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Studies on the filament of tasar silkworm, *Antheraea mylitta* D (Andhra local ecorace)

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***Antheraea mylitta* ecorace exclusively found in the northern part of Andhra Pradesh, South India was taken for Scanning Electron Microscope studies of its filament with reference to total indoor and outdoor rearings. Compared to the outdoor-reared cocoons, loose filament structure and wide gaps between filaments were found in indoor cocoons. A comparison was also drawn on the effect of various levels of gamma-irradiation on the silk filament. A dose-dependent decrement in the diameter of the filament was observed.**

Keywords: *Antheraea mylitta*, gamma irradiation, indoor rearing, silk fibre diameter, tasar silk filament.

THE major tropical and commercial tasar varieties of India mainly include Daba tv, Raily, Sukinda, Bagai, Sarihan, Bhandara, etc. Andhra local ecorace is also a tropical tasar variety available only in Andhra Pradesh. It is well known for its superior commercial qualities like hard and compact cocoons, high reelability (69%), high shell ratio (16.85) and low denier (7%)[1,2]. However, it shows heavy mortality of larvae due to predators, parasites[3] and climatic hazards[4].

Outdoor rearing of wild silkworm predisposes larvae to the vagaries of climatic conditions. It makes them more vulnerable not only to pests and diseases, but also to the impacts of temperature, photoperiodism, humidity, etc. In order to overcome these hurdles, several attempts were made to conduct indoor rearing. An artificial diet has been developed for the tropical tasar silkworm containing *Asan* leaf powder, the principal food plant and some success has been achieved[5]. Standardization of chawki-rearing to prevent the early stage larval loss has resulted in 20% increase in the effective rate of rearing[2,6].

In the present investigation, an attempt has been made at Kakatiya University, Warangal at total indoor rearing of the ecorace. Rearing of silkworm was undertaken from brushing stage to cocoon under controlled conditions for three crops. Simultaneously, outdoor rearing was also carried out in the field of *Terminalia arjuna* plantation. The structure of the filament was compared in both outdoor and indoor cocoons.

There is a dearth of appropriate technologies in the post-cocoon sector which has certain drawbacks like lack of uniformity in cocoon structure and silk deposition due to its hardness and this accounts to 50% loss in spinning. The technique of ionizing radiation has been employed to study eggs, larvae and pupae of silkworms to various doses of X-, gamma and UV rays for genetic mutations. Earlier, a study was carried out on the impact of insecticides following irradiation[7]. The effect of UV and gamma irradiation on heartbeat of *Bombyx mori* was investigated[8]. Since the environment has a direct influence on the cocoon surface, sun rays, which are known to possess ultraviolet, X-, gamma rays, etc. at different levels, in the present studies, cocoons are studied by exposing them to gamma rays and observing their effect on the filament.

While studying the cocoon characters, silk fibre diameter was also measured for the outdoor, indoor and irradiated cocoons using a microhardness tester. Earlier, silk thread size measurements were seen using CCD linear line sensor[9].

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Immediately after brushing, young worms were released on the branches of host plants which were cut and placed in conical flasks containing water. A hardbound sheet was placed above the neck of the flask exposed. A paraffin sheet was covered on the hardbound sheet to collect the faecal pellets and to maintain cleanliness and healthy atmosphere in the rearing set-up. Proper ventilation, temperature and humidity were ensured in the rearing room. Tender and juicy leaves were provided to the young larvae and mature leaves to the adult worms. Brushing was done according to improved technique of using only one-day hatching to maintain uniformity and avoid overcrowding. During moulting, care was taken not to disturb the arrangement. When the worms were out of moult, the branches were replaced (Figure 1).

In order to irradiate the cocoons, Gamma Irradiation Chamber 900 model of the Bhabha Atomic Research Centre, Mumbai was used. Three cocoons (which were selected from field/outdoor rearing) at a time were selected and kept in the sample chamber and irradiated at three dosage levels – 500 RADS (40 s), 750 RADS (60 s) and 1000 RADS (80 s).

Scanning Electron Microscope (SEM) model S-250 MK-III of Cambridge was used to study filament structure on the cocoon surface. For specimen preparation, drying and metal coating of the samples, which involved soaking 1 mm size cocoon shell pieces in acetone overnight, was followed by keeping them in an oven at 50°C for about 24 h for dehydration. After drying, the sample was coated with a high molecular weight conductive material like gold. The metal coating was done in a sputter coater which gave uniform and continuous coating on the surface. It was observed in SEM at various degrees of magnification.

In the present investigation, the diameter of silk fibre is measured using Microhardness Tester HMV-2000 Shimadzu (Figure 2). This is generally used to study the microstructures of various materials, plated layers, fine ceramics, etc. The silk fibre is affixed on the mirror-like polished metallic specimen and placed under the objective lens. After setting up all the parameters, the silk thread was observed through the eye piece. To measure the diameter, the image through the eyepiece was adjusted between two parallel lines by observing through the eye piece and measurements were taken. As the tester is an automatic machine, the diameter is directly displayed on the screen in terms of microns.

The outdoor, indoor and irradiated cocoons were reeled to obtain the silk filament. Fragments of the filament were observed under SEM. Filaments of the outdoor cocoon shell showed cross-bindings and bifurcation of filaments forming an intricate network and Y-shaped structures were clearly formed. In the indoor cocoon shell, the filaments are loosely arranged and slightly visible with wider gap. The number of filaments in the outdoor cocoons is found to be more than that of indoor cocoons. The sericin content, which forms a cementing substance between the filaments, is found to be more in the indoor cocoons than the outdoor ones (Figures 3 and 4).

From the literature, it is seen that the shell of Andhra local ecorace is superior to Daba tv due to cross-binding

Figure 1. Spinning stage of indoor rearing *Antheraea mylitta*. D (Andhra local).

Figure 2. Microhardness Tester used for measuring silk fibre diameter (photo: REC Warangal).

of shell filaments forming a close network[2]. The hardness of Andhra local cocoon is attributed to calcium oxalate crystals present between the filaments and the fibrils[10].

The silk protein fibroin is fibrous in nature, forming the main silk filament content, while sericin is a sticky coating substance between the layers of fibroin. Thus, the quality of cocoons depends both on sericin and fibrin which are controlled by atmospheric conditions[11]. The presence of more cementing substance (sericin) and less filament (fibrin) in the indoor cocoons suggests the role of environmental factors on the synthesis of these two proteins by the silk gland.

Figure 3. Outdoor (192X) tasar cocoon shell showing cross-binding vacuoles and Y-shaped structures.

Figure 4. Indoor (192X) tasar cocoon shell showing loose filaments and vacuoles.

The sericin content as being the deciding factor in the quality of the cocoon and raw silk reeled was reported by Singhvi and Bose[11]. However, filament length and quality of the shell are based on the fibroin content[12]. This is corroborated by the finding of a reduction in the filament length of indoor cocoons as observed in the present investigation (Table 1).

Matsuzaki[13] and Garel and Mendel[14] have reported that tRNA population in the silk gland is correlated with the composition of amino acids in the silk proteins. It is interesting to note that some of the amino acids (glycine, alanine and serine) are abundantly found in fibroin, which indicates changes in the amino acid acceptor activity of tRNA in the posterior and middle silk glands at various stages of larval development in *B. mori*[15]. Such changes are also likely to take place in *A. mylitta*, which cause changes in the composition of fibroin and sericin.

In the untreated outdoor cocoon shell, there is evidence of cementing substance between the filaments and the filaments are continuous. In cocoon irradiated at 500 RADS, the cementing substance/sericin is not seen and the continuity is broken at some points (Figure 5).

Figure 6 shows filaments of irradiated cocoon of dosage 750 RADS, with more fragmentation of the filaments which has resulted due to irradiation. Figure 7 shows the

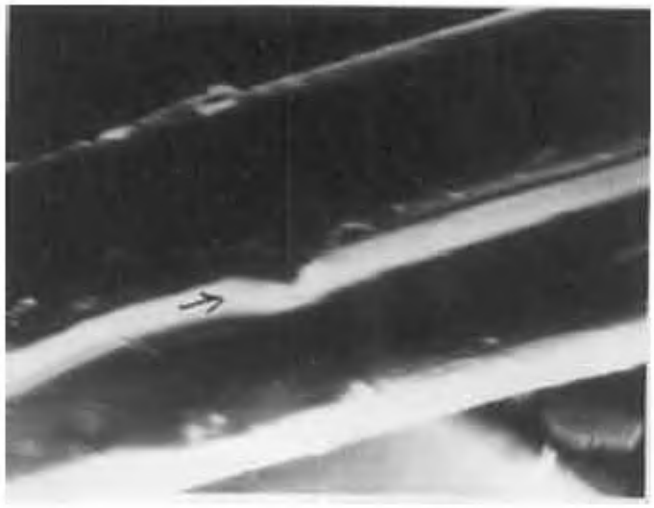

Figure 5. 500 RADS (3400X) filament of irradiated tasar cocoon. Dents shown at a higher magnification.

Table 1. Filament length (in m)

Rearing	Crop 1	Crop 2	Crop 3
Outdoor cocoon	250.25	385.92	460.16
Indoor cocoon	160.15	280.25	390.39
Dosage	500 RADS	750 RADS	1000 RADS
Irradiated cocoon	175	160	150

Table 2. Comparison of silk fibre diameter of outdoor and indoor-reared cocoons of tasar silkworm *Antheraea mylitta* D (Andhra local)

Cocoon category	Diameter of silk fibre
Outdoor	19 microns
Indoor	19 microns

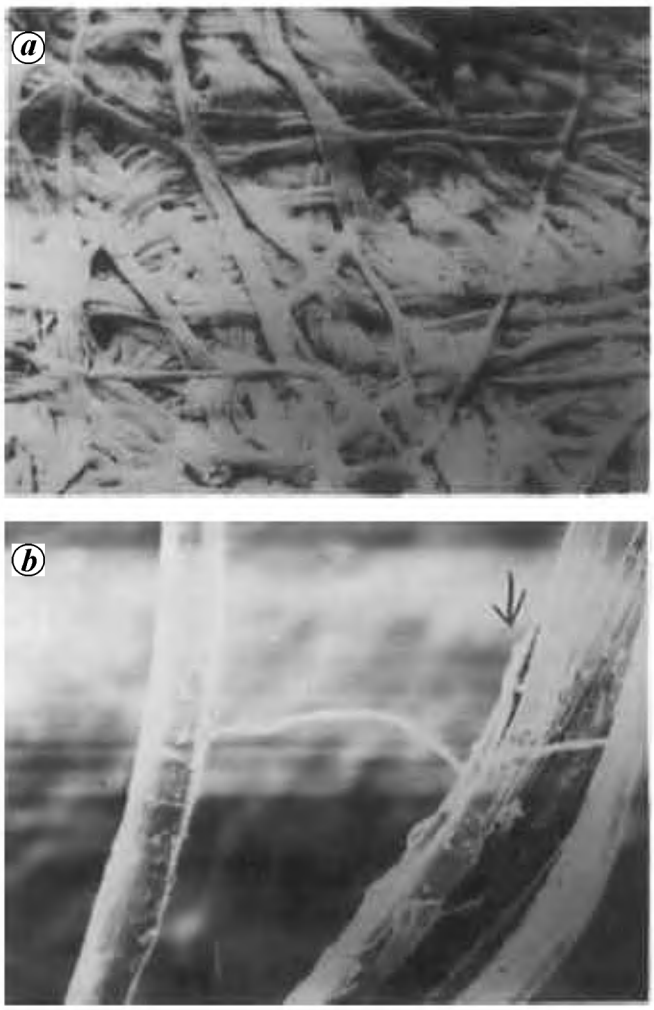

Figure 6. *a*, 750 RADS (100 X) irradiated tasar cocoon shell showing more fragmentation caused due to irradiation; *b*, 750 RADS (860 X) filament of irradiated tasar cocoon. Clear breakage of filament at peripheral region is caused at higher dose of irradiation.

Figure 7. 1000 RADS (360X) irradiated tasar cocoon showing coarse texture of the filament and damage of filament due to irradiation.

Table 3. Effect of irradiation on silk fibre of outdoor-reared cocoon of tasar silkworm *A. mylitta* D (Andhra local) as observed using a microhardness tester

Cocoon category	Diameter of silk fibre
Untreated	19 microns
500 RADS	14 microns
750 RADS	13 microns
1000 RADS	12 microns

cocoon shell given a dosage of 1000 RADS. It is seen that the texture of filament has become coarse due to radiation and has ultimately resulted in the damage of silk filaments. Moreover, the filament structures at 500 RADS have shown dents, while those at 750 RADS have shown more fragmentation and 1000 RADS have shown clear breakages.

The diameter of the silk filament in both outdoor and indoor-reared cocoons was found to be 19 μm. In spite of large differences in the cocoon characters between the two methods of rearing, the silk fibre shows uniformity. The diameter of irradiated cocoons in the three dose levels is 14, 13 and 12 μm respectively. Thus, there is a dose-dependent gradual decline compared to the normal fibre (Tables 2 and 3).

Kawahara *et al.*[16] have observed swelling behaviour of silk fibre due to X-ray scattering. Changes in the hue in raw silk fibre of *Antheraea yamamai* due to UV irradiation and heat treatment were reported[17]. A change in the fine structure of silk fibroin fibres due to gamma irradiation was reported by Tsukada *et al.*[18]. It is also observed that the effect of radiation is evaluated mainly by exposing the live material of silkworm during egg stage[19–22] and larval stage[23,24].

Change in colour and loss of lustre were the visible changes found following irradiation, where reeled filament turned dark in colour. Filaments have shown dose-dependent changes regarding level of dents and decrement in diameter. In the filament at 500 RADS, breakages are from the periphery to the lumen of the filament. At 750 RADS, fibrils of the filaments are found to be loose with breakages from the periphery and lastly at 1000 RADS, the fibrils are separated out with localized disintegration of fibroin, giving a granular appearance.

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Wolbachia endosymbiont in some insect pests of sericulture

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The polymerase chain reaction method was applied to screen some insect pests of sericulture for the presence of *Wolbachia*, a rickettsial alpha-proteo-bacterium. The results revealed that out of 16 insect species representing six major orders, viz. Lepidoptera, Diptera, Hemiptera, Coleoptera, Hymenoptera and Dictyoptera comprising fifteen families tested, four were positive for *Wolbachia* infection. Two among these are insect pests of *Morus* species, one of *Terminalia* species and another major dipteran endoparasite of tasar silkworm, *Antheraea mylitta* harbours *Wolbachia*. These results indicate that the occurrence of *Wolbachia* is widespread among insect pests of sericulture.

Keywords: Endosymbionts, insect pests, sericulture, *Wolbachia*.

CONVENTIONAL biocontrol involves the establishment of food chain against the target pests through introduction/release/inundation of parasites and parasitoids. Exploitation of microbial pathogenesis against insect pests includes specially designed control agents to suit the particular requirements of insect-to-insect and insect-to-host plant relationship[1]. Keeping this in view, the use of *Wolbachia* has been considered as a mechanism, with the necessity to screen all the insect pests of sericultural importance. Earlier, Puttaraju *et al.*[2–6] have screened different silkworm races and its pest uzifly, *Exorista sorbillans*. They found the presence of *Wolbachia* in *Exorista* species and its absence in silkworm *Bombyx mori*. The present communication further records the presence of *Wolbachia* in certain insect pests of silkworm, *B. mori* and its host plant *Morus* species; so also of tasar silkworm, *Antheraea mylitta* and its food plant *Terminalia* species.

Symbiotic association between microorganisms and higher eukaryotes is extremely common and ranges from mutualistic to commensal and parasitic[7]. Rickettsial member of the genus *Wolbachia* belongs to α-proteobacteria and has been identified as an intracellular, obligate endoparasite in several taxa of arthropods. *Wolbachia* is known to cause a number of reproductive abnormalities in its hosts, including cytoplasmic incompatibility[8–10], parthenogenesis[11] and feminisation[12,13]. It has a wide host range and multiple infection sites within the host and plays a role in the ageing of insects by degrading different tissues[14]. Its presence in the host is detected by PCR-based DNA diagnosis using a set of primers that specifically amplify

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