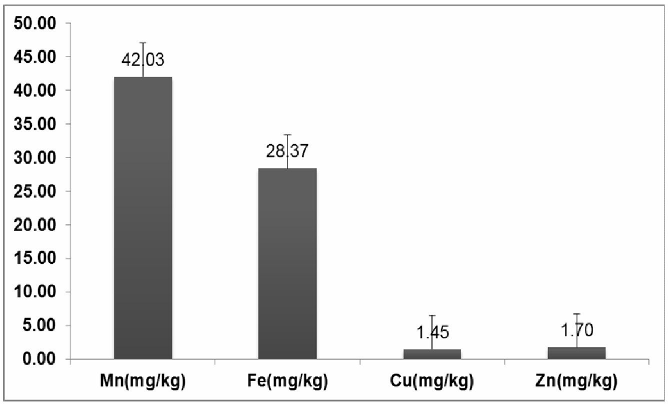

Fig. 6: Status of available micronutrients of transition metals in the soil of tasar producing area

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.

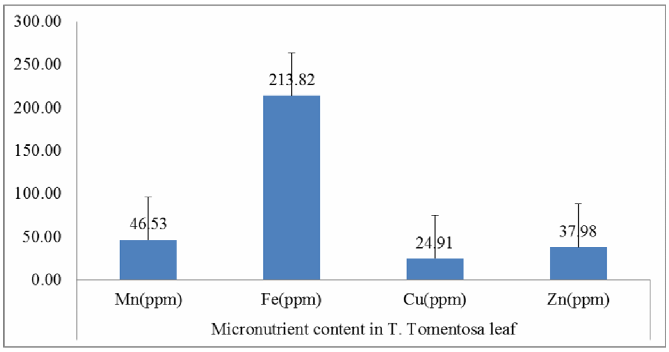

Fig. 7 : Micronutrient content of trasition metals in *T. tomentosa* leaf

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

Intake quantity of transition metal contents by the tasar silkworm larva of Daba bi-voltine 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.

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

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

Nutrition 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 important role in the life process which come under essential metals. 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 hymolymph of silkworm, *Bobmyx 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 2 : Content of transition metals in pupa and cocoon character of some important eco-races of tasar

Eco-Race	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	C.W. (g)	S.W. (g)	S.R.%	F. L. (metre)	Voltinism
Daba BV	10.91	178.71	16.10	294.58	13.18	1.87	14.19	1040	2
Modal	3.71	163.40	14.15	346.51	18.48	3.84	20.78	1625	1
Raily	10.94	149.33	34.07	257.47	17.24	3.14	18.21	1410	1
Bogai	10.25	461.81	14.09	175.47	9.26	1.33	14.23	715	2
Laria	12.72	186.42	133.43	216.57	8.92	1.21	13.62	690	2
STV	7.91	176.32	16.58	224.69	12.15	1.48	12.17	816	3

Abbreviations: C.W.: Cocoon Weight; S.W.: Shell Weight; S.R.: Silk Ratio; F.L.: Filament Length BV: Bi-voltine; STV: Sukinda Tri-voltine.

Micronutrient contents of transition metals in pupa of different eco-races of tasar silkworm

Content of Mn, Fe, Cu and Zinc were estimated in the pupa of six different eco-races i.e. Daba, Sukinda, Modal, Raily, Bogai and Laria. It was found that content of zinc is more in pupa of all the eco-races than compared to other micro nutrients except Bogai. It was also observed that higher content of zinc retained by the pupa of the eco-races having high silk ratio percentage than compared to pupa having low silk ratio percentage (Table-2). This result indicated that higher content of zinc in pupa supported the tasar insect for prolonged life period and less voltinism. Racial and economic character of tasar cocoons greatly influenced by the content of transition metals which is evident from the data of Table -2. Malik *et al.* (2009) reported that haemolymph zinc in *Bombyx mori* were relatively higher in pupae than larva. Orr *et al.* (1994) reported that over expression of Superoxide dismutase (SOD), zinc containing metallo enzyme extended the life span of *Drosophila melanogaster.* SOD has been considered to be involved in life span extension (Parkes *et al*., 1999). Analytical results of metal contents of transition elements in tasar silkworm pupa support that zinc has greater role in diapausing the pupa for longer time and also for longer life span of the insect being uni-voltine or bivoltine.

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

Parameter	Cu(ppm)	Fe(ppm)	Mn(ppm)	Zn(ppm)	C.W.(g)	SW(g)	SR%	F.L.(metre)
Cu(ppm)	1							
Fe(ppm)	-0.017	1						
Mn(ppm)	0.387	0.474	1					
Zn(ppm)	-0.122	-0.387	-0.222	1				
C.W.(g)	-0.391	-0.651	-0.480	0.476	1			
SW(g)	-0.297	-0.607	-0.464	0.430	0.943	1		
SR%	-0.166	-0.462	-0.352	0.300	0.762	0.927	1	
F.L.(metre)	-0.319	-0.636	-0.449	0.432	0.966	0.981	0.872	1

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.

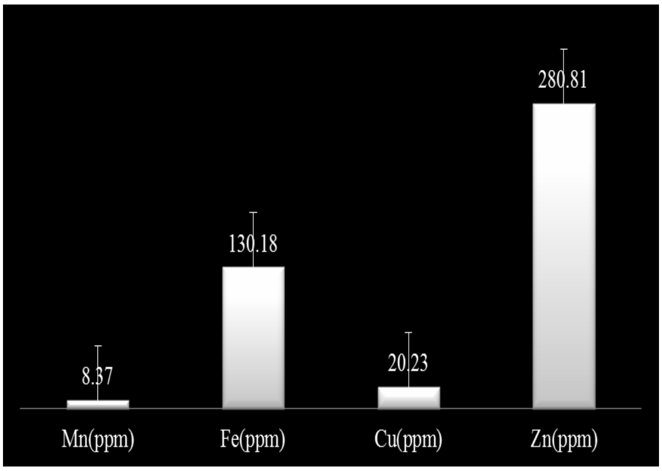

Fig. 8 : Content of transition metals in pupa of Tasar silkworm

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

Agarwal, S.C.; Jolly, M.S. and Sinha, A.K. (1980). Foliar constituents of secondary food plants of tasar silkworm *Antheraea mylitta D. Indian Forester.* 106 (12): 847-851.

Das, A.K. (2007). Bioinorganic Chemistry, Books & Allied (P) Ltd., 1-400.

Giri, S.; Dubey, O.P.; Das, S.; Sharma, S.P. and Sahay A.(2015) Efficacy of Tasar silkworm rearing on distinctly different soil and leaf nutrient status of its host plants: a case study. *International Journal of Analytical and Bioanalytical Chemistry*. ISSN 2231-5012:5(3): 65-70.

Jolly, M.S.; Chaturvedi, S.N. and Prasad, S. (1968). A survey of Tasar crops in India, *Indian Journal of Sericulture,* 7(1): 56-67.

Jolly, M.S.; Sen S.K. and Ahsan M.M. (1974). Ethology of Tasar Silkworm Chapter-8, Tasar Culture, 1st Edition: 125-133.

Kar, P.K.; Srivastava, A.K. and Naqvi, A.H. (2000). Changes in voltinism in some population of *Antheraea mylitta*: Response to photoperiod. *Int. J. Wild Silkmoth and Silk,* (5): 176-178.

Krishnaswami, S.; Kumarraj, S.; Vijayaraghawan, K. and Kasiviswanathan, K. (1971). Silkworm feeding trials for evaluating the quality of mulberry leaves as influenced by variety, spacing and nitrogen fertilization. *Indian J. Seric*, 10(1): 79 -89.

Lindsay, W.L. and Norvell, W.A. (1978). Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal,* **42:** 421-428.

Mahadeva, A. and Nagaveni, V. (2012). Evaluation of nutritional quality in spiraling whitefly (*Aleurodicus dispersus* Russell) infested mulberry (Morus sp.) foliage, *International Journal of Environmental Science,* 3(3): 1065-1071.

Malik, F.A. and Malik, M.A. (2009). Variation in metal ion concentrations in the hymolymph of silkworm, *Bobmyx mori* during development. *Academic Journal of Entomology,* 2(1):10-15.

Ojha, N.G.; Rai, S. and Pandey, P.N. (1998). Studies on the Consumption of Leaves of Different Food Plants By Different Ecoraces of Tropical Tasar Silkworm, *Antheraea mylitta* D.: 10th All India Congress of Zoology (14th- 18th October) Edited by B.N. Pandey and B.K. Singh Daya Publishing House, Delhi: 95-104.

Orr, W.C. and Sohal, R.S. (1994). Extension of life span by over expression of superoxide dismutase and catalase in *Drosophila melanogaster*, *Science* (263): 1128-1130.

Parkes, T.L.; Hilliker, A.J. and Phillips, J.P. (1999). Motor neurons reactive oxygen and life span in *Drosophila.* Neurobiol. Aging., (20): 531-535.

Prasad, A.S. (2008). Zinc in human health: effect of zinc on immune cells. *Mol Med*.: 14(5-6): 353-357.

Singhal, B.K.; Chakraborti, S.; Rajan, M.V. and Thippeswamy, T. (2004). Nutritional disorders, analytical diagnosis and nutrient guide for mulberry, *Morus indica* L.*;* a review, *Int. J.Indust. Entomol,* 8(1): 1-15.

Singhal, B.K.; Mala, V.R.; Sarkar, A. and Datta, R.K. (1999 a). Nutritional disorders of mulberry (*Morus* spp.) III. Leaf nutrient guide for secondary nutrients. *Sericologia*, 39: 599-609.

Singhvi, N.R.; Kodandramaiah, J.; Reddy, Munirathanam, M.; Katiyar, R.S. and Dutta, R.K. (2002). Study on nutrient deficiency in mulberry variety S36 under field conditions. *Sericologia*, 42(1): 53-58.

Sinha, A.K. and Jolly, M.S. (1971). Foliar constituents of the tasar food plants of tasar silkworm A. *mylitta. Indian Forester*, 97(5): 261-263.

Sinha, A.K.; Sinha, U.S.P.; Brahmachari, B.N. and Sengupta, K. (1989). Studies on the constituents of hairy and non-hairy leaves of *Terminalia tomentosa* W. & A. *Indian J. Seric*., 18(1): 115-117.

Sinha, U.S.P.; Sinha, A.K.; Chaudhary, S.K.; Prasad, J. and Suryanarayana, N. (2005). Comparative study on the nutritive value of the food plants of the tasar silk worm

Antheraea mylitta Drury Proceedings of the 20th Congress of the International Sericultural Commission, 15th-18th Dec. at Bangalore, 2, 281-284.

Sinha, U.S.P.; Sinha, A.K.; Srivastava P.P. and Brahmachari B.N. (1992). Studies on the variation of chemical constituents in relation to maturity of leaves in three primary food plants of tropical tasar silkworm *Antheraea mylitta* D. *Indian J. Seric*., 31(1): 83 - 86.

Tandon, H.L.S.; Ed (2001). Methods of Analysis of Soil, Plants, Waters and Fertilizers: 1-143

Yamamoto, K.; Zhang P.; He, N.; Wang, Y.; Aso, Y.; Banno, Y. and Fujii, H. (2005). Molecular and biochemical characterization of manganese containing superoxide dismutase from the silkworm, *Bombyx mori*. Science Direct, Comparative Biochemistry and Physiology, Part B 142; 403-409.