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Aflotoxin: A Higher Threat of Health Hazards for the Bivoltine Silkworms in Temperate Zones - A Review

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A B S T R A C T

The main aim of this review study was to know the effects by aflatoxin of mycotoxin fungal diseases for the health hazards of silkworm *Bombyx mori* and series of events that led to the discovery of aflatoxin as a potent carcinogen. Aflatoxins B_1, B_2, G_1 & G_2 may be referred as the toxic and produced by the Aspergillus fungal diseases, among all the Aflatoxins, B_1 and G_1 are highly toxic to the silkworm, which is carcinogenic in nature and can be occurred immediately higher incidence in bivoltine silkworms than in multivoltine and cross breed. The outbreak of aflatoxin was maximum in early instar larvae compare to later instar larvae & in the area of high humidity & temperature during rainy, winter seasons & also in the unhygienic condition of silkworm rearing rooms and mulberry gardens; etc. The symptoms of toxicity are larvae stop feeding, become lethargic, show body tension, lustrousness, chronic diseases, reproductive interference, immune suppression, finally die soon due to aflatoxin produced by the fungus in the host and will impact on the economic loss of crop productivity of the silkworm *Bombyx mori*. Hence, a review was framed to prevent the aflatoxin producing fungal attack as a precautionary high alert for the sericulturists of temperate region and may overcome by maintaining optimum temp, RH & hygienic condition in silkworm rearing climate, minimizes utilization of chemical fertilizers & insecticides in mulberry garden, developing silkworm breeds or races tolerant to aspergillosis could be a worthwhile attempt by silkworm breeders. Apart, utilization of advanced biotechnological and nano-technological tools for proper control and eradication of aflatoxin contamination and proper care should be taken for persistence and crossing of aflatoxins from other insects to/from mulberry garden & rearing room in the temperate bivoltine producing zones of the country and abroad.

Keywords

Aflatoxin, Aspergelous fungal disease, Temperate region, Bivoltine, *Bombyx mori*

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Introduction

The insect *Bombyx mori* gained important not only in sericulture economy stabilization but also in its biological importance. Hence, this insect is considering as one of the model organism for its genetics and molecular, physiological, behavioral, morphological features, *etc*. The China is the dominated country in the world raw silk production, which was discovered and introduced the silkworm/sericulture to the world-wide and the rearing of silkworms have been cultivating for over 5000 years in China for purpose of silk production (Nagaraju and Goldsmith, 2002). Moreover, even though presently China (146,000 MT), India (28,708 MT) and Uzbekistan (1,100 MT) are the three countries have occupied first, second and third positions respectively in the raw silk production. Totally, 21 traits play an important role in qualitative and quantitative of silk yield is influenced by healthy developmental stages of silkworm *Bombyx mori* (Chatterjee *et al.*, 1993). Hence, nourishment of silkworm during its developmental stages required because life traits, which are the effect on qualitative aspects of silk productivity (Ohi *et al.*, 1970). The qualitative and quantitative aspects of silk effects productivity by several silkworm diseases among them the fungal diseases are very dangerous to silkworm. This is a prime focusable disease caused by numerous *Aspergillus* species of fungi. It has been reported by Nomura, (1897) that, since the latest part of the 19^{th} century, the *Aspergillus* species have been known to pathogenic to silkworms. Generally, in Japan, it is called as "Kojickabi" (Ayuzawa *et al.*, 1972) and also called as brown muscardine. It has been accepted fact, that, more than 10 species of *Aspergillus* pathogenic to the silkworms are *A. flavus, A.tamari, A.oryzac, A.niger, A.ochraceus, A.sojae, A.fmigatus, A.nidulans, A.flavipes, A.clavatus, A.terreus, A.melleus, A.elegans, A.parasiticus*, etc from India, Indonesia, Thailand and Srilanka (Govindan and Devaiah, 1995).

Apart, more interesting to know that, the *Aspergillus* species produce a kind toxic substance called as "Aflatoxin", the aflatoxins can be defined as difuranocyclopentano-cumarines/ difuranolidocumarines. It has a dihydrofuran or a tetrahydrofuran ring. In general phenomenon, it is most prioritized vital effects especially on postharvest decays of fruits, vegetables and in particularly seed and feed deterioration by fungi, which produces mycotoxicosis. A report by Agrios, (1978) and Moss, (1989) and Talebi *et al.*, (2011) suggested that the most of the animals, dairy, poultry, livestock, and humans are causes diseases by consumption of feeds and foods invaded by fungi that produce toxic substances called mycotoxins.

However, few interesting investigations showed that aflatoxins are serious health hazard to human beings as well as animals through contamination of food (Diener *et al.*, 1987, Lubulwa and Davis, 1994, Cardwell and Miller, 1996) are correlated between insect damage and aflatoxin contamination (Bowen and Mack, 1991, Lynch and Wilson, 1991, Lynch *et al.*, 1991 and Gorman and Kang, 1991) and influenced of insect infestation on aflatoxin contamination of stored maize was studied in four agroecological zones in Benin (Hell *et al.*, 2000). Apart, observations were made by Sinha and Sinha, (1991 &1992) together revealed that the incidence of fungi of the *A. flavus* group and aflatoxin contamination was high by insect-damaged in maize than in insect free samples in India. Furthermore, few investigations also suggested that insects could act as a vector by transporting spores of fungal on their bodies, then contaminating grains as they moved about (Lynch and Wilson, 1991). According to Lussenhop and Wilcklow, (1991), finding revealed that few vital insects are acting like victors of *A. flavus* are carporphilus lugubris Murry and C.freemani Dobson on maize. Apart, Sauer and Burroughs, (1980) and Mills, (1983) reported that the environmental effect like in increased relative humidity (RH) also provides support to *A. flavus* growth through which seed germination almost reduced in the corn plant. Moreover, the accumulation of aflatoxin, the relative humidity, and temperature, as well as agroecological zones, are playing a very vital role, in addition to that for the development of aflatoxin is influenced by insects related fungi, maize varieties, polished & brown rice (Barry *et al.,* 1992 and Mousa *et al.,* 2013).

However, in sericulture industry mulberry, which is a primary source of food plant for silkworms and these plants are generally attacked by numerous fungal diseases like leaf spot, leaf rust, powdery mildew, root rot, root-knot, nematode, and intern ultimately causes of silkworm fungal diseases by consuming fungal infested mulberry leaves. The aflatoxin *viz*., B_1, B_2, G_1 & G_2 are may be referred as the *Aspergillus* agent or toxic substances produced by silkworm *Aspergillus* fungal diseases & among all above said aflatoxins B_1 & G_1 are highly toxic & carcinogenic to the *Bombyx mori*. Through the review of literatures, it has been digested and cleared that, the food, variability in environmental condition, stagnant aeration, unhygienic condition in the silkworm rearing rooms and mulberry garden, excess utilization of chemical fertilizers & insecticides, environmental factors and while transporting agents like insects and other sources are key factors to cause the fungal diseases. Hence, keeping the above concepts in our mind, it has boosted our interest to glance in this direction to present a review on the aflatoxin, which are toxic substances produced by *Aspergillus* fungal disease a threat of health hazards for the bivoltine silkworm *Bombyx mori*.

Discovery of aflatoxin and its process of distribution in different animals/livestock/others

The aflatoxin was primarily discovered 50 years ago in England as the causative agent of the "Turkey X" disease in 1960. Hence, the outbreak of the aflatoxicosis popularly known as the Turkey X disease. Because of this disease, there was the death of a large population of livestock (Blount, 1961 and Vander Zijden *et al.,* 1962) and this is the main reason, which helped for the discovery of aflatoxin in groundnut meal contaminated by *A. flavus* (Hesseltine, 1979). Further, this is also investigated in maize (Shotwell, 1977, Chakrabarty, 1981) and cottonseed meal (Lillehoj, 1979 and Sharma *et al.,* 1994). Apart, few other important findings like Busby and Wogan (1999), Eaton and Groopman, (1994), Wild and Turner, (2002) were suggested the carcinogenic potency of AFB_1 in several species *viz*, rodent, nonhuman primates, fish, and birds. However, the name aflatoxins are generally obtained from the first letter in *Aspergillus* and three letters from the flavus (Schoental, 1967). But it is fact that basically aflatoxins are known to be produced by *Aspergillus flavus* (Agrios, 1978) and later on other suggested that it is also produced by some species of *Aspergillus* too and may be referred as aflatoxins, viz., B_1, B_2, G_1, and G_2. In addition to these, there are two more metabolic products like M_1 and M_2, these are significance for direct contaminates of foods, feeds, and animals (Fig.1). Further, M_1 & M_2 are originally extracted from the milk of lactating animals fed an aflatoxin preparation that is the reason M designation and for B designation because of blue fluorescence under UV light, while G refers for the yellow-green fluorescence under UV light.

Expected avenues for spreading aflatoxin diseases to the sericulture industry

Sericulture is an agro-based rural oriented industry. The silkworm rearing and mulberry cultivation are considered as two prominent activities of sericulture industry. Historical account of this industry date backs to five thousand BC and it is originated in China and it is now established that domesticated silkworm *Bombyx mori* is evolved from its progenitor *Bombyx mendarina*. During mulberry cultivation, it is of common practice to come across with several bacterial, viral and fungal diseases. These diseases are either airborne (foliar) or soil borne (root) in nature and reported to decreases the leaf yield around 20%, besides deteriorating the leaf quality (Dandin and Giridhar, 2014). Several reports are also available where insects invade the mulberry and cause mulberry diseases, pathogens and insects. Several pesticides belong to either organochlorine, organocarbomates and organo phosphorous compounds. The environmental factors *viz*., temperature, humidity, and rainfall play an important role in the spread of mulberry diseases. It is of common practices to notice that, because of humid and low temperature in the environment, the fungal diseases of mulberry are commonly perpetuated in temperate climatic condition (Sengupta *et al.*, 1991), whereas, such problems are clear evidence during winter seasons of the tropical climates (Dandin and Giridhar, 2014 and Sengupta *et al.,* 1991).

However, among the broad categories of fungal diseases of mulberry, powdery mildew is very common during rainy and winter seasons and feeding of mildew affected leaves to silkworm adversely affects on silkworm growth and development resulting in poor cocoon yield and silk quality (Nomani *et a1*., I970 and Sullia and Padma, 1987), leaf spot is more during rainy season (Siddaramaiah *et al.,* 1978) and leaf rust are predominant during winter season (November-February) and matured leaves are more susceptible to this disease and in the presence of rust there will be rapid premature defoliation of leaves resulting in a shortage of leaves during late age rearing (Dandin and Giridhar, 2014), *etc*. The above major diseases commonly controlled (or) minimized using by common conventional practices, closely watch, timely control measures and regular monitoring are essential for managing different diseases is need of the hour rather than utilizing different commercially available fungicides, namely diathium 45, bavistain, benlite (Govindaiah *et al.,* 1989a and Govindaiah and Sharma 1994) and commercial fertilizers, chemicals as plant growth hormones. The environmental fluctuations and *Aspergillus* is a facultative fungus and is able to live saprophytically in the silkworm rearing environment like soil surface and rearing appliances, silkworm feces, *etc* (Aoki, 1971; Ayuzawa *et al.*, 1972). The extends of diseases are minimized to a large extends in the mulberry garden because of the recommendation of the above pesticides but it is important to note that though on one side, the fungicides minimize the diseases yet the important of aflatoxin produced by the fungus is very important to be noted it down. Such a contaminated food with aflatoxin diseased leaves feeding will affect on the growth and development in mulberry silkworm and non-mulberry silkworm (Figs. 2 & 3), cocoon yield and ultimately silk quality.

In regard to silkworm rearing because of domestication since several years/centuries, silkworm are exposed to vagaries of environmental fluctuation (RH, Temperature, winter & rainy seasons), chemical fertilizers, chemical plant growth hormones, chemical insecticides, crossing with other insects/pests to mulberry and silkworm rearing environments, unhygienic conditions in rearing environments and as a result it is accepted as a carriers of aflatoxin fungal through common practices in mulberry garden and silkworm rearing environments (Fig.4). Further, aflatoxin productions on rice and oral toxicity to silkworm larvae of extracts of larvae infected with A. flavus (Tables: 1&2) was well documented by Ohtomo *et al.,* (1975).

Apart from, several other diseases like bacterial, viral and microsporidia (protozoan) diseases are also predominant in the mulberry garden and silkworm rearing environments. In addition, infestation caused by uzifly (Exorista bombycis) inflicting 10-20% damage to the silkworm cocoon crop is noteworthy both in tropical and temperate climatic condition (Dandin and Giridhar, 2014). Several quick measures are practiced to reduce the pathogens caused by fungal disease, namely baveria basiana, red muscardin, *etc* (Aoki, 1971 and Aoki *et al.,* 1972). The utilization of fungicides not only minimizes the fungal diseases but also acts as aflatoxins. Keeping the important of aflatoxin in the light of the human health and welfare as well sericulture industry, the present review article contemplates the need of careful monitoring of aflatoxins both in the silkworm rearing and mulberry gardens too.

Table.1&2 Showing Aflatoxin productions on rice and oral toxicity to silkworm larvae of extracts of larvae infected with A. flavus. (Source from: Ohtomo et al., 1975)

TABLE 1. *Aflatoxin B_1 production on rice by A. flavus isolates from sericultural farms and by A. paraciticus var. globosus ATCC 15517 known as an aflatoxin-producing strain*

Aspergillus	Aflatoxins B_1 (μg/g)
A. flavus strains[a]	
K-29	2.0
K-35	14.5
K-135	25.0
K-136	23.5
K-199	170.0
K-204	50.0
K-208	4.5
K-224	90.0
K-246	150.0
K-274	60.5
A. paraciticus var. *globosus* ATCC 15517	55.0

[a] All strains isolated from sericultural farm.

TABLE 2. *Oral toxicity to silkworm larvae of extracts of larvae infected with A. flavus*

No. of larvae	Mortality of silkworm larvae at incubation days:				
	2	3	4	5	7
Control	20	20	20	20	20 (5 instar)
$CHCl_3$ added	20	20	20	20	20 (5 instar)
Extracts from normal larvae	20	20	20	20	20 (5 instar)
Extracts from affected larvae	16	4	0	0	

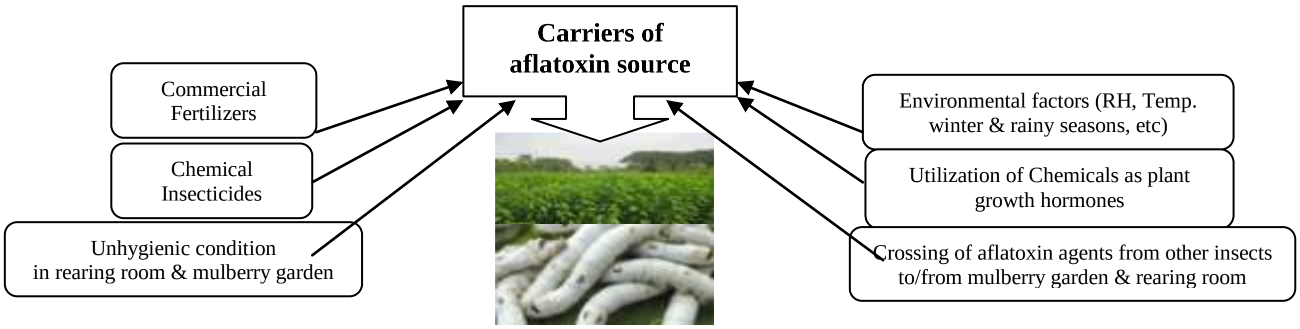
(Source from Ohtomo *et al.,* 1975)

Fig.1 Aflatoxin and its process of distribution in different food chains and animals, etc.

(Source: Pradeep kumar *et al.,* 2017)

Fig.2&3 Before and after infection by aflatoxin in mulberry silkworms and non mulberry silkworm

Fig.4 Come across with aflatoxin carriers (source) and infection to the silkworms *Bombyx mori* L.



Management and controlling measures of aflatoxin production in silkworm rearing environment

First of all, as per the silkworm safety is concerned or being protected from or unlikely to cause danger diseases by aflatoxins and prevention of aflatoxin-producing fungal attack as a high alert for the sericulturists of temperate region and may overcome by maintaining optimum temp, RH & hygienic condition in silkworm rearing climate conditions. It is suggested that minimization of chemical fertilizers & insecticides utilization regularly in mulberry garden. Biological control of aflatoxin production in crops in the US has been approved by the Environmental Protection Agency and two commercial products based on a toxigenic A. flavus strains are being used (Afla-guardR and AF36R) for the prevention of aflatoxin in peanuts, corn, and cottonseed (Dorner, 2009). Hence, development of silkworm breeds or races/hybrids tolerant to aspergillosis could be a worthwhile attempt by silkworm breeders. In addition, races/breeds resistant to aflatoxin are yet to be evolved in several popular silk producing countries of the world. Apart, efficient biochemical markers and genes for resistance in maize against *Aspergillus* could also be utilized (Chen *et al.*, 2007).

Moreover, biotechnological methods have already be utilized for aflatoxin management (Yu, 2012) and advanced genomic technology-based research and decoding of the *A. flavus* genome have supported identification of the genes responsible for production and modification of the aflatoxin biosynthesis process (Bhatnagar *et al.*, 2003; Cleveland, 2006; Holbrook *et al.*, 2006; Ehrlich, 2009). Further, it is suggested that aflatoxin accumulation can be reduced by utilizing transgenic Bt maize with insect resistance traits (Wu, 2010). These are the many advanced initiations to be focused for the sericulture advanced countries for control and eradication of aflatoxin-producing fungal in bivoltine producing temperate zones of the world.

In conclusion, all the studies made till now is only restricted to the *Aspergillus* fungal diseases with relevant to the silkworm *Bombyx mori* but in-depth aflatoxins relevant approaches are yet to be initiated in temperate climatic zones of the sericulture industry. As per this review work is concerned, relative humidity, temperature, rainy and winter seasons are the main weapon for quick spread of aflatoxin and the temperate zones are the sources and feasible climate for healthy growth and development of aflatoxin fungal. Hence, eradication of toxic materials aflatoxin (B_1 and G_1), which are the main infection source to the silkworm in the rearing house should be thoroughly investigated. The developing new breeds/races/hybrids are yet to be initiated for tolerant and resistant to the aflatoxin and utilization of advanced biotechnological and nano-technological tools are badly needed to the bivoltine silk producing counties of the world. In addition, precautionary measures of feasible prevention and management strategies should be taken up especially in bivoltine producing temperate climatic zones are need of the over through numerous approaches as discussed herein.

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