Causal Pathogens of *Aspergillus* Disease of Silkworm and Its Control

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Aspergillus disease, a fungus disease caused by *Aspergillus* fungi, is one of the important diseases of silkworm. Muscardine disease, also known as a fungus disease of silkworm, is distinguished from *Aspergillus* disease, because it differs in various characteristics.

More than ten species of *Aspergillus*, i.e., *A. flavus*, *A. oryzae*, *A. tamarii*, *A. ochraceus*, *A. sojae*, *A. oryzae* var. *fulvus*, *A. flavipes*, *A. terreus*, *A. melleus*, *A. clavatus*, *A. fumigatus*, *A. nidulans*, *A. elegans*, *A. parasiticus*, etc. have been reported to be parasitic to silkworm. Among them, important pathogens are included in the *A. flavus* group (Raper and Fennel, 1965), and the species detected at the highest frequency (higher than 90%) is *A. flavus*.

In recent silkworm rearing, silkworm larvae hatched from eggs are grown in "co-operative rearing houses for young silkworms" for about 1 week, and then they are distributed to sericultural farmers for the rearing up to cocooning. As the *Aspergillus* disease occurs particularly in the young stage of silkworm, the control of this disease is extremely important in co-operative rearing houses where a large number of larvae to be distributed to several—several tens and sometimes several hundreds of sericultural farmers are grown.

In Japan, large scale co-operative rearing of young silkworm with improved facilities and mechanization has been promoted for the purpose of increasing productivity and stability of cocoon production. As a result, the number of sericultural farmers joined the co-operative rearing was increased from 51.9% in 1965 to 86.9% in 1975 and 91.0% in 1979. The number of co-operative rearing houses reached as many as 2,652 in 1979, and they are equipped with automatic temperature and humidity regulating facilities, enabling easily to maintain high temperature and high humidity (28–30°C, 85–95% of RH). This temperature and humidity condition was set-up from the viewpoint of silkworm growth and preventing withering of mulberry leaves supplied. However, this condition is quite favorable for growth and multiplication of *Aspergillus* fungi, causing a high risk for the occurrence of *Aspergillus* disease. As a matter of fact, the disease damage was increased since about 1965. However, the damage at present is suppressed to a low level owing to the improvement and spread of control measures developed by researches made over a period of ten years.

However, the possibility of occurrence of this infectious disease is not disappeared. As silkworm faeces and litter offer a suitable substratum for growth of this fungus, control measures, including hygenic management, for this pathogen will continuously be of importance. As the *Aspergillus* fungi prefer high temperature and high humidity, the control may be more important in silkworm rearing in the subtropics and the tropics than in Japan.

Detection of *Aspergillus* fungi

It was in about 1965 that disinfection of *Aspergillus* fungi was recognized as the most difficult one to be overcome among various pathogens of silkworm diseases, and can not be done satisfactorily. For example, after the

Table 1. Characteristics of *Aspergillus* fungi isolated from co-operating rearing houses for young silkworm

Source of fungus isolated	Number of isolates tested	Percentages of resistant isolates		Percentages of virulent isolates to larvae of the silkworm
		to formaldehyde	to mercuric fungicide	
Rearing houses for young silkworm	395	87%	48%	79.8%
Stock culture preserved for long period by subculture	24	18	0	33.0
Mixed feed for livestocks and fowls	35	14	17	22.8

disinfection of rearing rooms and tools, the *Aspergillus* disease occurred, causing a doubt on the effectiveness and method of use of disinfectants. The major reason was regarded that characteristics of the pathogen such as degree of resistance to fungicides, are not sufficiently known.

Consequently, characteristics of the fungi distributed in the co-operative rearing houses for young silkworms located throughout the country, particularly their resistance to disinfectants routinely used and their pathogenicity to silkworm were surveyed.

At first, dusts in the silkworm rearing rooms, such as in co-operative rearing houses for young silkworms, were collected to isolate and collect pathogenic fungi. To facilitate the detecting culture of *Aspergillus* fungi, Rose Bengal agar was deviced as the selective medium[1]. It is composed of $NaNO_3$ 1 g, K_2HPO_4 1 g, sucrose (or glucose) 10 g, Rose Bengal 50–70 mg, and agar 15 g, dissolved in 1,000 m*l* of distilled water. By this selective medium, presence or absence of the *Aspergillus* fungi can be known after 3–4 days of culture. If needed, transfer to pure culture can be done conveniently to examine various characteristics of them.

From a total of 681 samples of dust (source for isolation) collected from 175 co-operative rearing houses for young silkworms in the country (mostly in eastern Japan), *Aspergillus* fungi were detected at a rate of 54.4%[6]. The rate was higher, 72–84%, in farmer's rearing rooms. Thus, the rate of detection was very high, likely to be abnormal. Isolates detected were *A. flavus-oryzae* group, *A. tamarii*, *A. ochraceus*, etc., and 90% or more of them was fungi of *A. flavus-oryzae* group[6].

Characteristics of pathogenic fungi

With about 400 isolates collected from various materials (dusts, silkworm faeces, litters, etc.), resistance to fungicides and pathogenicity to silkworm were examined. As a result, it was made clear that there exist large variations in their characteristics: variation of 10–100 times in LC_{50} of virulency to silkworm, 40 times (0.01–0.4%) in MIC of resistance to formaldehyde, 200 times (0.1–20 ppm) in MIC of resistance to mercuric fungicides, and 170 times (0.019–3.5 g/100 m*l*) in productivity of kojic acid[3,6,7].

Characteristics of *Aspergillus* fungi isolated from co-operative rearing houses for young silkworms were compared with those of other isolates. As shown in Table 1, the former exhibited markedly higher percentage of iso-

Table 2. Relation between aldehyde dehydrogenase activity and formaldehyde resistance of *Aspergillus* fungi

Eungus isolates	Formaldehyde resistance	Enzyme activity (unit/min.)
1016	Susceptible	324.0
2262	Susceptible	323.0
1366	Resistant	585.0
2129	Resistant	570.0

Table 3. Relation between virulence to silkworm larvae and enzymatic activity of *Aspergillus* fungi

Isolates tested	Degree of virulence to silkworm larvae	Lipase activity (0.05N, NaOH, m*l*)	Cellulase activity (unit/m*l*)	Chitinolytic activity	
				Liquefying (unit/m*l*)	Saccharifying (unit/m*l*)
907	Moderate	0.16	23.1	0.90	0.50
1016	Moderate	0.38	1.3	0.54	0.35
2262	Small	0.42	14.1	0.23	0.20
2475	Moderate	0.0	28.9	0.91	0.50
2634	Small	0.06	17.0	0.25	0.56
2705	Moderate	0.0	2.6	2.90	1.80
1366	Virulent	0.08	16.1	2.90	1.80
1398	Virulent	0.04	17.9	3.33	2.12
2129	Virulent	0.10	13.5	4.00	1.40

lates resistant to formaldehyde and mercuric fungicide (routinely used), and highly virulent to silkworm than isolates from stock cultures preserved for long period by subculture in research institutes like university or from feeds for livestocks and fowls. Of these charteristics, a correlation was found between formaldehyde resistance and virulency: isolates with higher formaldehyde resistance are more virulent to silkworm[3,6,7].

As the isolates resistant to formalin, used habitually, can grow well on the media with formaldehyde, and amount of formaldehyde in the media decreases with growth of the fungi, the formaldehyde metabolic system was examined. It was found that formaldehyde was oxidized to formic acid by aldehyde dehydrogenase in the isolates resistant to formaldehyde[9], and the activity of this enzyme coincided with the degree of formaldehyde resistance (Table 2)[10].

Furthermore, the formaldehyde resistance of the isolates coincided with their degree of virulence to silkworm larvae[6]. In the larvae inoculated with *Aspergillus* fungi, a large amount of glucosamine and N-acetylglucoasamine were released by the dermal intrusion of the fungi, showing the decomposition of chitin of larval cuticle. Activity of chitinolytic enzyme of the fungi also coincided with the degree of their virulence to silkworm larvae, but no definite relation was observed between activities of lipase and cellulase of the fungi and their virulence (Table 3)[11].

Silkworm growth stage and susceptibility to *Aspergillus* fungi

Susceptibility of silkworm to *Aspergillus* fungi varied with larval instars: the 1st instar showed the highest susceptibility, followed by the 2nd instar. With the advance of instar, the susceptibility of larvae decreased, but it slightly increased in mature larvae. The susceptibility expressed by LC_{50} (medium lethal concentration) is shown in Fig. 1, which indicates the susceptibility of the 1st instar larvae is 100 times that of the 3rd instar larvae. Mortality of larvae in the period from the 1st day of the 1st instar to the 1st day of the 4th instar showed that the susceptibility was high at the stage of the 1st and 2nd day of the 1st instar and the 1st day of the 2nd instar, while it was low in other stages (Fig. 2).[7] Infections at these susceptible stages must be prevented in the control of the disease.

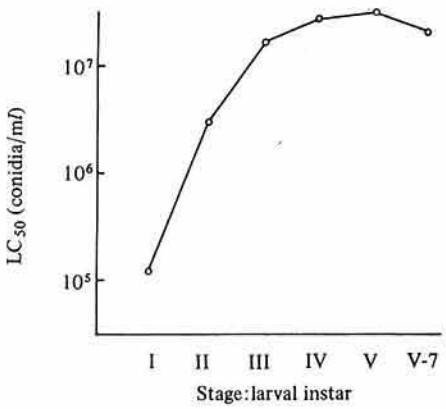

Fig. 1. Susceptibility shown by median lethal concentration in each larval stage of the silkworm to the infection with *Aspergillus* fungi

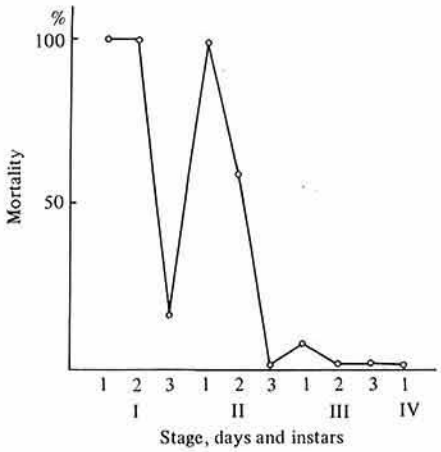

Fig. 2. Mortality of several days-old larvae in each instar of the silkworm to infection with an *Aspergillus* fungus

Practices of disease control

There are two methods of controlling *Aspergillus* disease: the one is disinfection of rooms and tools for silkworm rearing, and the other is disinfection of rearing beds and body surface of silkworm larvae. In either case, disinfectants effective to fungi which are resistant to formaldehyde are needed. In the former method, benzalkonium chloride, iodine disinfectant, benzalkonium chloride + dodecyl diaminoethylglycine (amphoteric surfactant), didecyl dimethyl ammonium chloride (cationic surfactant), etc. were proved to be effective[2,4].

For the latter method, dithiocarbamate fungicides were found to be effective[5]. Their effectiveness to three fungicide-resistant isolates (K1, K431 and S85) is shown in Table 4. Disinfection by organo-sulfurous fungicides

Table 4. Control effect of disinfectants on *Aspergillus* disease of the silkworm, *Bombyx mori* L.

Disinfectants	Content (%)	Per cent survival after dusting against following fungi*		
		K-1	K-431	S-85
Not disinfected	–	0%	0%	0%
Talcum	100	0	0	0
Lime	100	0	0	0
Paraformaldehyde	3.3	6	6	0
Ceresan (phenylmercuric acetate)	5.0	0	0	44
Chlorinated lime	10.0	30	58	34
Sorbic acid	10	0	0	0
Salicylic acid	5.0	60	66	56
Maneb	2.0	100	92	96
Zineb	2.0	92	96	96
Mancozeb	2.0	92	96	100
Cufram Z (octabithio carbamate)	2.0	100	100	96

* Larvae of first instar of the silkworm were disinfected by dusting chemicals after the fungus inoculation.

such as Maneb, Zineb, etc. resulted in 92–100% of survival rate of silkworm larvae. They showed far higher effectiveness of disinfection than paraformaldehyde, and, of course, much higher survival rate than the control without disinfection.

As a help to hygenic management for more efficient control of the disease, a simple fungi-detecting method (Plate 1) was deviced by using Rose Bengal agar, mentioned earlier. Rose Bengal agar is crammed aseptically into polyethylene cylinder to produce "stamp agar". The stamp agar is a column of Rose Bengal agar medium, enclosed by polyethylene cylinder. Surface of the agar column is pushed onto a place to be examined, and sliced off at the thickness of about 0.5 cm. The sliced agar plates are incubated at 30°C for 2 days with its original surface oriented upside in order to examine the presence of *Aspergillus* fungi in that place. Diameter of the stamp agar is 3.6 cm giving just 10 cm^2 of area, which makes quantitative estimation of the fungi possible. For the users of the stamp agar, preparation of agar medium is not necessary and no skill is needed in the use, so that any farmer can detect the contamination by *Aspergillus* fungi easily and quickly[8]. Thus, this method has made a contribution to preventive management of the epidemics in the co-operative rearing houses for young silkworms.

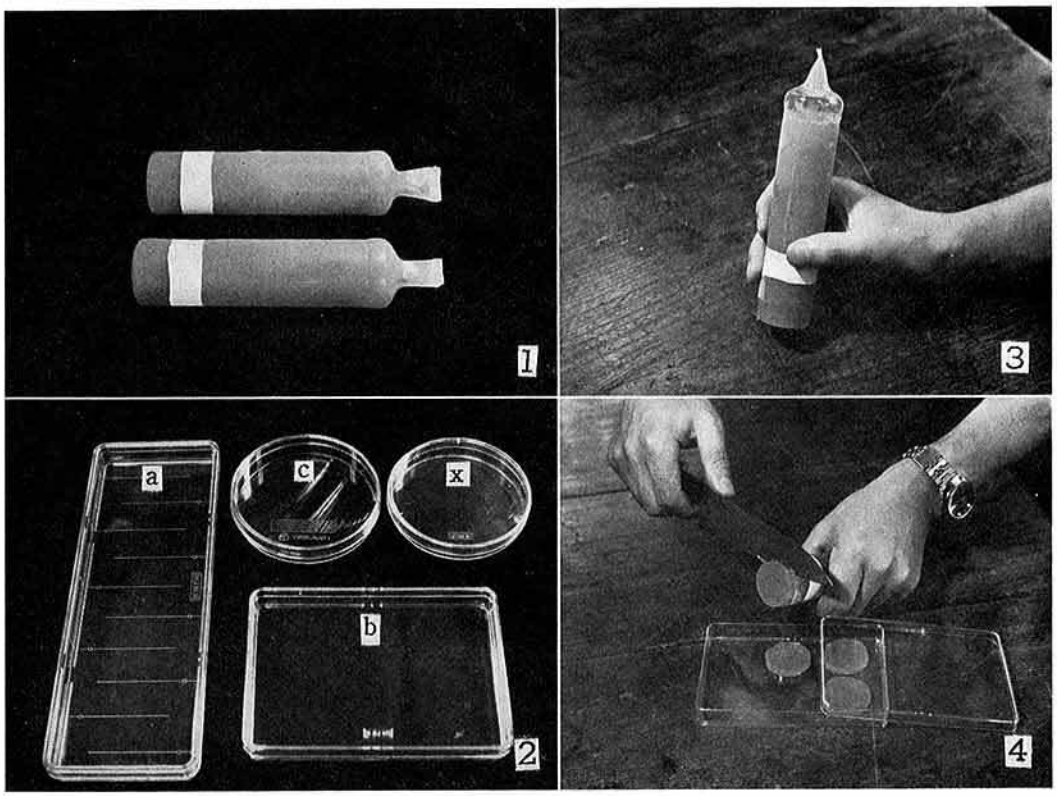

Plate 1. Easy detection method for *Aspergillus* fungi in silkworm rearing house by means of the stamp agar (= agar stamp method)
1. Stamp agar
2. Some kind of dishes for culture of slices of agar
3. Stamping
4. Cutting of stamped agar

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