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Cereal Scab Causing Mycotoxicoses in Korea and Present Status of Mycotoxin Researches*

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韓國에서의 眞菌中毒症을 일으키는 麥類 붉은 곰팡이병 및 眞菌毒素 硏究의 現況

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In 1963, a severe epidemic of cereal scab caused by *Gibberella zeae* occurred in southern Korea and to a less extent in central and northern Korea. In some areas losses were 80~100 percent. The epiphytotic was due to heavy rainfall during the heading and flowering season which provided a favorable environment for this severe epidemic. Yield losses resulted a great social problem because of the resultant food and feed grain shortage, lose of seeds for planting the following crops and mycotoxicoses to man and animals.

In the same year, a nationwide research committee was organized including plant pathologists, microbiologists, agronomists and biochemists under the juristiction of the Ministry of Agriculture and Forestry. The committee initiated research on etiology, epidemiology, and control of the disease and on the toxic effects of infected grains to man and animals. The present paper will review some research carried out in Korea on cereal scab with special reference to epidemiology and mycotoxicoses to animals and man. In addition, the present status of research in Korea on aflatoxins in foods and toxic moldy rice will be briefly reviewed.

1. Cereal scab

(1) Differences in isolates of *G.zeae*

Ten isolates of *G. zeae* including 7 from infeced barley grains and 3 from wheat were tested on barley (cultivar. Kantorisaki No. 1) and wheat (cultivar. Norin No. 2). The percent seed germination and seedling blight was recorded. Test seeds were surface disinfected with 1% NaOCl solution and followed by a water rinse, one group of seeds were inoculated with a conidial suspension for seed germination tests in Petri plates. The other disinfected seeds were sown in pots in which the soil was previously infested with the different isolates for seedling blight tests (Chung et al., 1963).

Percentages of seed germination varied with the isolates. In general wheat seeds germinated better

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than barley seeds with one exception regardless of the isolates obtained from barley or wheat. More seedling blight of wheat occurred than barley did with one exception. The isolates from barley, B—1, B—3 caused severe seedling blight to both wheat and barley. The other isolates were moderate to light (Fig. 1).

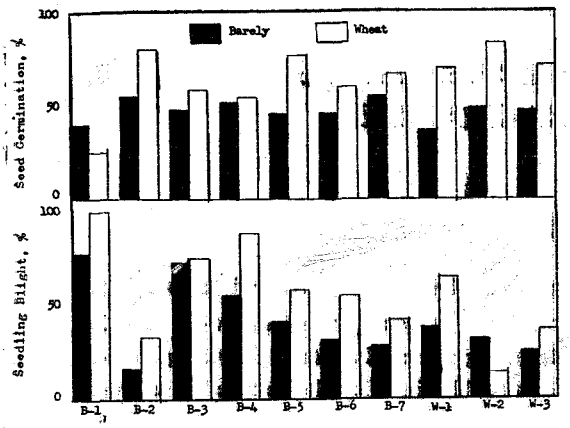

Fig. 1. Percentage of seed germination and seedling blight of barley and wheat by 10 isolates of *Gibberella zeae* (Chung et al., 1963)

(2) Overwintering of *G. zeae*

It is well known that ascospores overwintered from perithecia are known to be an important primary inoculum of the disease. Lee et al. (1865) also found perithecia on the infected straw of wheat, barley and rice stubble as a primary inoculum in 1963. Soil-borne *G. zeae* is also capable of causing seedling blight as well as head blight of cereals. This was demonstrated by Chung et al. (1964) when soil infested artificially with *G. zeae* resulted in varing degrees of pre-and post emergence seedling blight of wheat, barley, corn, soybean, cotton and buck wheat.

Thus when populations of overwintered soil-borne *G. zeae* are abundant they then may serve an important role as a primary inoculum. Survival of the organism from buried samples in soil was examined on modified PCNB agar by the dilution plate method (Kim et al., 1972).

The percentage of colonies recovered from surviving *G. zeae* decreased rapidly in December, and increased gradually beginning in March regardless of soil moisture (Table 1). Survival was highly affected by temperature fluctuation of soil through the winter. However, the organism showed remarkable ability to multiply once returned to favorable temperature.

Table 1. Percentage of recovered colonies on modified PCNB agar by survived *Gibberella zeae* at different soil moistures through the winter

(Kim & Chung, 1972)

Soil moisture (%)	Percentage of recovered colonies*					
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
10	100	74	6	10	35	60
30	100	56	40	13	35	80
50	100	120	44	40	39	70

* Based on two 1-gram samples (2×10^4 gram soil/plate) from each of three vials with 3 replicates.

2. Mycotoxicoses due to cereal scab

(1) Mycotoxicoses to animals

As previously reported, the toxic substance produced by *G. zeae* on grains caused vomiting in live stock depended upon animal species. Oh et al. (1963) reported that chicks and cows were not sensitive to the toxin while vomiting, dizziness, decreasing body weight occurred with pigs and dogs. In pigs an increased amout of scaby grains resulted in less feeding and increased vomiting (Table 2).

Table 2. Mycotoxicoses due to scabby barley infected with *Gibberella zeae* to pig

(Oh et al., 1963)

Amount infected grains, %	Amount of feed rejected %	Mycotoxicoses*			
		Vomiting	Dizziness	No vigor	Diarrhea
5	20	—	—	—	—
10	25	+	+	+	—
20	30	++	+	+	—
92	45	+++	+	+	—

* — : None, + : Light, ++ : Moderate, +++ : Severe

Toxicity of scaby grains to experimental animals has been shown to wary by many authors. Among these in 1963, Oh et al. (1963) found that suckling

mice were lethal when compared to rats, rabbits and chicks with scaby grains. Water extracts of scaby barley bran injected intraperitonaly was the most lethal to suckling mice, followed by extracts from ground kernels and from intact kernels in that order(Table 3). These results indicate the location of the toxic substance within the scaby kernels to be in the bran.

Table 3. Lethal effect of the water extracts of scabby barley infected with *Gibberella zeae* to suckling mice*

(Oh et al., 1963)

Extracts from	No.death with injection,**ml		
	0.1	0.2	0.3
Kernels	0/6	3/6	6/6
Kernels, ground	0/6	1/6	5/6
Barley bran	3/6	6/6	6/6
Control, Kernels	—	—	0/6
Control, Barley bran	—	—	0/6

* No toxicity with mice, rats and rabbits
** Intraperitoneal injection

(2) Mycotoxicoses to man

Extensive surveys on mycotoxicoses to man due to scaby grains at Kyongsang-namdo where severe epidemics of cereal scab occurred were made by Chun et al. (1963). Results summarized in Table 4 that nausea and vomiting occurred frequently within 5~15 minutes after consuming a meal containing immatured infected grains. Diarrhea, headache, dizziness and throat irritation occurred during the period from several hours to 24 hours. Symptoms varied depended upon the consumption of scaby grains as to percent infection, quantity and duration, generally reduction of body weight weakness were found when they consumed meals prepared with scaby barley for a two month period. However, dry cured infected grains followed by milling and soaking in water caused a mild toxicoses or no symptoms to man during a two month feeding period.

3. Mycotoxin researches in Korea

Mold induced deterioration of grains and foodstuff has centered principally upon the resultant of economic losses or other attributes of the commodity until recently. Since the potential significance of food-borne mycotoxins has become apparent, mycofloral studies in relation to mycotoxin, especially with aflatoxins, on grains and other foodstuffs are now being made in Korea as in many other countries.

Table 4. Mycotoxicoses to man after consuming a meal containing immature barley grains infected with *Gibberella zeae*

Chun et al. (1963)

Symptoms	Frequency* (%)	Symptoms occurred within	Duration (hr)
Nausea	66	5~15 min	1.5
Vomiting	60	5~15 〃	1.5
Diarrhoea	44	1~6 hr	5.0
Headache	18	Several min	Several
Dizziness	23	10~30 〃	24.0
Throat irritation	13	Several 〃	Several

* Percentages among 62 people surveyed in each symptom

(1) Mycoflora on deteriorated rice and aflatoxin

Cho et al. (1972) and Kim and Cho (1973) examined microflora of deteriorated rice samples collected all over Korea. The media used were Czapek agar medium for storage fungi, *Aspergillus* and *Penicillium*; 20% sucrose Czapek agar medium for the *A. glaucus* group.

Predominant fungi among the total 27 samples (Cho et al., 1972) were *A. glaucus* group, especially species of *A. amstelodami*, *A. chevalier*, *A. montevidensis* and *A. ruber*. Of 62 samples with 448 grains examined (Kim et al.,1974), fungi associated with deteriorated rice were *A. glaucus* group, *A. orzae*, *A. candidus*, and *A. versicolor*. Mycotoxin producing fungi, *A. flavus*, *A. ochraceus*, *A. fumigatus*, *P. citrinum*, *P. islandicum* were also detected, but their frequencies were so low that they might not be severe problem (Table 5). One sample of deteriorated rice in both studies was heavily infected with *P. islandicum* which might be a problem of mycotoxicoses. Additionally *Aspergillus* species on deteriorated milled rice were frequently associated with *Penicillium* and bacteria (Ki-

m et al., 1974).

Table 5. Frequency of toxin producing fungi obtained from deteriorated stored rice

Adapted from Cho et al. (1972)& Kim et al. (1974)

Fungi	Cho et al. No. among 448 grains	Kim et al. No. among 2976 grains
Aspergillus flavus	10	15
A. ochraceus	8	1
A. fumigatus	3	58
Penicillium citrinum	—	9
*P. islandicum**	14	30

* 10 out of 16 isolates obtained from imported milled rice in each study.

Seven isolates of *A. flavus* obtained from previous studies (Cho et al., 1974; Kim et al.,1974) were subjected to isolation and quantitation of aflatoxin by Lee et al. (1974). Polished rice was inoculated then chloroform extracts of each culture filtrate was identified by thin layer chromatography on silica gel H and derivative formation of water and acetate aducts. All isolates tested were capable of producing aflatoxins, preferentially B_1 but no G_1 at all. The isolate producing the most aflatoxin was *A. flavus* var. *columnaris*, excerting to 1 ppm on rice. Shaking cultures of *A. flavus* group on milled rice produced more than 10 times the aflatoxin than by stationary culture. Color change of the culture was closely related to production of the toxins during the cultivation period.

Table 6. Production of aflatoxin on rice by *A. flavus* strains isolated from deteriorated rice in Korea[1] Lee et al., (1974)

Strain	Aflatoxin in shaking culture (ppb)[2]					Aflatoxin in stationary culture (ppb)[3]				
	B_1	B_2	G_1	G_2	total	B_1	B_2	G_1	G_2	total
A. flavus var. columnaris	1081.7	25.8	nil	11.7	1119.2	116.4	nil	nil	3.5	119.9
A. flavus SN-7	705.6	7.4	nil	7.1	720.1	83.2	nil	nil	2.4	85.6
A. flavus SN-8	trace	nil	nil	7.7	7.7	—	—	—	—	nil
A. flavus SN-9	trace	6.8	nil	trace	6.8	—	—	—	—	nil
A. flavus SN-10	trace	nil	nil	trace	trace	—	—	—	—	nil
A. flavus SN-11	285.4	nil	nil	8.2	293.6	15.2	nil	nil	nil	15.2
A. flavus SN-12	5.8	nil	nil	trace	5.8	—	—	—	—	nil

1) Aflatoxin was assayed by eliminating column chromatography process.
2) Incubated on a rotary shaker (180rev/min) at 28°C for 6 days.
3) Allowed to stand but shaking once a day by hand at 28°C for 6 days.

(2) Mycoflora on foodstuffs and aflatoxin-like substance.

Since fungal fermented foodstuffs such as soybean paste and others have been used extensively for a long time in Korea, it is likely that mycotoxin producing fungi could be contaminated during the process of fermentation. Recently mycoflora on various fermented foodstuffs with special reference to aflatoxin has been studied.

Chloroform extracts obtained from 15 samples of fermented soybean foods were subjected to investigate the possible occurrence of aflatoxins by Lee et al. (1969). With thin layer chromatography, Rf value was the same as aflatoxin B_1, but none of the absorption curve of eluates showed accordance with the curve of aflatoxins. Lee et al. (1971) collected 486 samples of fermented foodstuffs in Korea during the period from 1965 to 1970. Among 117 isolates of *Aspergillus*, 64 isolates were *A. flavus* with 64% of the totals, 12 *A. oryzae*, 12 *A. kawachii*, 9 *A. niger*, 4 *A. versicolor*, 3 *A. fumigatus* and followed by other species. Of the isolates of *A. flavus*, 3 tested were known to produce aflatoxin-like substances by the methods of previous investigators (Kim et al., 1974). Similar results were also obtained from 3 isolates of *Aspergillus* on cereal grains by Koh et al. (1973).

A screening test for toxicogenic fungi isolated

from various food stuffs was made with HeLa cells and mice (Cho et al., 1973). The fungi tested were 9 isolates of *Aspergillus*, 9 *Penicillium* and 2 *Alternaria*. Of 20 isolates tested, 14 isolates were toxic to both HeLa cells and mice, 17 to HeLa cells alone and 14 to mice alone. In most instances, the results obtained by HeLa cells were in good accordance with those obtained by mice (Table 7).

Table 7. Grade of toxicity on HeLa cells and on ICR mice

(Adapted from Cho et al., 1973)

Strain	on HeLa cells with % of filtrate		on ICR mice
	10	1	
Aspergillus sp.			
A−8	2	1	++
A−35	2	1	+
A−49	3	2	+++
A−66	2	1	++
N−25−5	0	0	−
N−1−2	1	0	−
N−1−7	2	1	+
N−25−1	0	0	−
N−25−2	1	1	+
Penicillium sp.			
P−32	1	0	−
P−39	0	0	−
P−63	3	2	+++
P−66	1	0	+
P−67	1	0	+
P−68	1	0	+
P−71	1	0	−
P−75	1	0	+
RC−8	1	1	+
Alternaria sp.			
N−1−11	2	1	+
C−10	1	1	+

4. Conclusion

Severe epidemics of cereal scab caused by *G.zeae* in Korea resulted in not only a considerable reduction of yield and quality of the crop but also severe mycotoxicoses to man and animals. Although differences in susceptiblity of barly and wheat varieties to scab are known to exist, no commercially satisfactory, highly resistant varieties have been developed. The nature of resistance to cereal scab both genetically and physiologically should be studed for breeding of resistant varieties in an environment favorable for disease development.

Some molds, growing on deteriorated rice and fermented foodstuffs are known to produce aflatoxin or aflatoxin-like substances in Korea. Because stored grains, foods and feeds may be exposed to undesirable fungi, the mycotoxic problem is not only of an academic interest but also of important concern to the health of man and animals. Some of the probems of mycotoxicoses can be minimized by the cooperative research of mycologists, chemists, medical scientists etc. Some problem areas require improved processed, storage and enforcement of quality control programs to insure consumer protection from exposure to contaminated foodstuffs.

摘　　要

1963年에 麥類붉은 곰팡이병(*Gibberella zeae*)이 大發生하여 莫甚한 收量減少를 가져왔고, 병든 穀類를 먹은 人畜에 中毒症을 일으켜서 큰 社會問題를 일으켰다. 本 綜說은 當時 農林部의 主管으로 植物病理學, 醫學, 獸醫學을 專功하는 對策委員들이 硏究한 內容을 中心으로 그 大要를 紹介한 것이다.

그리고 眞菌으로 因한 穀類 및 여러 食品의 變質과 眞菌毒素에 대한 關心이 學術的인 面으로나 人畜의 保健面으로 提高되고 있음에 비추어 最近에 우리나라에서 硏究한 쌀을 變質시키는 貯藏菌 및 메주 其他 食品에서 分離한 곰팡이와 「아플라톡신」 分泌에 關해서 그現況을 收錄하였다.

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