INDUSTRIAL UTILIZATION OF SILKWORM PUPAE – A REVIEW

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Abstract

The art of silk production is called sericulture that comprises cultivation of mulberry, silkworm rearing and post cocoon activities leading to production of silk yarn. Apart from silk, there are several other by-products from sericulture. Silkworm pupae is an immediate by-product of reeling industry, obtained after reeling. Also, with increasing cost of production in sericulture, there is a need to augment alternative income sources by adding value to by-products to keep the sericulture sector viable. Silkworm pupal wastes are used as organic manure and increases the growth of crop plants. Silkworm pupae contains well-balanced amount of nutrients *viz.,* protein and essential amino acids, fats included polyunsaturated fatty acid (PUFA), carbohydrate, vitamins and minerals so it can be used as a protein supplement for animals. The presence of high value amino acids in silkworm pupa is used in the field of pharmacy and pharmacology. Chitin and chitosan derived from silkworm pupae are used in various industrial applications including pharmaceuticals. Pupal oil extracted from silkworm pupae are used in manufacture of industrial products such as paints, varnishes, pharmaceuticals, soaps, candles, plastic and bio-fuels respectively.

Keywords: Silkworm Pupa, Organic Manure, Protein Supplement, Chitin, Chitosan, Pupal Oil, Pharmaceutical Uses.

Introduction

Silk is one of the costliest natural fibers as its production is labor intensive in both sericulture and post-cocoon processing stages. Hence, the subsidiary activities, which utilize the waste generated from the primary activity, have also gained prominence. Sericulture is a well-established rural agribusiness in India and produces about 40,000 MT per year of silkworm pupae (SWP) on dry weight basis.

It is also estimated that from the cocoons obtained by rearing from one acre of rain fed mulberry, 76 kg fresh or 47 kg dry pupae can be obtained upon reeling. Similarly 192 kg fresh or 119 kg dry pupae were obtained from one acre of irrigated mulberry rearing. One hectare of mulberry field yields 2472.80 kg of mulberry leaves as dry matter, of which only 620.70 kg are digested by the silkworm larvae and further transformed into 211.20 kg of silk/cocoon shells. Hence, the silk production is only 8.54 per cent from the dry matter of mulberry leaf yield. On the other hand, the different wastes such as leaf remnants, excrements and the pupae is about 2061.60 kg *i.e.,* 83 per cent of all dry matter are transformed as waste products.

The pupae, which are obtained after reeling the silkworm cocoons, are generally thrown away though they are very rich in amino acids, oil, carbohydrate and minerals. Spent silkworm pupae are a fresh, highly degradable product. In silk producing areas, the disposal of large quantities of pupae can cause serious environmental problems (Wang Jun *et al.,* 2010). The utilization of this resource for feed and food or for the production of valuable biological substances such as chitin, protein, oil and fatty acids (α-linolenic acid) is a way to reduce the environmental impact of silk production. For 1 kg of raw silk, 8 kg of wet pupae (2 kg of dry pupae) are produced. About 6 to 8 kg of silk cocoons are required to produce 1 kg of raw silk. In doing so, about 6 kg of pupae are discarded as waste. About three to four decades back, these pupae would have been disposed off outside the dwelling regions as waste. However, recent years have witnessed the use of the pupae waste as a source for high-protein food and pupae oil for various applications (Patil *et al.,* 2013). With these views, the present review deals with the commercial applications of silkworm pupae and it paves the way for scope and commercial ventures available for the utilization of silkworm pupae.

Silkworm pupae as organic manure

Dried silkworm pupae contains about 8 per cent nitrogen, 0.29 per cent calcium and 0.58 per cent phosphorus. Silkworm pupae is used as manure to the mulberry in three different forms *viz,* raw pupae, raw pupae powder and de-oiled pupae powder. Krishnaswamy *et al.,* (1973) reported that silkworm pupae were very rich in protein and fat content and felt that it can be used as manure. Application of pupae resulted in significantly greater root and shoot weight. Maximum root, shoot and leaf yield was recorded in de-oiled pupal powder with increased crude protein and reduced mineral content in mulberry leaf. The minerals present in the pupae and de oiled pupae are as follows.

Minerals	Pupae	De-oiled pupae
Ca (%)	0.65	0.97
P (%)	1.22	1.70
Na (%)	0.30	0.38
K (%)	0.80	1.10
Mg (mg/Kg)	0.325	0.465
Fe (mg/Kg)	230.00	300.00
Zinc(mg/Kg)	285.00	371.00

Spent silkworm pupae are a waste material often discarded in the open environment or used as fertilizer (Wei ZhaoJun *et al.,* 2009). Sangeetha *et al.* (2012) reported that the application of SLPW (Silkworm litter-pupal waste) + Vermicompost recorded significantly higher leaf yield (32,098.5 kg) and NPK content (3.11%, 0.39% and 2.48 %) respectively in mulberry.

Silkworm pupae as feed

The protein concentration of pupae can be used to supplement the poultry feed and in cattle feed and reported that this meal might be an important substitute for fish meal (Nataraj and Basavanna, 1969). Joshi *et al.* (1980) studied the effect of substitution of various levels of deoiled silkworm pupae meal (both quantitative and isonitrogneous) replacing fishmeal on performance of layers, they also reported substitution of fishmeal by silkworm pupae improved feed efficiency over the control. In Japan, silkworm pupal cakes are being prepared and used as feed for cattle, pig and fowls. Rabbits fed with the silkworm pupae resulted in increased fat deposition and fur growth rate significantly (Aruga, 1994). Hens improved their egg laying capacity with impact on the colour of the egg yolk when it is used as protein supplement (Hisao Aruga, 1994).

The pisciculture sector used the silkworm pupae powder as food for fisheries. Studies on the nutritive value of untreated, acid, and water-treated deoiled silkworm pupae meal as a substitute for fish meal with respect to body weight gain, feed, and protein conversion ratio have concluded that untreated silkworm pupae can replace 50% and 75% fish meal protein in starter and finisher diets, respectively (Virk *et al.,* 1980).

Jun Zhou and Dingxian Han (2006) reported that silkworm pupae contained eighteen known amino acids, including all of the essential amino acid (EAAs) and sulphur containing amino acids. Methionine is an essential amino acid for animals particularly poultry. Rich methionine component of spent silkworm pupa makes it an ideal candidate for poultry feed which could enhance the egg quality.

An appropriate fermentation ensiling process has been developed in the laboratory to prepare a pathogen free SWP silage product (Yashoda *et al.,* 2001). Fermented silkworm pupae (SWP) silage or untreated fresh SWP pastes were incorporated in carp feed formulations replacing fishmeal. Survival rate, feed conversion ratio and specific growth rate, respectively, were 84.2%, 2.10 and 2.39 for fermented SWP silage, 65.8%, 2.98 and 2.26 for untreated SWP and 67.5%, 3.16 and 2.20 for fishmeal indicating clearly that the fermented SWP silage was nutritionally superior to untreated SWP or fishmeal (Rangacharyulu, 2003). Swamy (1994) reported that silkworm pupae that contain fat and protein were superior to plant proteins when fed to carp fish.

Dutta *et al.* (2012) treated the three days old chicks with five dietary treatments of silkworm pupae meal. The result showed that the silkworm powder meal (SWPM) increased broiler growth performance and has potential to replace the costly and contaminated fish meal, as the protein source, used in poultry industry.

Silkworm pupae as protein supplement

Silkworm pupae are highly nutritious and possess genuine food of high nutritive value. Silkworm pupae enriched protein biscuits contained six per cent nitrogen and 37 per cent protein (Anon., 1967). Pury is a yellowish fine powder made from the silkworm pupae which is a sericultural by product. It has a well-balanced amount of nutrients (protein and essential amino acids, fats included polyunsaturated fatty acid (PUFA), carbohydrate, vitamins and minerals). Trina *et al.,* (2014) developed four kinds of snacks based Pury *i.e.,* Likury, Sempry, Makary, and Krupy.

Pharmaceutical and pharmacological uses of silkworm pupae

The pupae were used in medicinal wine since ancient days and for lowering fat, BP and blood sugar levels. They also used for treating liver hepatitis, pancreatitis, leukocytopenia, neurological, ophthalmic, anti-bacterial, anti - fungal, anti-histaminic, gastric ailments and in preparations of vitamins A, E and K. The silk worm pupae are equal to meat and better than the protein of soya bean, fish or beef in terms of protein, fat, vitamins and calories. The glucosamine extracted from silkworm pupae can be used for treating osteoarthritis (Datta *et al.,* 2007).

In South Korea pupae are used for growing medicinally important fungi for heart diseases four species of fungi *viz., Paecilomyces japonica*, *Paecilomyces farinose, Paecilomyces* sp. 1300 and C*ordyceps militaris* are cultured on silkworm pupae and late age worms in sterilized conditions. These fungi are used for the manufacture of medicine for heart ailments. Silkworm pupal powder can be easily digested and absorbed by human bodies. It also can promote the physiological functions of the gastrointestinal tract (Ryu *et al.,* 1997).

Pharmacological studies show that silkworm pupae are alimental for increasing immunity, protecting the liver and preventing cancer. Consumption of silkworm pupae could supplement Vitamin B2 intake, which can be important to prevent the serious effects of Vitamin B2 deficiency (Kwon *et al.*, 2012). Silkworm pupae are good resources of edible oil that is especially rich in unsaturated fatty acids and considered an excellent dietary supplement for hyperlipidemia (Zou *et al.,* 2013). Silkworm pupae have a great antioxidant potential with potent polyphenolic contents to scavenge free radicals and a good antityrosinase activity (Deori *et al.,* 2014).

Extraction of chitin and Chitosan and its pharmaceutical uses

Chitin

Pupa skin is made up of chitin, which is a polysaccharide and its structure is similar to that of cellulose. Chitin is the second most abundant material available on earth. It is found on the exoskeletons of arthropods such as insects, spider and some fungi and in crustaceans such as crabs and shrimps. In USA over 500 million pounds of crabs are processed for meal. This yields about 80% of waste. This waste is simply dumped into coastal wastes. Pupa skin too is being thrown as a waste, causing pollution problem. Chitin can be converted into various useful products like Chitosan, Chitin sulphate, Chitin nitrate, Chitin xanthate, Sodium carboxymethyl chitin, *etc*. It can be hydrolyzed by enzymes and acids. Chitin and chitosan have immense applications in various fields such as food industry, cosmetics, agriculture, water treatment, biomedicine, textile, biotechnology, paper industry and wound healing (Ahamed and Sastry, 2011).

Chitosan

Chitosan is a well known derivative of chitin and it is a deacetylated derivative of chitin. It has also been reported that chitin can be 100 per cent deacetylated acetone (Zikakis *et al.* 1982). It is chemically known as poly (2amino-2-deoxyglucose) the degree of deacetylation refers to the relative amount of acetyl groups removed from the molecule of chitin. Ramachandra Naik *et al*. (1993). It is not a single substance, but a group of partially decacetyalated products of various degree of polymerization chitosan is insoluble in water, concentrated alkalies , alcohol and acetone unlike chitin ,it is soluble in dilute acids it can be converted into chitin by n-acetylating it because of its solubility, it is available for commercial use.

Suresh *et al.* (2012) reported that, the pupae of silkworm are an alternative source of chitin which consequently yields chitosan. Among the different races of mulberry silkworm, multivoltine pure races contain higher chitin of 3.225 percent in male pupae and 3.078 per cent in female pupae. Similarly higher chitosan per cent was observed in male 2.449 per cent and 2.354 per cent in female pupae.

Pharmaceutical uses of chitin and chitosan

Chitin, a component of pupal skin used in different applications like additive to increase the loaf volume in wheat flour bread, in post operational treatments such as conchotomy, deviatomy, polypectomy because of its easy usability, less hemophase, greater pain relief and fastens healing of wounds. Chitin found as potent antimicrobial against *Staphylococccus aureus, Klebsiella pneumoniae, Aspergillus niger etc.,* anti fungal against *Trichophyton equinum*, its buffering activity against acids, as food additive to control carcinogenicity of food stuffs .Chitin was also used as immunoadjuvant (antiviral agent), bacteriostatic agent, fungistatic agent, anti-sordes agent in preventing carcinogenic bacteria from teeth and bio compatible membrane to check bleeding in major surgeries (Katti *et al.,* 1996).

The general uses of chitin and chitosan are in oil refinery, wastewater contaminant, anticoagulant, antimicrobial activity,vascular grafts sutures, aggregation of cells, artificial kidney membrane, feed supplement, digestive aid, promotion of growth of bifido bacteria (probiotic), cheese processing, breweries, flavor productions, food thickener, paper-making additive for surface strength, removal of radioactive metals, heavy metals, color and environment contaminants inhibition of plant growth, and suppression of plant parasites (Sharmila, 2008 ; Assainar and Nair, 2014).

Extraction of pupal oil and its uses

The oil content of the silkworm, *Bombyx mori* in the pupal stages is 4.8 per cent for the male and 9.0 per cent for the female respectively (Suresh *et al.*,2012). The yield of silkworm pupae oil is about 20 per cent on the dry weight of pupae. The oil extracted from silkworm pupae contains more than 70% unsaturated fatty acids, 43 particularly the α-linolenic acid and oleic acid accounting for a high percentage (Rao, 1994). The extraction of silkworm pupal oil is mainly performed by traditional methods, including mechanical pressing extraction, organic solvent extraction, aqueous enzymatic extraction and supercritical fluid extraction developed lately (Zhu, 2012).

Properties and applications of pupal oil

The freshly extracted pupal oil appears with brownish tinge with fishy odour, while the refined oil is with fluorescent yellow colour and odourless (Shivaprakash, 1988). The oil saponification number is 203, iodine number is 45.02, non-saponification matter is 3.078 per cent, acid value is 11.53g and RM value of 2.798.

Winitchai *et al.,* (2011) extracted oil from five native Thai silkworm varieties. The oil extracted by the Soxhlet method from silkworm varieties showed free radical scavenging activity. Pupal oil may contain a lot of antioxidants such as α tocopherol and other kinds of lipid soluble antioxidants. For the presence of rich unsaturated fatty acid, especially α-linolenic acid in the silkworm pupa oil, it can be used in medicine, health food, nutritional food and high-end cosmetics and other industries except for eating. The silkworm pupal oil is considered a good source of raw material for various applications including food, medicines and cosmetics (Longvah *et al.,* 2011).

Silkworm pupae oil extracted by boiling is used in the cosmetic industries for making soaps and moisturizers (Kotake-Nara *et al.,* 2002 and Winitchai *et al.,* 2011). The total fatty matter increased from 25-50 per cent. This soap was used for degumming of silk. The pupae oil can be used in jute industry for lubricating (presently rice bran oil is being used) and in leather processing (presently, fish oil is being used).

Pupal oil also contains DNJ, which is a potent alpha glucosidase inhibitor used to treat diabetes (Kotake-nara *et al.,* 2002). Silkworm pupae and pupal oil are important materials as they are a rich source of essential omega-3 fatty acid and ALA (alpha lenoleic acid), which are of great importance for human health (Tomotake *et al.,* 2010). The valuable pupae oil is used in industrial products such as paints, varnishes, pharmaceuticals, soaps, candles, plastic and biofuels (Wang *et al.,* 2013).

CONCLUSION

The pupa which is available abundantly in the silk reeling, grain age and silkworm egg production industries as a waste can be utilized as a high potential raw material for various industries including cosmetics, animal nutrition and pharmaceuticals. Distinctive characteristics of silk by-products have a variety of important applications ranging from food supplements to pharmaceutical ingredients. There lies a vast scope for utilization of silkworm pupae in many advanced fields of science of commercial importance.

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