The Bioscan 6(4) : 627-629, 2011
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

ANTIOXIDANT STATUS OF PRIMARY HOST PLANTS OF *ANTHERAEA* SP.

G. C. PATRA*, N. MOHANTY AND D. G. DEY[1]
North Orissa University, Takatpur, Baripada, Mayurbhanj - 757 003, Orissa
[1]Udala College, Udala, Mayurbhanj - 757 041, Orissa
E-mail: gcpatranou@gmail.com

KEY WORDS
Antheraea
Host plant
Protein
Ascorbic acid
Glutathione
Total phenolics

Received on :
31.08.2011

Accepted on :
10.11.2011

***Corresponding author**

ABSTRACT
The tropical tasar silkworm, *Antheraea* sp. feeds primarily on *Shorea robusta* (Sal), *Terminalia tomentosa* (Asan) and *Terminalia arjuna* (Arjun). As it has to counter the extremities of ecoclimatic conditions during its life cycle, to know the antioxidant source from outside, the antioxidant contents, like ascorbic acid (Vitamin C), glutathione and total phenolics including protein content of leaves of these host plants were estimated. Analyses showed that, among these three plants, *Shorea* is the best one containing highest antioxidant contents compared to the other two. So, *Shorea* is the highly recommendable feed for silkworm (*Antheraea* sp.) to increase the antioxidant status of the silkworm and thereby its silk productivity.

INTRODUCTION

Silk is one of the nature's gifts to mankind produced by silkworm. Among silkworms the most commercially exploited one of non-mulberry silkworm belongs to *Antheraea* sp. Silk is a natural fibre secreted by the larvae for the protection of pupae in the process of completing their life cycle. The larva of *Antheraea* sp. is an elongated caterpillar commonly called as silkworm. These are polyphagous and feed primarily on the leaves of *Shorea robusta* (Sal), *Terminalia arjuna* (Arjun) and *Terminalia tomentosa* (Asan) plants (Mahapatra, 2009). These are the sole food plant for the silkworm, *Antheraea* sp. The fresh and nutritive quality of leaf plays an important role on the development of silkworm stabilizing the cocoon production and silk productivity. There is a relation between nutrition and voltinism. Low food value of host plants synchronizes the life cycle of wild tasar silkmoth (Dey *et al*., 2010a, b). Production of tasar cocoons mainly depends upon the eco-climatic conditions, food plants and altitude of rearing (Nayak, 1994).

The food plant population of this species is reducing year after year with depletion of forest coverage. So, to meet the demand of food plants additional creation of new plantation for food plants may fulfil the gap of reduced plant population. Further, evaluation of nutritional quality of host plant leaves and their antioxidant status will help to choose the suitable food plants for better production of silk.

Nutrition is a factor of paramount importance that regulates growth, development and reproduction of animals. Intake and growth targets are important to reach the functional optima in an insect (Raubenheimer and Simpson, 1999). The various chemical constituents of host plant leaves are responsible for successful cocoon harvest and silk productivity, thus the leaf quality of food plants plays a predominant role in healthy growth of silkworm. Hence, nutrition of silkworm, *Antheraea* sp. is of primary importance as the cocoon production is directly influenced by chemical substances especially the antioxidant status of the host plant leaves. Ascorbic acid (ASA) is a redox catalyst which can reduce, and thereby neutralize, reactive oxygen species (ROS) such as hydrogen peroxide (Padayatty *et al*., 2003). In addition to its direct antioxidant effects, ascorbic acid (Vitamin C) is also a substrate for the redox enzyme ascorbate peroxidase, a function that is particularly important for stress resistance in plants (Shigeoka *et al*., 2002). Glutathione (GSH) is considered as one of the most important cellular antioxidants (Meister and Anderson, 1983). There are numerous reports on phenolic compounds having prooxidant effects (Hodnick *et al*., 1989; Pardini, 1995) and/or antioxidant effects (Hagerman *et al*., 1998; Aherne and O'Brien, 2000).

The larvae of the silkworm feed on the leaves of their host plants. Naturally the antioxidants present in leaves will supplement to the antioxidant status of the larvae to fight adverse situations. Therefore, the objective of this study is to screen antioxidant contents of leaves of three host plants of *Antheraea* sp. (viz. *Shorea robusta*, *Terminalia tomentosa* and *Terminalia arjuna*) to find out the best one.

MATERIALS AND METHODS

Sample collection: The freshly green leaves of three primary host plants (*Shorea robusta*, *Terminalia tomentosa* and *Terminalia arjuna*) of tasar silkworm, *Antheraea* sp. were collected from the local sericulture rearing field of the State Government, Baripada, Orissa during 2010-11. Samples were placed in polyethylene bags and transported under refrigerated conditions to the laboratory. Samples collected were washed under running tap water to remove the adhering dirt and then stored under -20ºC until analyzed. Analysis was completed within 24 h of sample collection.

Tissue preparation: Each leaf sample was homogenized in 25 mL ice-cold extraction buffer (0.1M Tris-HCl, pH 8.0, 1mM EDTA, 5 mM $Na_2S_2O_3$ and 20 mM â-mercaptoethanol). Soluble protein concentration was determined in the supernatant after centrifuging the homogenate at 10, 000 x g for 10 minutes at 4ºC. The ascorbic acid, GSH and total phenolics contents were determined in the supernatant.

Biochemical estimation: The amount of protein was determined by the method of Lowry *et al*. (1951) with bovine serum albumin as standard. Ascorbic acid content was measured according to the method of Jagota and Dani (1982). The GSH content in the tissue samples was determined according to the method of Ellman (1959). Total phenolics content was measured according to the method of Slinkard and Singleton (1977). The concentration was expressed per gram tissue wet weight.

Statistics: To know the difference between means of three samples, one-way analysis of variance (ANOVA) was employed (Chainy *et al*., 2008).

RESULTS AND DISCUSSION

It was observed that the concentration of the protein, ascorbic acid, glutathione and total phenolics of different tissues were not similar.

The concentration of protein was more in Asan leaf than those of the Sal and Arjun. The latter two host plants showed almost equal concentration of protein in their leaves (Table 1). The ascorbic acid, glutathione and total phenolics contents were more in Sal than that of the other two (Table 1). The leaves of Arjun showed less glutathione than the Asan (Table 1). The total phenolics content was least in Asan (Table 1). As mentioned earlier, the contents of ascorbic acid (ASA), glutathione (GSH) and total phenolics were found to be the highest in Sal as compared to Asan and Arjun (Table 1). So, it may be concluded that the leaves of Sal plant have the highest antioxidant quality. The ascorbic acid content of Asan was slightly more than the Arjun whereas Arjun showed less glutathione and more total phenolics contents than that of the Asan (Table 1). One-way analysis of variance (ANOVA) of

Table 1: Concentrations of antioxidants in the leaf tissue of three host plants (Sal, Asan and Arjun). Data are Mean ± SD (n = 8 each)

Host Plant	Concentration Protein (mg/g)	ASA (mg/g)	GSH (*i* mol /g)	Total phenolics (mg/g)
Sal	172.94 ± 24.63	2.19 ± 0.44	1.88 ± 0.51	9.29 ± 0.73
Asan	204.88 ± 23.91	1.50 ± 0.33	0.78 ± 0.11	1.64 ± 0.56
Arjun	159.74 ± 19.00	1.36 ± 0.26	0.44 ± 0.11	2.76 ± 0.36

Table 2: Summary of computations for one-way analysis of variance (f-test) of data of protein concentration (mg/g) in the leaf tissue of three host plants (Sal, Asan and Arjun)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Between groups	3-1 = 2	BSS = 8618.857	BMS = 4309.428	F_c = BMS/WMS = 0.124
Within groups (or Error)	23-2 = 21	WSS = 762006.51	WMS = 34636.66	
Total	23	TSS = 770625.367		$F_{0.05}$ = 3.47

Table 3: Summary of computations for one-way analysis of variance (f-test) of data of ascorbic acid (ASA) concentration (mg/g) in the leaf tissue of three host plants (Sal, Asan and Arjun)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Between groups	3-1 = 2	BSS = 3.2	BMS = 1.6	F_c = BMS/WMS = 13.19
Within groups (or Error)	23-2 = 21	WSS = 2.67	WMS = 0.1213	
Total	23	TSS = 5.87		$F_{0.01}$ = 5.78

Table 4: Summary of computations for one-way analysis of variance (f-test) of data of GSH concentration (i mol/g) in the leaf tissue of three host plants (Sal, Asan and Arjun)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Between groups	3-1 = 2	BSS = 9.27	BMS = 4.64	F_c = BMS/WMS = 50.43
Within groups (or Error)	23-2 = 21	WSS = 2.03	WMS = 0.092	
Total	23	TSS = 11.3		$F_{0.01}$ = 5.78

Table 5: Summary of computations for one-way analysis of variance (f-test) of data of total phenolics concentration (mg/g) in the leaf tissue of three host plants (Sal, Asan and Arjun)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Between groups	3-1 = 2	BSS = 228.15	BMS = 114.07	F_c = BMS/WMS = 48.13
Within groups (or Error)	23-2 = 21	WSS = 52.08	WMS = 2.37	
Total	23	TSS = 280.23		$F_{0.01}$ = 5.78

protein was not significantly different (Table 2), whereas ascorbic acid (ASA), glutathione (GSH) and total phenolics of leaf tissue among Sal, Asan and Arjun were significantly different ($p < 0.01$) (Tables 3, 4 and 5).

As discussed earlier (Meister and Anderson, 1983, Hodnick *et al*., 1989, Pardini, 1995, Hagerman *et al*., 1998, Aherne and O'Brien, 2000; Shigeoka *et al*., 2002; Padayatty *et al*., 2003), antioxidants help in fighting ROS, H_2O_2, stress resistance etc. Therefore, besides own antioxidant status of the larva itself, the antioxidants present in its food (leaves of host plants) will be helpful in fighting extremities of climatological factors like temperature, relative humidity etc. during different instars and pupation. So, a better harvest is expected.

Finally, basing on the antioxidant contents of the leaves, it is concluded that Sal is the best host plant due to highest antioxidant contents among the primary host plants of tasar silkworm, *Antheraea* sp. followed by Asan and Arjun.

REFERENCES

Aherne, S. A. and O'Brien, N. M. 2000. Mechanism of protection by the flavonoid, quercetin and leutin, against Tert-butylhydroperoxide and menadione-induced DNA single strand breaks in Caco-2 cells. *Free Radic. Biol. Med*. **29:** 507-514.

Chainy, G. B. N., Mishra, R. and Mohanty, P. K. 2008. Basic Biostatistics, Kalyani Publishers, New Delhi.

Dey, D. G., Mohanty, N., Guru, B. C. and Nayak, B. K. 2010a. Tasar Silkmoth of Similipal. Indian Academy of Sericulture, Bhubaneswar, Orissa, India.

Dey, D. G., Nayak, B. K., Mohanty, N. and Guru, B. C. 2010b. Reproductive characteristics of modal ecorace of wild tasar silkmoth, *Antheraea paphia* Linn. in different altitudes of Similipal Biosphere Reserve, Orissa, India. *The Bioscan.* **5(1):** 41-45.

Ellman, G. L. 1959. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **82:** 70-77.

Hagerman, A. E., Riedl, K. M., Jones, G. A., Sovik, K. N., Ritchard, N. T., Hartzfeld, P. W. and Riechel, T. L. 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J. Agric. Food Chem*. **46:** 1887 -1892.

Hodnick, W. F., Kalyanaraman, B., Pritsos, C. A. and Pardini, R. S. 1989. The production of hydroxyl and semiquinone radicals during the autoxidation of redox active flavonoids. In: *Oxygen Radicals in Biology and Medicine*, Simic, M. G., Taylor, K. A., Ward, J. F. and von Sonntag, C. (Eds.): Plenum press, New York. pp. 149-152.

Jagota, S. K. and Dani, H. M. 1982. A new colorimetric technique for the estimation of vitamin-C using Folin phenol reagent. *Clin. Biochem*. **127:** 178-182.

Lowry, O. H., Resebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. **19:** 265-275.

Mahapatra, H. C. 2009. Tropical tasar biodiversity and forestry. In : *Proceedings of National Workshop on Seri-Biodiversity Conservation*. March 7-8, CSGRC, Central Silk Board, Hosur, India. pp. 163-167.

Meister, A. and Anderson, M. 1983. Glutathione. *Annu. Rev. Biochem.* **52:** 711-760.

Nayak, B. K. 1994. The wild tasar (*Antheraea paphia* Linn.) of Similipal hill forest of Orissa, India, Int. Conference on wild silk moths. Hotaka, Japan. pp. 18-22.

Padayatty, S., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J., Chen, S., Corpe, C., Dutta, A., Dutta, S. and Levine, M. 2003. Vitamin C as an antioxidant: Evaluation of its role in disease prevention. *J. Am. Coll. Nutr*. **22(1):** 18-35.

Pardini, R. S. 1995. Toxicity of oxygen from naturally occurring redox-active prooxidants. *Arch. Insect Biochem. Physiol*. **29:** 101-118.

Raubenheimer, D. and Simpson, S. J. 1999. Integrating nutrition: A geometrical approach. *Entomologia Experimentalis et Applicata*. **91:** 67-82.

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa , Y., Takeda ,T., Yabuta, Y. and Yoshimura, K. 2002. Regulation and function of ascorbate peroxidase isoenzymes. *J. Exp. Bot.* **53(372):** 1305-1319.

Slinkard, K. and Singleton, V. L. 1977. Total phenolics analysis: Automation and comparison with manual method. *Am. J. Eno. Vitriculture.* **28:** 49-55