Kernel : Maize kernel of 80~119 days were sampling. Infection inside the kernel of 0.5 % was first found in 112 days growing stage. The infection increases after 119 days. Surface contamination of A. flavus was first found in immature kernel of 88 days and the population increases as maize become more mature.

Silk seems to have important role of the infection of A. flavus in the field. Silk contamination and infection may lead A. flavus to contaminate kernels inside the cob, remain on the surface and ready to infect the kernel in a favorable condition.

Studies on the population of A. flavus and aflatoxin content in trade maize

Studies on A. flavus infection and aflatoxin contamination during sun dry in the middleman scale and laboratory scale

Code No. III-1-(3), 3-1-2

Thai maize is mainly cultivated and harvested in rainy season in that time weather condition is unstable and occasionally thunder storm attack the maize production area. Drying of maize is usually accomplished by sun dry in Thailand. Therefore, once rain comes drying has to be stopped and undried maize is heaped up covered with tarpaulin to wait recover the weather condition. If the maize contains high moisture, mold might be grown not only surface but also inside of maize kernel even an overnight. So far, it is not well known that relations between sun dry and A. flavus infection, or influence of stoppage of drying for aflatoxin contamination owing to rain. Hence, Microbe group has planned to investigate the actual condition of sun dry of maize in middleman level and effect of various thickness of maize by laboratory scale on the drying floor in the sun.

Material and method

Investigations have been carried out four times in 1990 maize season. A middleman, Koktoom, Phraphutthabat in Lopburi, was selected for the large scale drying experiment. Small experiment, laboratory scale, has been carried in Phraphtthabat Field Crops Experiment Station at the same time of large scale experiment.

1. First experiment was carried out on 18th September. Maize, Ciba-Hybrid, was harvested at a farmer (A) on 17th and heaped up in the farmers compound an overnight. Next morning, maize was shelled and transported to the middlemans drying yard and basketball court in Phraphuttabat FCES.

1-1. Middleman scale

One ton of shelled maize were spread on the concrete floor by usual manner using rake attached on tractor. If moisture content did not reach to 14 %, maize is collected by hand scraper or a bulldozer, and heap up covered with tarpaulin on the floor. Drying was continued again in next day.

During sun dry following items were observed in every hour. ① sampling time, ② thickness of maize, ③ air temperature, ④ surface temperature of maize, ⑤ bottomm temperature of maize, ⑥ temperature of the concrete floor, ⑦ weather condition of observing time, ⑧ moisture content of maize (by Steinlite moisture meter). Air born fungi was captured on PDA media using SAS air sampler in appropriate time when starting and stoppage of drying. Maize sample for A. flavus check was extracted every starting and stoppage of drying. Immediately after sampling, agar test for the maize kernels was made using PDA media in the laboratory of Phraphuthabat FCES, and incubated at 28 ℃ for 3~5 days. Count number of fungi individually, for instance, A. flavus, A. niger, Penicillium sp., Botryodiplodia and other fungi. Rest sample was dried to 14 % for aflatoxin analysis.

1-2. Laboratory scale

Two hundred Kg of shelled maize was transported to the basketball court in the station on 18th and started dry from noon. Drying was carried out in wooden frame 2 × 2 m × 5 cm, varing thickness 1, 3 and 4 cm. Investigation items were recorded as same as in the large scale experiment.

2. Both second large scale and laboratory scale experiments have been carried out on 19th September. Maize, Pioneer-Hybrid, was harvested at farmer (B) on 24th, and shelled in the next morning. The experimental conditions were same as in the first experiment.

3. Both third large scale and laboratory scale experiments have been carried out on 24th September. Maize, Ciba-Hybrid, was harvested at farmer (A) on 24th, and shelled in the next morning. The experimental conditions were same as in the first experiment.

4. Both fourth large scale and laboratory scale experiments have been carried out on 27th September. Maize, Pioneer-Hybrid, was harvested at farmer (B) on 26th, and shelled in the next morning. The experimental conditions were same as in the first experiment.

Result and discussion

1. Middlemen scale
Data is now under processed.

2. Laboratory scale
Four experiments have been carried on 18th, 19th, 25th and 26th September. Changes of moisture content (drying curve) are shown in Figure 1~4 respectively. In the figures, total time indicating the accumulative time required during drying. Real time express actual time from start to stop in a day and lowest is date of drying experiment.

2-1. In the first experiment, harvesting of ear maize, Ciba-Hybrid variety, was harvested on 17th and shelled in the next morning. Drying started at noon. It was good weather condition from noon to evening during drying experiment. Drying curves and infection ratio of A. flavus was shown in Figure 1. Initial moisture content was 27 %. It took 2 days, only 12 hours required till moisture content reaching to 14 % in 1 cm thickness. In 3 cm thickness, it took 3 days, about 19 hours required till 14 % of moisture content. In case of 4 cm thickness, it took 3 days, totaly 23 hours required till 14 % moisture content. Initial infection ratio by A. flavus was 30 %, but there was no increase infection by A. flavus in 1, 3 and 4 cm of thickness during drying.

2-2. In the second experiment, harvesting ear maize, Pioneer-Hybrid variety, was harvested on 18th and shelled in same day. Shelled maize was put in gunny bags and kept overnight at the farmers compound. Next morning gunny bags were transported to the Phraphthabat FCES and started drying experiment at 10 AM as same manner as above. The weather condition was very good during experiment. Drying curves and infection ratio of A. flavus were shown in Fig. 2. Initial moisture content was 23 %. In 1 cm it took 5 hours required till 14 % of moisture content. In 3 cm thickness, it took 2 days, about 10 hours required till 14 % of moisture content. In 4 cm thickness, it took 2 days, totally only 12 hours till 14 % of moisture content. Initial infection ratio by A. flavus was 15 %, but there was no clear changes was found among the 3 thickness during drying.

2-3. In the third experiment, harvesting ear maize, Ciba-Hybrid, was harvested on 24th and shelled at farmers compound in the next morning, then transported to Phraphthabat FCES and started drying experiment at 12:10 PM as same manner as the above. The weather condition was very bad during experiment. Initial moisture content was 23~24 %. Drying curves and infection ratio

of A. flavus were shown in Fig. 3. There were 3 times rain during experiment and it took 5~6 days to complete dry. In 1 cm thickness, it took 5 days, about 28 hours required till 14 % of moisture content due to 2 times thunder shower. When drying was stopped by rain, the maize samples were collected and kept in gunny bags overnight. In 3 cm thickness, it took 5 days, 30 hours required till 14 % of moisture content due to 2 times thunder shower. In 4 cm thickness, it took 6 days, totally 36 hours due to 3 times rain. Intial infection by A. flavus was 0 %. Inspite of 3 times rain during experiment, low infection increased were observed in all 3 thickness.

2-4. In the forth experiment, harvesting ear maizePioneer-Hybrid varity, was harvested on 26th and shelled in the next morning at farmers compound, then transported Phraphthabat FCES and started drying experiment at 14:30 PM as same manner as above. The weather condition was very bad in this experiment too. Initial moisture content was 28~29 %. Drying curves and infection ratio of A. flavus were shown in Fig. 4. There were 2 times thunder shower during experiment. in 1 cm thickness, it took 4 days, about 14 hours required till 14 % of moisture content due to one time rain. In 3 cm thickness, it took 4 days, about 17 hours required till 14 % of moisture content. In 4 cm thickness, it took 4 days, totally 23 hours required till 14 % of moisture content. Initial infection of A flavus in 1 cm thickness was 18 %, but it was icreased to 35 % after 16 hours, during 3 days. In 3 cm thickness, initial infection was 30 %, but but only 5 % increased after 19 hours, during 4 days. In 4 cm thickness, initial infection was 17 %, but it was increased to 65 % after 19 hours, during 4 days.

It is observed that there is no increase A. flavus infection in first and second drying experiments because of good weather condition. However, very low increase in infection was observed in 3 cm thickness thickness. It might be due to low initial infection of A. flavus and rather low moisture content. In 4 cm thickness, high infection, 65 %, was observed after 19 hours. It must be due to high initial moisture content and required long time till 14 % of moisture content.

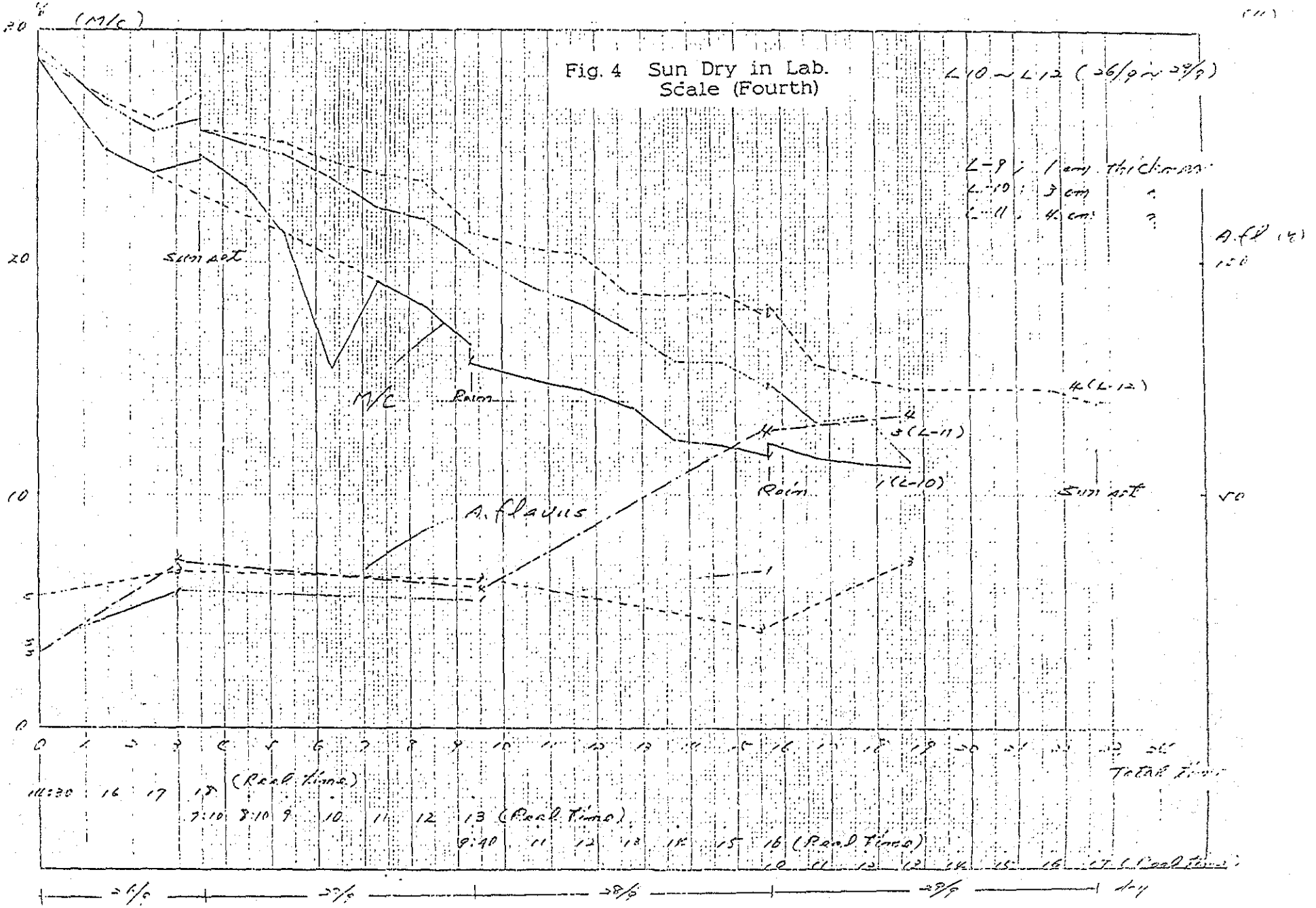
Fig. 4 Sun Dry in Lab.
Scale (Fourth)
(M/C)
Sun dry
M/C
Rain
A. flavus
Rain
Sun dry
A.fl (%)
Total Time

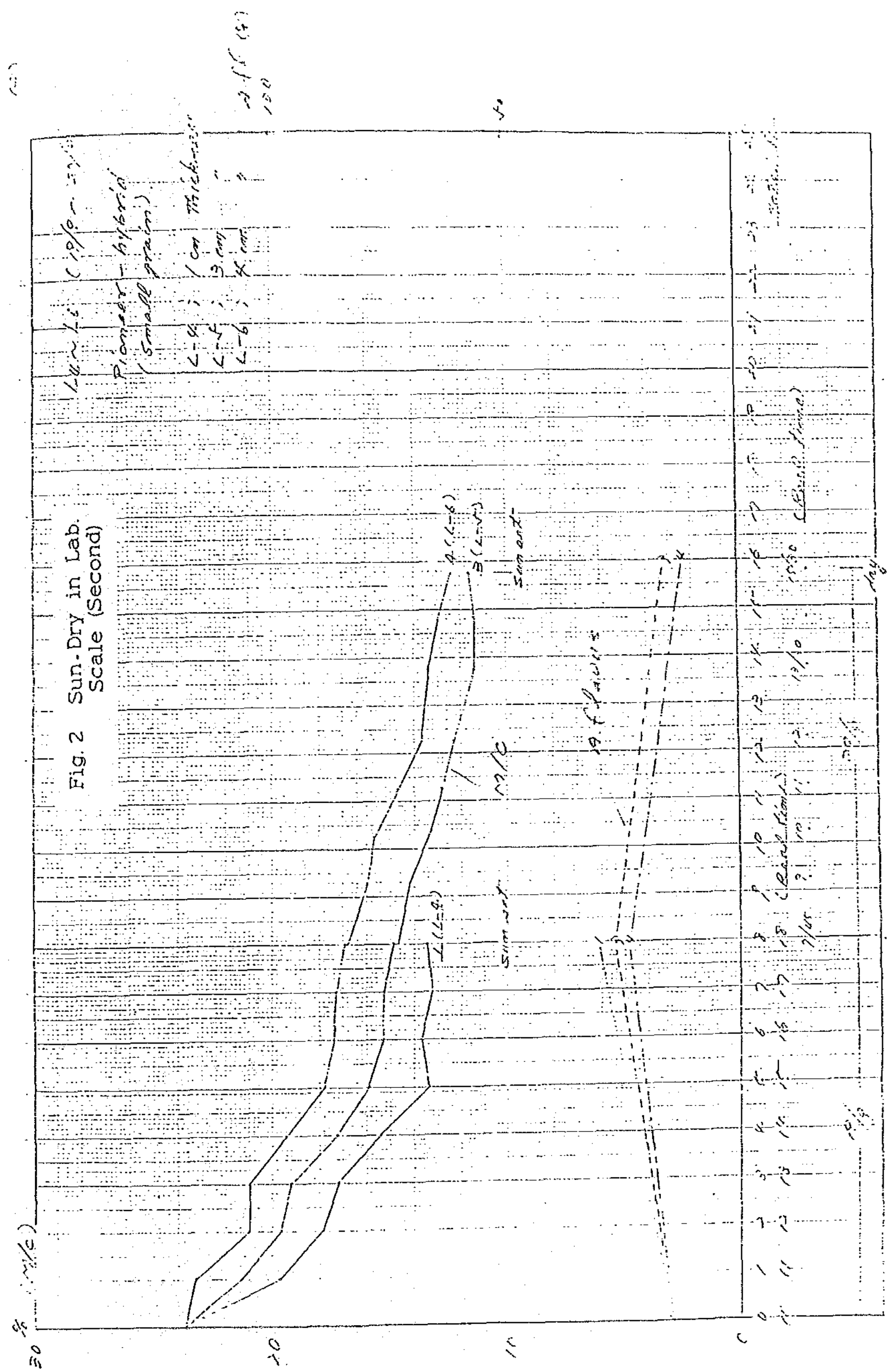

Fig. 2 Sun-Dry in Lab. Scale (Second)

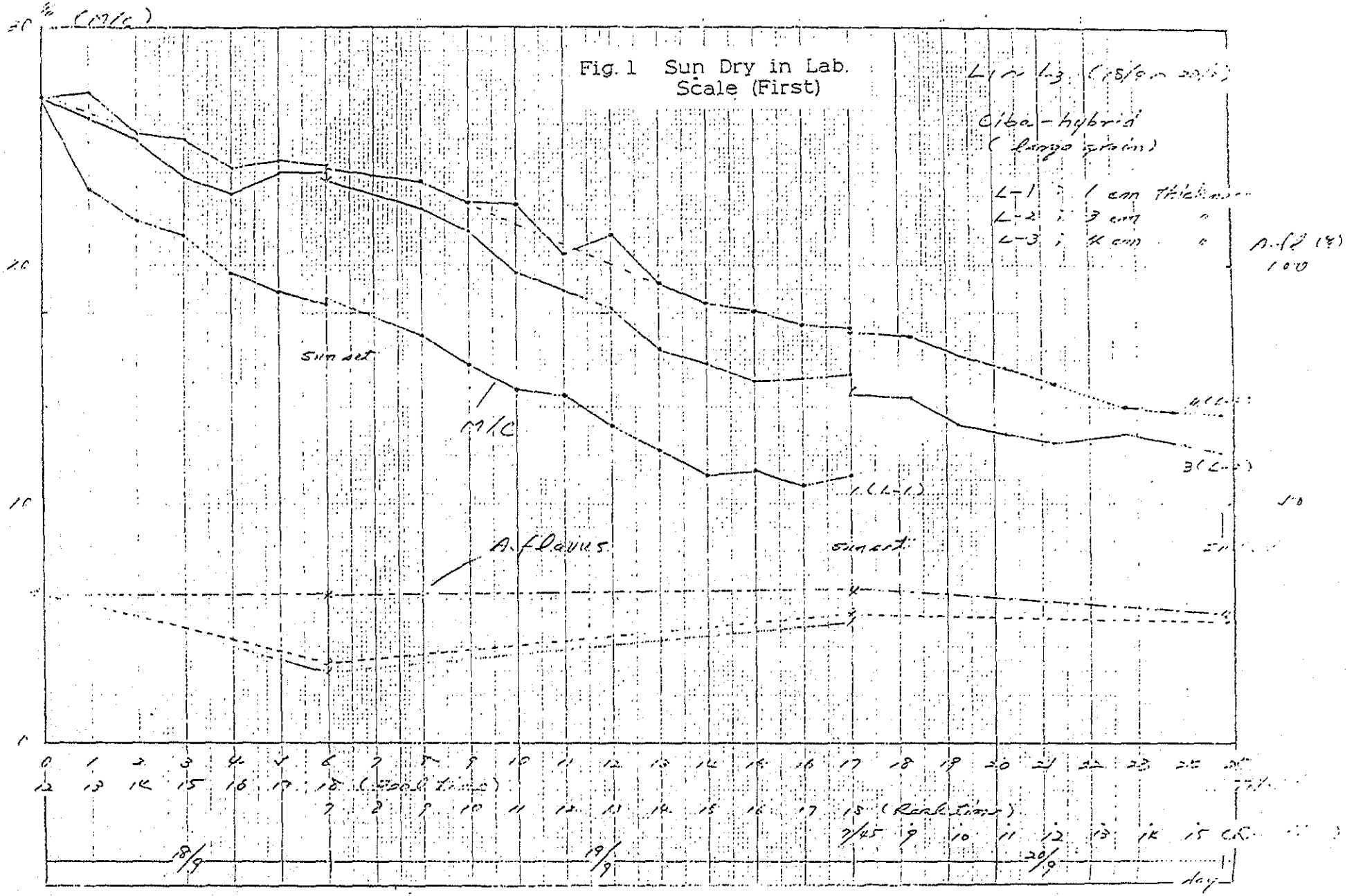
Fig. 1 Sun Dry in Lab.
Scale (First)
Ciba-hybrid
sunset
A. flavus
sunset
day

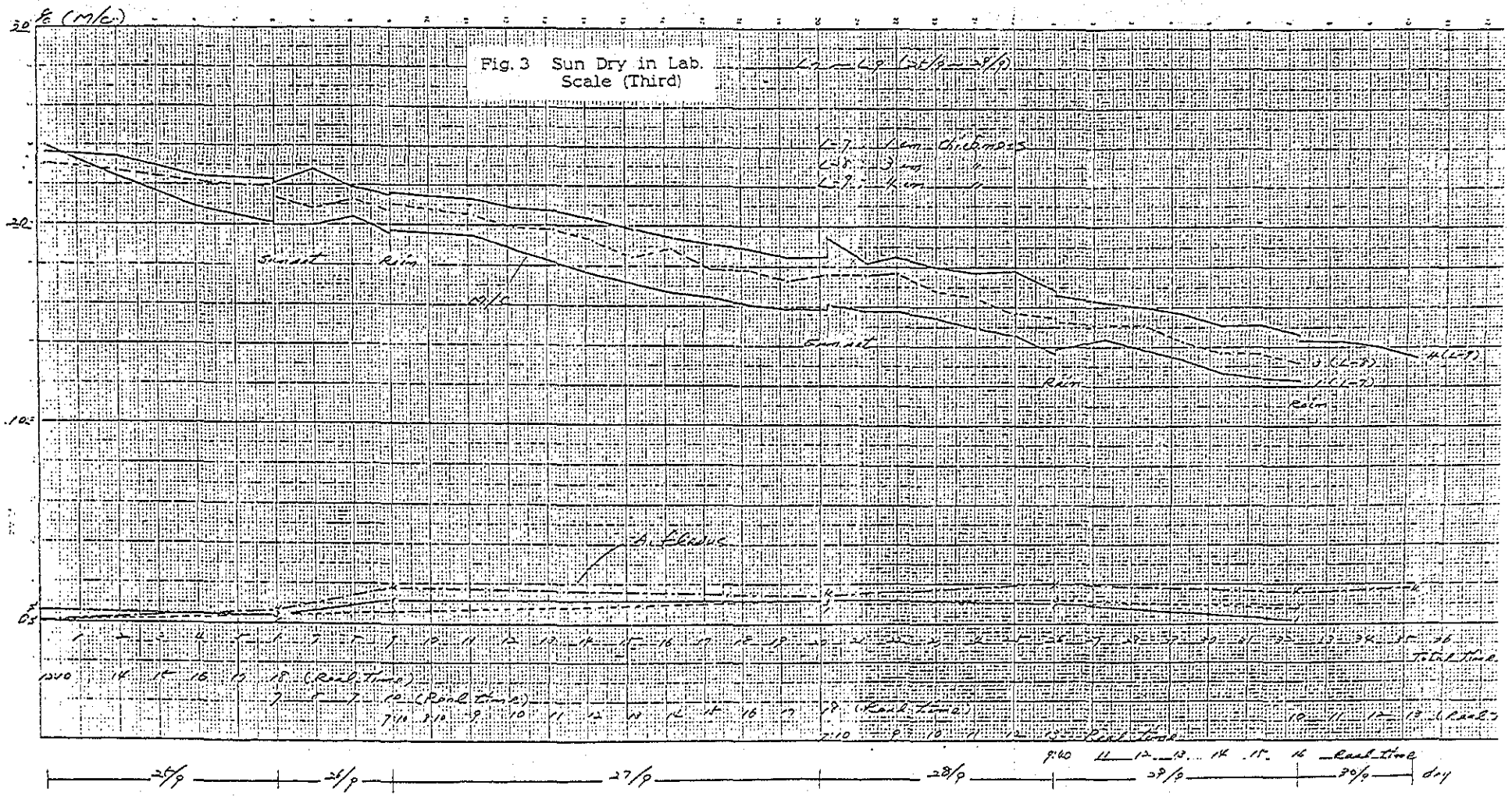
Fig. 3 Sun Dry in Lab.
Scale (Third)
Rain
Rain
Rain
Rain
Real time
Real time
Real time
Real time
Real time
dry

Identification of aflatoxin producing ability of A. flavus by coconut powder agar and coconut cream agar

Code No. III-1-(3), 3-2-1

Coconut powder agar and coconut cream agar were tried to identify the aflatoxin producing ability of A. flavus isolated from maize, soil and air.

Method

160 gm of commercial coconut powder and coconut cream were dissolved in 1 lit. of distilled water separately [CPA(-) and CCA(-)].

In addition to, 4 gm of Na-desoxycholate was dissolved in the another half of same solutions individualy [CPA(+) and CCA(+)]. Sterilize the agar solutions by autoclave at 121 ℃ for 15 min. then spread into the petri dishes. Inoculate A. flavus spores in one or three point on the agar with hook. Incubate at 25 ℃ for 3~4 days. Measure the diameter of colony surface under the room light. At the same time, reverse side of petri dish was exposured under UV light, 365 nm, and observe the fluorescent color and also measure the diameter of colony.

Result and discussion

Selected 12 A. flavus strains known aflatoxin producing ability determined by TLC or HPLC method on GY culture fluid were used in this experiment. Results are shown in Table 1. ① shows the aflatoxin B1 content in the culture fluid determined by TLC and ② shows the aflatoxin B1 and G1 content in the culture fluid. Figures under the agars shows the diameter of colonies, upper one is diameter of colony under the room light and bottom one is diameter of reverse side of colony under the UV light. Right column mentioned the characteristics of fluorescent color of reverse side.

Non aflatoxin producing type of A. flavus, A2, A3 and S4, and low producing type, M63 did not occur any fluorescent illuminant and could not observe shape of colony under UV light. Aflatoxin producing type, such as M8, S10, S50, S43, S69 and S57 occurred fluorescent illuminant more or less under the UV light. However, aflatoxin producing type, M56 and I9 did not show any fluoresent illuminant under the UV exceptionally.

Na-desoxycholate added in the media as growth inhibitor for fungi other than A. flavus, CPA(+) and CPA(+), has effect of control growth for A. flavus. Colony of A. flavus grown in the above media did not show fluorescent illuminant. It seems to have higher correlation between non Na-desoxycholate agar, CPA(-) and CCA(-), and aflatoxin producing ability.

Some differences were observed influorescentcolor between A. flavus slants, especially S50 which produce aflatoxin G1 showed bright violet fluorescent color.

This method must be useful for detection of aflatoxin producing ability of A. flavus, however, it is necessary to continue the additional experiment.

Table 1. Aflatoxin Producing Ability Test by Coconut Agar Medium

Isolate	CPA (+)	CPA (-)	CCA (+)	CCA (-)	
A2	1.7 cm*		1.0	2.0	
① B_1 = N D	N D**	-	N D	N D	
② B_1 = N D					
A3	1.9	2.5	1.0	2.2	
① B_1 = N D	N D	N D	N D	N D	
② B_1 = N D					
M8	1.9	3.1	1.1	2.0	
① B_1 = 201 ppb	2.0	2.0	N D	2.5	Blight
② B_1 = 2027					
M56	2.1	3.2	0.9	2.2	
① B_1 = 203	N D	N D	N D	N D	Yellow
② B_1 = 9513					
M68	1.6		1.1	2.3	
① B_1 = 9	N D	-	N D	N D	
S4	1.8	2.9	1.0	2.0	
① B_1 = N D	N D	N D	N D	N D	
② B_1 = N D					
S10	2.0	3.0	1.2	2.0	
① B_1 = 50	N D	2.0	N D	1.8	Blight
② B_1 = 1066					
S50	2.1		1.2	2.2	
① B_1 = 280					Blight
② B_1 = 703	2.0	-	1.0	3.0	very
② G_1 = 9392					strong

S69	2.1	3.2	1.1	2.0
① B_1= 67	±	±	±	2.1
② B_1= 1269				
S87	2.2	3.0	0.7	2.1
① B_1= N D	±	2.7	N D	N D
② B_1= 1528				
I9	1.8	3.0	1.2	2.1
① B_1= 299				
② B_1= 718	N D	N D	N D	N D
② G_1= 6				
S43	1.8	3.0	0.9	2.2
① B_1= 6	N D	2.2	N D	2.3
② B_1= 409				

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* Colony diameter (Average of 3 colonies)

** Fluorescent zone diameter

① : AFB_1 production (ppb) TLC

② : AFB_1 (or AFG_1) production (ppb) HPLC

ND : Not Detected

CPA (+) : Coconut powder agar (16% W/V) + 0.8% Na-desoxycholate

CPA (-) : Coconut powder agar (16% W/V)

CCA (+) : Coconut cream agar (16% W/V) + 0.8% Na-desoxycholate

CCA (-) : Coconut cream agar (16% W/V)

Culture : 25 ℃, 3 days

Coconut powder : Chao Thai Brand

Coconut cream powder : Korn Thai Co. Ltd.

(Ingredient : Natural coconut, Dextrin, No additives, No presenatives)

Coconut cream : HAWAII coconut cream, (The Thai Dairy Industry Co. Ltd.)

(No artificial flavering, coloring and preservatives)

Studies on the population of A. flavus and aflatoxin content in ear maize stored with and without husk in the farmer's cribs

Code No. III-1-(3), 3-2-2
Related Code No. III-1-1-(1)

This subject was jointly carried out with Agronomy section.

To know the effect of storing condition in the farmer's cribs for ear maize with and without husk, Microbe group has been studied on the population of A. flavus and aflatoxin content during storing. Agronomy group has been carried out on measuring moisture content of maize, temperature of inside pile of ear, temperature and humidity in the cribs. Also, Agronomy group measured the carbon dioxide gas concentration inside pile of ear.

Material and method

Maize cultivated and harvested in Phraphuttabat, Lopburi, was purchased, and stored in 3 farmer's cribs (A, B and C) with and without husk. Each cribs were divided to rooms in which one for ear with husk and the other one for with husk. Sampling has been made every week, toally continued ten weeks. Samples were brought to MQIRC in Bangkhen and examined on infection and contamination by fungi and analyzed aflatoxin content by TLC method.

Results and discussion

1) Comparison of fungus infection in maize with and without husk.

Both maize kernels, surface sterilized (with 1 % NaOCl) and no treatment, were cultivated in PDA media at 28 ℃, for 3~5 days.

Percentage of infected kernels by fungus was mentioned in Table 1~3. It seems to be different infection ratio by the type of cribs. A. flavus infection in A-crib showed 0~6 % in maize with husk (+) and 0~30 % in without husk (-). B-crib showed 0~8 % in maize with husk and 0~29 % in maize without husk by A. flavus infection. C-crib showed 0~2 % in maize with husk and 0~8 % in without husk by A. flavus infection. Commonly 3 cribs showed lower infection by A. flavus in maize with husk than that of without husk.

However, there was no clear relations between type of crib and storing period. Size of crib, height of floor from the ground, aeration ratio in the crib, volume and height of maize in the pile, distance from the top of pile to roof etc. may affect on the infection ratio. The appearance of maize kernels with husk was worth than without husk.

Among the other fungi, Botryodiplodia sp., Rhizopus sp., Curvularia sp., A. terreus, A. glaucus, other Penicillium sp. and Aspergillus sp., and also yeast were observed.

2) Comparison of aflatoxin content in maize with and without husk

Results are shown in Table 4.

Clear differences were observed in aflatoxin content between maize with and without husk. In case of A-crib, aflatoxin content of B1 and B2 in Maize with husk were within safety level even in 2 weeks, but high B1 content was found in maize without husk. In B-crib, maize with husk showed still low level of aflatoxin contamination even in 3 weeks, but high contamination was found from 2 weeks in maize without husk. In C-crib, maize with husk was in safety level in 2 weeks. Detected aflatoxins were almost B1 and B2 except in Crib-A at 2 weeks sample in which G1 was found. After 4 weeks, aflatoxin contents were remarkably

increased. Some differences were observed in starting time of aflatoxin contamination, however, it may be due to the crib structure.

TABLE 1 Changes infection and contamination by fungus in maize during storing in Crib - A

Storage time	Code	A. flavus		A. niger		F.moniliforme		P.funiculosum		other fungi		Note
		no	wash	no	wash	no	wash	no	wash	no	wash	
start	+H	98	4	27	1	-	5	-	5	1	3	Botryodiplodia sp.
	-H	47	1	99	3	-	11	5	8	2	2	Botryodiplodia sp. Rhizopus sp.
1 week	+H											(no sample)
	-H											
2 weeks	+H	46	-	100	4	2	-	-	6	4	10	Rhizopus sp. Botryodiplodia sp.
	-H	6	30	30	24	-	14	-	38	8	6	Curvularia sp. A.Terreus Rhizopus sp. Botryodiplodia sp.
3 weeks	+H	58	2	100	2	-	-	-	12	-	14	Rhizopus sp. Botryodiplodia sp. Curvularia sp.
	-H	58	8	90	12	-	9	-	40	2	8	Botryodiplodia sp. Rhizopus sp. p.citrinum
4 weeks	+H	48	-	100	2	-	14	50	40	-	6	Rhizopus sp. Botryodiplodia sp.Curvularia sp.
	-H	68	6	100	4	-	18	86	66	-	4	Rhizopus sp. A.terreus P.citrinum

Storage line	Code	A. flavus		A. niger		F.moniliforme		P.funicilosum		other fungi		Note
		no	wash	no	wash	no	wash	no	wash	no	wash	
5 weeks	+H	2	4	100	6	-	4	52	22	6	12	Botryodiplodia sp.
	-H	60	2	100	8	-	14	66	64	2	12	Botryodiplodia sp. A.terreus A.glaucus
6 weeks	+H	4	-	100	2	-	-	34	14	10	10	Botryodiplodia sp.
	-H	4	-	100	4	-	8	32	38	8	10	Botryodiplodia sp. P.citrinum
7 weeks	+H	4	2	100	2	-	-	-	18	78	12	Botryodiplodia sp. P.sp. Rhizopus sp. P.citrinum
	-H	12	2	100	8	-	8	-	32	102	10	P.sp. Rhizopus sp. Botryodiplodia sp.
8 weeks	+H	24	2	100	8	-	2	-	4	26	10	Botryodiplodia sp. P.sp.
	-H	10	-	100	14	-	12	-	46	36	24	Rhizopus sp. P.citrinum Botryodiplodia sp. P.sp. yeast A.terreus A.glaucus
9 weeks	+H	-	-	100	4	-	4	-	16	34	10	Botryodiplodia sp. P.sp.
	-H	14	4	100	-	-	10	-	22	24	12	Botryodiplodia sp. P.sp., Bacteria
10weeks	+H	78	6	98	-	-	26	-	16	30	28	A.sp. Alternaria sp. Rhizopus sp. P.sp. Botryodiplodia sp. A.terreus
	-H	64	14	100	4	-	20	-	26	44	12	P. citrinum Rhizopus sp. P.sp. Botryodiplodia sp. A.terreus

TABLE 2 Changes infection and contamination by fungus in maize during storing in Crib - B

Storage time	Code	A flavus		A niger		F.moniliforme		P.funiculosum		other fungi		Note
		no	wash	no	wash	no	wash	no	wash	no	wash	
start	+H	77	-	29	-	17	15	18	-	-	-	Dacteria Neurospora sp.
	-H	60	-	56	-	3	12	49	1	1	4	Alternaria sp. A.terreus bacteria Neurospora Rhizopus sp. Botryodiplodia sp.
1 week	+H	54	4	100	2	-	12	2	8	4	4	Rhizopus sp. Botryodiplodia sp.
	-H	16	10	98	4	-	24	16	10	4	46	Curvularia sp. A.terreus Rhizopus sp. Botryodiplodia sp.
2 weeks	+H	70	8	74	10	-	24	12	40	10	12	P.citrinum Botryodiplodia sp. Rhizopus sp. Curvularia sp.
	-H	70	4	100	6	-	24	4	54	6	10	Botryodiplodia sp. Curvularia sp. A.terreus
3 weeks	+H	62	2	100	-	-	4	40	16	-	26	Rhizopus sp. Curvularia sp. A.terreus A.sp.
	-H	72	-	100	2	-	36	32	50	-	8	Rhizopus sp. Curvularia sp. A.terreus
4 weeks	+H	24	2	100	-	-	-	46	32	-	22	Botryodiplodia sp. A.terreus A.sp.
	-H	12	-	100	2	-	16	50	26	2	24	Curvularia sp. Botryodiplodia sp. A.terreus P.citrinum

Storage time	Code	A.flavus		A.niger		F.moniliforme		P.funiculosum		other fungi		Note
		no	wash	no	wash	no	wash	no	wash	no	wash	
5 weeks	+H	2	2	100	4	-	4	26	18	22	42	A.terreus Botryodiplodia sp. Rhizopus sp. P.sp. A.sp.
	-H	42	28	100	6	-	8	40	18	4	10	Botryodiplodia sp. Rhizopus sp.
6 weeks	+H	62	2	100	-	-	-	-	6	28	14	A.terreus P.sp. Rhizopus sp. Botryodiplodia sp. Rhizopus sp.
	-H	32	6	100	2	-	36	-	34	88	12	P.sp. Rhizopus sp. Botryodiplodia sp. Rhizopus sp.
7 weeks	+H	20	2	100	-	-	-	-	-	24	34	Rhizopus sp. P.sp. Yeast A.glaucus.A.terreus A.sp.
	-H	46	2	100	4	-	14	-	26	20	13	Botryodiplodia sp. P.sp. A.glaucus
8 weeks	+H	88	4	100	-	-	4	-	4	12	38	P.citrinum A.sp. Botryodiplodia sp. P.sp. Rhizopus sp. A.terreus
	-H	48	4	100	6	-	28	-	24	14	15	P.sp. A.terreus
9 weeks	+H	4	2	100	4	-	-	-	10	44	12	Rhizopus sp. P.sp. Botryodiplodia sp. A.terreus
	-H	8	8	100	16	-	18	-	50	40	42	P.sp. Yeast P.citrinum
10weeks	+H											[no sample]
	-H											

TABLE 3 Changes infection and contamination by fungus in maize during storing in Crib-C

Storage time	Code	A.flavus		A.niger		F.moniliforme		P.funiculosum		other fungi		Note
		no	wash	no	wash	no	wash	no	wash	no	wash	
Start	+H	28	-	100	16	-	26	3	6	-	-	Rhizopus sp.
	-H	94	8	76	4	-	16	4	6	8	4	Rhizopus sp. Botryodiplodia sp.
1 week	+H	56	-	86	2	-	8	14	10	10	10	Botryodiplodia sp. Rhizopus sp.
	-H	98	6	98	4	-	36	-	14	6	6	Botryodiplodia sp. Rhizopus sp.
2 weeks	+H	70	-	98	-	-	-	-	10	10	10	Botryodiplodia sp. Rhizopus sp.
	-H	92	8	100	2	4	36	18	18	6	20	P.citrinum Rhizopus sp. Botryodiplodia sp.
3 weeks	+H	68	-	100	-	-	10	32	32	2	10	Botryodiplodia sp. Rhizopus sp. A.glaucus A.terreus
	-H	46	4	100	-	-	40	32	28	-	6	Botryodiplodia sp. Curvularia sp.
4 weeks	+H	98	-	84	-	-	2	16	30	10	10	Botryodiplodia sp.
	-H	86	-	100	2	-	16	24	26	12	10	Botryodiplodia sp. A.terreus

Storage time	Code	A.flavus		A.niger		F.moniliforme		P.funiculosum		other fungi		NOTE
		no	wash	no	wash	no	wash	no	wash	no	wash	
5 weeks	+H	98	2	92	-	-	-	10	26	50	10	Botryodipoldia sp. Rhizopus sp. P.sp.
	-H	78	-	100	4	-	8	4	28	36	12	Botryodiplodia sp. P.sp.
6 weeks	+H	100	-	88	-	-	4	10	13	8	10	Botryodiplodia sp. Yeast
	-H	100	2	98	2	-	16	24	38	10	10	Botryodipoldia sp.
7 weeks	+H	-	-	100	-	-	-	32	18	6	12	Botryodiplodia sp. P.citrinun
	-H	100	6	54	-	-	-	14	18	8	12	Botryodiplodia sp. Bacteria Yeast P. Citrinum
8 weeks	+H	42	-	100	-	-	4	10	24	10	10	Botryodipoldia sp. Rhizopus sp.
	-H	30	4	100	-	-	6	10	36	10	18	Botryodiplodia sp. F.sp.
9 weeks	+H	44	-	100	-	-	-	18	12	10	10	Botryodiplodia sp.
	-H	28	6	100	4	-	-	24	20	4	12	Botryodiplodia sp. Rhizopus sp. P. citrinum
10weeks	+H											[no sample]
	-H											

TABLE 4 Changes aflatoxin content in maize with and without husk during storing Crib - A ~ C,

Storage time	1st Storage (A) [Farmer No.2]				2nd Storage (B) [Farmer No.1]				3rd Storage (C) [Farmer No.3]				NOTE
	+H		-H		+H		-H		+H		-H		
	B_1	B_2	B_1	B_2	B_1	B_2	B_1	B_2	B_1	B_2	B_1	B_2	
Start	ND		ND		ND		3	-	ND		3	-	
1 week	/	/	/	/	1	-	3	-	21	1	22	1	
2 weeks	ND		49	2	6	-	38	2	18	-	59	4	(A-H) G_1=6
3 weeks	58	4	134	7	3	-	138	12	112	9	219	18	
4 weeks	60	3	279	17	33	2	90	4	266	24	407	30	
5 weeks	140	7	909	43	72	6	149	13	73	5	304	22	
6 weeks	325	24	294	18	107	9	120	8	109	15	234	24	
7 weeks	84	6	551	36	27	-	287	23	15	-	553	34	
8 weeks	22	2	329	20	28	2	320	31	22	-	253	15	
9 weeks	63	5	254	12	337	32	524	34	476	33	499	32	
10 weeks	280	13	138	8	/	/	/	/	/	/	/	/	

/ = no sample

The simple and rapid method for detection of aflatoxin in maize

Code No. III-2-1, 4-1-1

Many report on analytical method of mycotoxins have been presented since aflatoxin, metabolized products of Aspergillus flavus, was found in England in 1960s.

These method for aflatoxins, however, have required not only much time to detect but also a high levelled technique and expensive instrument.

Therefore, it is necessary to develop the simple, rapid and inexpensive method for detection of quality inspection and control.

Method

Apparatus

1. Mini-chromatographic column
   Glass tube, 15 cm × 3 mm, × 4 mm and × 5 mm ID
2. UV light (365 nm)

Reagents

1. Florisil for mini-column chromatography
   Florisil, 100 mesh
2. Silica gel for mini-column chromatography
   Silica gel (merck)
3. Alluminum oxide (reagent grade) for mini-column chromatography
4. Extraction solution
   $CHCl_3$:MeOH (97:3)
5. Aflatoxin standard

Extraction and chromatography

Weigh 50 gm of ground (using 1 mm size mesh) maize sample into 300 ml beaker, add 100 ml $CHCl_3$-MeOH (97:3), extract 5 min. using Ultra-sonicator. Immediately after the extraction, put a thimble filter paper in beaker for filtration.
Insert the mini-column to the thimble filter paper in the extract solution and develop to the top. After developing observe under UV light for fluoresent band.

Experiment 1.

Investigation of various kinds and volumes of absorbent, and various inside diameter (ID) of column.

1) Preparation of mini-column for aflatoxin as follow:
Stuff with absorbent cotton in bottom of glass column in 5~7 mm thicness (using 3 mm, 4 mm and 5 mm ID).
Silica gel column: put 1.5 cm or 4 cm in thickness of silica gel, 1 cm of Florisil and 8.5 or 6 cm of silica gel.
Aluminum column: put 1.5 or 4 cm of alumina and 1 cm of Florisil and 8.5 or 6 cm of silica gel.
Before being used, activate the mini-column at 120 ℃ for 30 min. and keep in a desicator.

2) Sample
Each sample spiked 100 ng of aflatoxin B1 were extracted by a blender, shaker and ultra-sonicator, then developed and observed fluorescent band under the UV light. Four mm ID of silica gel and alumina column, 1.5 cm of silica gel and 4 cm of alumina layer in the bottom of column were chosen for the following experiment.

Experiment 2.
Investigation of detection limit
1) Preparation of spiked sample of maize as follows:

				Bl 1 ug/ml
①	5 ppb	50 gm +	250 ng	0.25 ml
②	10		500	0.5
③	20		1000	1.0
④	30		1500	1.5
⑤	40		2000	2.0
⑥	50		2500	2.5
⑦	100		5000	5.0

2) Mini-column
1.5 cm of silica gel layer (3, 4 and 5 cm ID) and 4 cm alumina layer (3, 4 and 5 cm ID) columns were prepared.

Investigation of detection limit mixed aflatoxin
Preparation of spiked sample of maize as follows:

			Total AF
①	Bl only	5 ng/g	5 ng
②		10	10
③		20	20
④		30	30
⑤	Bl & B2	5, 0.5 each	5.5
⑥		10, 1	11
⑦		20, 2	22
⑧		30, 3	33
⑨	Bl, B2 & Gl, G2	5, 0.5 each	11
⑩		10, 1	22
⑪		20, 2	44
⑫		30, 3	66

Results and discussion

Result of detection limit are shown in Table 1

Table 1. Detection limit of aflatoxin Bl spiked

		Aflatoxin Bl spiked (ng/g)							
Absorbent	ID mm	0	5	10	20	30	40	50	100
Alumina (4 cm)									
	3	–	+	+	+	++	++	+++	++++
	4	–	+	+	+	++	++	+++	++++
	5	–	+	+	+	++	++	/	/
Silica gel (4 cm)									
	3	–	–	+	+	++	++	+++	++++
	4	–	–	+	+	++	++	+++	++++
	5	–	–	+	+	/	/	/	/

1) Absorbent: Alumina 4cm, Florisil 1 cm and silica gel 6 cm column showed best result for refining of extract and detection limit of aflatoxin. It is confirmed that the above column can detect 10 ng/g (ppb) and if the concentration step is hired to original extract it can detect 5 ng/g also.

2) Fluorescentband of mixed aflatoxin, B1~G2, were appeared on the Florisil layer in one band. Detection limit was almost same as in case of B1 only.
3) To simplify and speed up for the analytical operation, capillary phenomena is utilized to develop of extract from the bottom of column.
As a result, it is recognized that the new method can save time and more simplified.
4) Extraction method using Sonicater can be appllied for lots of samples in one time. Extraction ratio was almost same as other extractor, such as blender or shaker.
5) Chloroform-Methanol (97:3) has more higher extraction ratio than other solvent and it does not bring much out interference substances for the chromatography.
6) Analysis cost may be about 50 Baht (250 Yen) for one sample which about 1/3~1/5 of other analytical method.

Control of A. flavus and aflatoxin contamination of high moisture content of maize in anaerobic condition

Code No. III-1-(3), 5-1-1

Thai maize is mainly cultivated and harvested in rainy season. Moisture content of maize just harvested is usually ranging from 25 to 35 %. Drying of maize is generally accomplished in the sun at the middlemen drying yard. If middlamen accepted the fresh shelled maize beyond their drying capacity, drying will not be able to achieved in a short time and the maize might be contaminated by mold. To avoid such incidence, several treatments have been examined so far.

TARC and microbe group has found the fact that anaerobic condition in plastic bag has an effect to control growth of A. flavus especially high moisture of maize.

In this year, large scale experiment was attempted and examined microbiological aspects. Also, feeding effect of maize stored in plastic bag for chicken is tested in cooperation with Kasetsart university.

Material and method

Maize, Ciba-Hybrid, of 3.5 tons harvested and shelled in Koktoom, Muang district, Lopburi, on 8th October, 1990 was used for the studied. The maturity of maize was 100 days after planting with the moisture content of 36.7 %. Fifty kg of shelled maize were packed in a plastic bag lining in jute bag. As control sample, shelled maize was packed in the gunny bag. Both lots, totally 70 bags were prepared. Bags were transported to Bangkhen immediately and kept in the store room. Sampling was made at 0, 1, 2, 4, days, 1, 2, 3, and 4 weeks for the microbiological experiment and aflatoxin analysis. At the same time, gas composition and temperature inside the bag was investigated.

Rest samples were immediately dried by mechnical dryer in the/ Division for the feeding sample. Engineering

Oxygen and carbon dioxide gas inside bag were measured by Gastech sampler using No. 31 (for oxygen) and 2H (for carbon dioxide) detection tubes. pH of the samples were measured on slurry, homogenized 25 gm of sample with 100 ml of distilled water by Stomacher using Hitachi-Horiba pH meter. Fungi population was examined by DRBC media. Surface was sterilized by 3 % NaOCl solution. Also, both aerobic and anaerobic bacteria, and yeast population were investigated. Aflatoxin content in both plastic bag samples and control samples were analyzed by TLC method.

Results and discussion

1) Temperature of inside bag

Temperature of inside gunny bag was vigourously raising up to maximum 55 ℃ only an overnight whereas maximum temperature in the plastic bag showed only 33 ℃. This may be due to the plastic bag cut off supplying oxygen for respiration by maize kernel.

2) Oxygen and carbon dioxide gas in the plastic bag

Oxygen concentration was dramatically decreased to 1~2 % within 2 hours immediately after packed and maintained same level for 4 weeks. Carbon dioxide gas was increased to maximum 24 % after packed within 12 hours, then gradually decreased to 5% within one week, and maintained almost same level for 4 weeks.

3) Contamination by A. flavus and other fungi

Contamination by total fungi in the gunny bag sample was rapidly increased to maximum 100 % since store started and maintained same level for weeks. Contamination by A. flavus also

sharply increased to maximum 100 % in 2 weeks since store started, and then decreased to 10~20 % level after 3 or 4 weeks.

Total fungi in the samples of plastic bag store gradually decreased and reached to 3 % in one week, and maintained same level for 4 weeks. A. flavus contamination was increased from 0 to 10 % within 2 days, then decreased to 1 % level after 4 days, and maintained for 4 weeks.

4) Bacteria and yeast

On the other hand, population of some lactic acid bacteria and yeast increased during storage may have and some role in inhibiting the growth of A. flavus and other fungi.

5) pH of the gunny bag samples were not much changed during store, ranging from 5.5 to 6.2. On the other hand, pH of plastic bag samples were changed from 5.8 to 4.3 within 2 days, and maintained same level for 4 weeks.

6) Feeding test is now under going.