| No. | Source | Morphology* | AFPA medium* | | | | UV-FL | Immuno assey | TLC gualt. | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| 61 | M-6 | dark green | 40 | OY | - | | ± | >>80 | +++ | |
| | | SC(+) | 41 | OY | - | | ± | | | |
| 62 | M-7 | green | 38 | OY | - | | + | 3 | ND | |
| | | | 42 | OY | - | | + | | | |
| 63 | M-8 | dark green | | | | | | ND | ND | |
| | | | 43 | OYP | - | | + | | | |
| 64 | M-9 | dark green | 35 | OY | - | | + | ND | ND | |
| | | | 37 | OYP | - | | + | | | |
| 65 | M-10 | dark green | 34 | OYD | - | | ± | 6 | ND | |
| | | | 35 | OY | - | | + | | | |
| 66 | M-11 | green | | | - | | | 5 | ND | |
| | | | 42 | OYD | - | | - | | | |
| 67 | M-12 | | 37 | OY | +++ | | ± | 39 | ND | |
| | | | 38 | OYP | +++ | | + | | | |
| 68 | M-13 | dark green | 46 | OYD | - | | - | >>80 | +++ B1 B2 | |
| | | | 33 | OY | - | | - | | | |
| 69 | M-14 | white-green | 39 | OY | +++ | | ± | >>80 | ++ B1 | |
| | | SC(+) | 42 | OY | +++ | | + | | | |
| 70 | M-15 | dark green | 37x39 | OY | +++ | | + | 11 | ND | |
| | | SC(+++) | 38 | OY | +++ | | + | | | |
| 71 | M-16 | dark green | | | | | | >>80 | +++ B1 B2 | |
| | | | 39 | OY | - | | + | | | |
| 72 | M-17 | green | 41 | OY | - | | ± | 17 | ND | |
| | | SC(++) | 40 | OY | - | | + | | | |
| 73 | M-18 | green | 36 | OY | - | | ± | >>80 | +++ B1 B2 | |
| | | SC(+) | 39 | OY | - | | ± | | | |
| 74 | M-19 | green | | | | | | 5 | ND | |
| | | SC(+) | | | | | | | | |
| 75 | M-20 | green | 42 | OY | - | | ± | 3 | ND | |
| | | SC(+) | 39 | OYP | - | | + | | | |
| 76 | M-21 | pale green | 44 | OY | - | | ± | >>80 | +++ B1 B2 | |
| | | SC(++) | 44 | OY | - | | + | | | |
| 77 | M-22 | green | | | | | | >80 | ND | |
| | | | 40 | OY | - | | + | | | |
| 78 | M-23 | green | | | - | | | 10 | ND | |
| | | SC(+) | 39 | OY | - | | + | | | |
| 79 | M-24 | dark green | 9 | Grey Brn | | | | ND | ND | |
| | | | 8 | Grey Brn | | | | | | |
| 80 | M-25 | green | 37 | OY | - | | + | >80 | + | |
| | | | 38 | OYD | - | | + | | | |

| No. | Source | Morphology* | AFPA medium* | | | | UV-FL | Immuno assey | TLC gualt. | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| 81 | M-26 | green | 39 | OY | + | | ± | ND | ND | |
| | | SC(++) | 40 | OY | + | | + | | | |
| 82 | M-27 | green | 42 | OY | - | | ± | ND | ND | |
| | | SC(++) | 39 | OY | - | | + | | | |
| 83 | M-28 | yellow | 43 | OYP | - | | + | >>80 | +++ | |
| | | | 44 | OY | - | | - | | | |
| 84 | M-29 | green | | | | | | 30 | ND | |
| | | SC(+) | 41 | OYP | - | | + | | | |
| 85 | M-30 | green | | | | | | >80 | + | |
| | | SC(+) | 37 | OY | - | | + | | | |
| 86 | M-31 | green | 39 | OYD | - | | ± | ND | ND | |
| | | Sc(+) | 38 | OY | - | | + | | | |
| 87 | M-32 | green | 38 | OY | - | | + | >80 | + | |
| | | | 38 | OY | - | | + | | | |
| 88 | M-33 | brown | 18 | Black | | | | 3 | ND | |
| | | | 16 | Black | | | | | | |
| 89 | M-34 | green | 41 | OY | - | | + | ND | ND | |
| | | SC(+) | 39 | OY | - | | ± | | | |
| 90 | M-35 | green | 38 | OYP | - | | + | >>80 | +++ B1 B2 | |
| | | | 39 | OY | - | | ± | | | |
| 91 | M-36 | green | 36 | OYD | - | | + | ND | ND | |
| | | SC(++) | 31 | OY | - | | + | | | |
| 92 | SP-3C | green | 34 | OYP | + | | + | ND | ND | Phrapht, field, |
| | | | 38 | OYD | + | | - | | | soil Jul/89 |
| 93 | CR-E-1 | green | 39 | OY | - | | + | ND | ND | ChianRai, farmer |
| | | SC(+++) | 37 | OY | - | | + | | | godown, earJul89 |
| 94 | PD-2b | green | | | | | | ND | ND | Pakchong, midman |
| | | SC(+) | 38 | OY | +++ | | + | | | godow, dustJul89 |
| 95 | SP-3b | green | | | | | | ND | ND | Phrapht, field, |
| | | | 38 | OY | +++ | | ± | | | soil Jul89 |
| 96 | SiL-1b | green | 42 | OY | - | | + | >80 | ND | Lopburi, air |
| | | SC(+++) | 38 | OY | - | | + | | | Jul/89 |
| 97 | SP-3a | green | | | | | | ND | ND | Phrapht, soil |
| | | SC(+) | 36 | OY | - | | + | | | Jul/89 |
| 98 | SL-1b | green | | | | | | 11 | ND | Lopburi, soil |
| | | | 39 | OY | - | | + | | | Jul/89 |
| 99 | SL-1a | green | 38 | OY | ++ | - | | ND | ND | Lopburi, soil |
| | | | 38 | OY | ++ | | + | | | Jul/89 |
| 100 | 35a | green | 31 | OY | - | | + | ND | ND | |
| | | SC(+) | 31 | OY | - | | + | | | |

Table 2
AF productivity of A.flavus (121 strains)

| | Toxin Productivity AFB1 (ELISA) | No. of Toxin Producer in 121 strains | No. of Sclerotium formation in 121 strains |
|---|---|---|---|
| | >80 ppb | 46(38%) | 23(19%) |
| | 79~50~21 | 14(12%) | 7(6%) |
| | 20~1 | 26(22%) | 9(7%) |
| | (total) | (86 72%) | (39 32%) |
| | ND | 35(29%) | 18(15%) |

ND : Not detected

| No. | Source | Morphology* | AFPA medium* | | | | UV-FL | Immuno assey | TLC gualt. | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| 101 | SL-2a | green | 43 | OY | - | | + | >80 | ++ B1 | Lopburi, soil |
| | | | 43 | OY | - | | + | | | Jul/89 |
| 102 | SL-1c | green | 36 | OY | - | | + | >80 | ++ B2 | Lopburi, soil |
| | | | | | | | | | | Jul/89 |
| 103 | SIL-1a | green | 37 | OYP | - | | + | >>80 | +++ B1 B2 | Lopburi, air |
| | | | 36x38 | OYP | - | | + | | | Jul/89 |
| 104 | CR-E1b | green | 36 | OYP | - | | + | 8 | ND | ChianRai,farmer |
| | | | 37 | OYP | - | | + | | | godown,earJul89 |
| 105 | PD-1a | pale green | 38 | OYD | - | | ± | ND | ND | Pakchong,midman |
| | | SC(+++) | 36 | OYP | - | | + | | | godown,dustJul8 |
| 106 | PD-1b | green | 36 | OY | - | | + | 3 | ND | " |
| | | SC(+) | 38 | OY | - | | + | | | " |
| 107 | PD-2a | green | 37 | OY | - | | + | ND | ND | " |
| | | SC(+) | 37 | OY | - | | + | | | " |
| 108 | FE-3a | green | 39 | OY | ++ | | ± | >80 | ND | Phrapht.kept in |
| | | SC(+++) | 36 | OY | ++ | | + | | | field,earJul/89 |
| 109 | FE-2b | green | 35 | OY | - | | + | ND | ND | " |
| | | | 37 | OY | +++ | | + | | | " |
| 110 | FE-1b | green | 40 | OYD | - | | - | ND | ND | " |
| | | SC(+) | 40 | OYD | - | | - | | | " |
| 111 | FE-2a | white,pale grn | 41 | OY | +++ | | + | >>80 | +++ B1 B2 | " |
| | | SC(+++) | 43 | OYD | +++ | | - | | | " |
| 112 | FE-1a | white,pale grn | 41 | OYD | - | | - | >>80 | +++ B1 B2 | " |
| | | SC(+) | 43 | OYD | +++ | | - | | | " |
| 113 | FE-3b | green | 34 | OYD | - | | + | 2 | ND | " |
| | | | 37 | OYD | - | | ± | | | " |
| 114 | FE-4a | green | 36 | OYD | +++ | | ± | 38 | ND | Phrapht,kept in |
| | | | 35 | OY | +++ | | + | | | farme,godow,ear |
| 115 | A | pale brown | 38 | OY | | | ± | >80 | +++ B1 B2 | Jul89 |
| | | SC(+++) | 38 | OY | | | - | | | |
| 116 | B | green | 34 | OYD | | | ± | ND | ND | |
| | | SC(+) | 36 | OYD | | | + | | | |
| 117 | C | pale green | 39 | OYD | | | - | >80 | +++ B1 B2 | |
| | | SC(+++) | 39 | OYD | | | - | | | |
| 118 | D | dark green | 24 | YG | | | Contami. | 16 | ND | |
| | | | 27 | YG | | | " | | | |
| 119 | 39C | dark green | 38 | OY | | | + | >80 | + B1 | |
| | | | 29 | OYP | | | + | | | |
| 120 | 34C | yellow brown | 22 | black | | | Contami. | 3 | ND | |
| | | | 23 | black | | | " | | | |
| 121 | 35C | green | 21 | OY | | | + | ND | ND | |
| | | SC(+) | 33 | OY | | | + | | | |

Table 3
AF productivity of A.flavus (in 24 strains)

| | Source | No. of Strain | Place & Month | Toxin Production AFB1 | ( ELISA ) |
|---|---|---|---|---|---|
| | Air | 2 | Phraphuttabat, field | >80 (1) | Soloro (1) |
| | | | (Jul/89) | 22 (1) | |
| | " | 2 | Lopburi, Silo | >80 (2) | " (1) |
| | | | (Jul/89) | | |
| | Soil | 3 | PPB, field (Jul/89) | ND (3) | " (1) |
| | | 4 | Lpburi, field | >80 (2) | " - |
| | | | (Jul/89) | 11 (1) | " - |
| | | | | ND (1) | " - |
| | Dust | 4 | Pakchong, middleman | 3 (1) | " (1) |
| | | | godown (Jul/89) | ND (3) | " (3) |
| | Maize | 2 | Chiang Rai, farmer's | 8 (1) | " - |
| | | | "Ear" Store house | ND (1) | " (1) |
| | | | (Jul/89) | | |
| | Maize | 1 | PPB, kept in farmer | 38 (1) | " - |
| | | | "Ear" Store house | | |
| | | | (Jul/89) | | |
| | Maize | 6 | PPB, "Ear" kept in | >80 (3) | " (3) |
| | | | the field (Jul/89) | 38 (1) | " - |
| | | | | ND (2) | " (1) |
| | Total | 24 | | | |

( ) show the number of strain

Remarks :

>80 as strong
79~21 medium
20~ weak

Toxin producer (in 24 strains)

strong 8 (33%)
medium 3 (13%)
weak 3 (13%)
No toxin 10 (42%)

Sources

A : from Air
B : from Soil
C : from Maize

Morphology

Sc : Sclerotium
Sc (+) : little
(++) : medium
(+++) : many

Color

OY : orange yellow
OYP : pale orange yellow
OYD : dark orange yellow
OYB : brownish orange yellow
Br : brown

Sclero (Sclerotium)

+ : little
++ : medium
+++ : many

UV-FL (check fluoressent of AFPA petri dish under UV light)

- : cannot identified colony
+ : slightly can identified colony
+ : clearly can identified colony

Immuno assey

>>80 : more than 80 ppb, ca. 500~600 ppb
>80 : more than 80 ppb, ca. 250~300 ppb
> 80 : nearly 80 ppb
ND : not detected

TLC qualitative test

+++ : strong toxin producibility
++ : medium toxin productivity
+ : weak toxin productivity
ND : not detected

Aflatoxin content in stored maize by means of Immununoassey
Code No. III-1-(3) (MIC/IV/89)
Code No. III-2-(1)-B

In the preliminary ammonia treatment and storing test of wet maize, interesting but incomprehensible phenomena, changing aflatoxin content was observed in the no treatment, viz, aflatoxin content showed highest in the early stage of storing, then gradually or suddenly decreased during storing.

To confirm these phenomena, storing test was made on various moisture content of maize by laboratory scale.

In this report, relationship between BGYF and ELISA for AFB1, determination will, also, be discussed.

## Materials and methods

Suwan - 1, typical Thai variety, cultivated at the Phraputtabat Field Crops Experiment Station in 1989, was used in this experiment. Four lots of maize samples, one handshelled and three machine shelled were prepared. Their initial moisture content were : 23.0% (P-22), 11.9% (P-26), 28.7% (P-28) and 20.5% (P-29), respectively, when storing test was started. One lot sample, 60 Kg. of shelled maize was divided into 20 cotton bags. Ten bags among them were kept in the laboratory and rest were stored in the semi-outdoor storage of the workshop building at the centre.

Details of sample preparation of each lot were; (1) P-22, harvested and handshelled at Phraputtabat FCES, (2) P-26, shelled by machine and store for one week in a gunny bag as very wet condition, then dried in the sun before storing, (3) P-28, shelled by machine and store immediately, (4) P-29, same maize of P-28 was kept in the gunny bag for overnight then started storing.

Three hundred grams of maize from each lot was extracted at every week and determined moisture content by means of the Single Kernel Moisture Meter, CTR-160-A (Shizuoka Seiki Co., Ltd., Japan), then dried in the sun. The maize samples were tested on fungi (A.flavus and others) by agar test, germination ratio, BGYF and aflatoxin B1 (AFB1) by means of ELISA as mentioned below;

(1) Agar test : A hundred gram of kernels of each sample was taken to 100ml volume of erlenmyer flask having glass stopper and sterilized surface of kernels with 50 ml. of 3% NaOCl solution for one minute by vigorous shake. Discard NaOCl followed by washing with sterilized water for several times. A hundred sterilized kernels were placed on the petri dish containing PDA-Rose Bengal media (five kernels in a petri dish), then incubated at 27 C for 3-5 days. Count number of

infected kernels on *A. flavus* and other fungi, and express as percent. At the same time, germination ratio was also observed.

(2) BGYF : Each maize samples were ground by means of RETCH MZ-1 atmizer using 2 mm seive. The grit maize sample was put into black vat (33x38 cm) in flat and exposured under UV ray, 365 nm of wavelength, in the dark box. Count number of BGYF particles and express average number of triplicate measurements.

(3) ELISA : . Powdered maize samples used for BGYF test were reground by means of same atmizer using 1 mm seive. Aflatoxin B1 was determined by the UBE EIA AFB -KIT which utilize the monoclonal antibody reacting with only aflatoxin B1.

## Results and discussion

Table 1 - 4 show the analytical data of maize stored in various moisture content and Figure 1 and 2 show the changes of moisture content in maize during 10 weeks storing. The moisture content of wet maize samples, P-22, P-28 and P-29, were gradually decreased during storing and finally reached to 13 to 16 %, even the experiment started from late rainy season. However, there was not much changes in moisture content of dried maize samples, P-26, during storing. BGYF count and aflatoxin B1 (AFB1) content in the samples determined by ELISA were shown in table 1 - 4. P-22, handshelled wet maize, showed very low BGYF and AFB1 in both laboratory and semi-outdoor storing even long term storage. However, P-26, P-28 and P-29 showed very high BGYF and AFB1 during storing, especially in P-28 and P-29. Their initial moisture content condition of storing were a little different before start, but all samples were machine shelled. The differences of BGYF and AFB1 content among the four series might be due to the shelling method, initial moisture content and pretreatment before storing.

Figure 3 and 4 shows the changes of AFB1 content in maize stored at different place, laboratory and semi-outdoor. In the laboratory storing, P-28 and P-29 showed considerably high AFB1 content after few days store and reached peak after one month, then decreased gradually. However, finally AFB1 content in P-29 was increased again.

In the semi-outdoor storing, clear phenomena was not observed among P-28 and 29. It seems to be increased high AFB1 in P-29 at early stage of storing, if the AFB1 content at the end of storing (20/Oct.) was neglected, due to sampling or analysis error. In case of P-26, the condition of sample preparation was very severe before storing, viz, wet maize sample was kept in the gunny bag for one week after shelled by machine and dried in the sun, then stored in the cotton bags in both laboratory and semi-outdoor storage. However, AFB1 content in P-26 was less than that of p-28 and 29. It was observed that maximum temperature in the gunny bag in pretreatment during a week was elevated to 54 C. It may not be able to say exactly, but high temperature before storing in the gunny bag might be affected on the production of AFB1 in that period.

P-22, handshelled maize could be stored safely even in high moisture content. This suggest that mechanical damage in maize is one of the biggest factor for aflatoxin contamination.

In this experiment, BGYF test was applied along with ELISA for analyzing aflatoxin in the maize samples. However, the clear relation between BGYF count and AFB1 content by ELISA in the samples was not found. It is necesssary to continue further study for the BGYF test.

Table 1
Analytical data of stored maize (p-22)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 22/Sep | M/C: 23.0 + 2.88 (%) | | | A.flavus 0 | Others (A.nig) 200 (in 200 kernels) | | | Germination 97% |
| | AFB1 (ELISA) : ND(ppb), BGYF : 0 | | | | | | | |
| | Stored at sample prep. Rm. | | | | Stored at Annex Bldg. | | | |
| | Moist | FUNGI | | Germ'tn | M/C | FUNGI | | Germ'tn |
| | AFB ppb | A.fl | Other | BGYF | AFB | A.fl | Other | BGYF |
| | | | | | | | | |
| 25/Sep | 21.2±2.09 | 7 | 93 | 99 | 21.6±1.41 | 4 | 96 | 95 |
| | | | | | | | | |
| | ND | | | 0 | ND | | | 0 |
| 29/Sep | 21.3±1.46 | 6 | 96 | 100 | 21.2±1.31 | 4 | 97 | 100 |
| | | | | | | | | |
| | ND | | | 0 | ND | | | 0 |
| 06/Oct | 20.2±0.96 | 4 | 98 | | 20.2±0.89 | 3 | 73 | |
| | | | | | | | | |
| | ND | | | 1 | ND | | | 3 |
| 13/Oct | 19.0±1.13 | 9 | 98 | | 17.7±1.15 | 11 | 95 | |
| | | | (2) | | | | (3) | |
| | ND | | | 0 | ND | | | 0 |
| 20/Oct | 19.3±0.75 | 8 | 94 | 90 | 18.0±0.88 | 7 | 94 | 94 |
| | | | (1) | | | | (2) | |
| | ND | | | 0 | ND | | | 4 |
| 27/Oct | 18.6±0.99 | 11 | 69 | 82 | 16.6±0.85 | 16 | 81 | 86 |
| | | | (5) | | | | (1) | |
| | ND | | | 0 | ND | | | 2 |
| 03/Nov | 17.6±1.17 | 5 | 96 | 91 | 15.7±0.95 | 13 | 86 | 91 |
| | | | (2) | | | | (2) | |
| | ND | | | 0 | ND | | | 0 |
| 10/Nov | 16.9±1.10 | 2 | 101 | 88 | 14.9±0.98 | 4 | 87 | 83 |
| | | | (2) | | | | (4) | |
| | ND | | | 0 | ND | | | 0 |
| 17/Nov | 16.7±0.82 | 4 | 72 | 83 | 14.7±0.63 | 3 | 96 | 87 |
| | | | | | | | | |
| | 10.8 | | | 1 | 9.3 | | | 0 |
| 24/Nov | 16.0±0.80 | 6 | 98 | 78 | 13.9±0.85 | 3 | 99 | 88 |
| | | | (2) | | | | (2) | |
| | 6.3 | | | 0 | 6.3 | | | 1 |
| 01/Dec | 15.3±0.80 | 6 | 84 | 62 | 13.0±0.80 | 12 | 88 | 74 |
| | | | (3) | | | | | |
| | 11 | | | 0 | 5.3 | | (3) | 0 |

Table 2
Analytical data of stored maize (p-26)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | A.flavus | Other(A.niger) | | Germ'tn | |
| | | | | 18 | 34 | | 25% | |
| 26/Sep | M/C: 11.9 + 1.32(%) (in 100 kernels) | | | | | | | |
| | AFB1 (ELISA) : 18(ppb) BGYF : 132 | | | | | | | |
| | Store at Sample prepn. Rm | | | | Store at Annex Bldg. | | | |
| | Moist. | FUNGI | | Germ'tn | M/C | FUNGI | | Germ'tn |
| | AFB | A.fl | other | BGYF | AFB | A.fl | other | BGYF |
| 27/Oct | 11.3±0.83 | 21 | 21 | 9 | 11.3±0.88 | 19 | 29 | 19 |
| | | | (4) | | | | (4) | |
| | 50 | | | 62 | 48 | | | 139 |
| 03/Nov | 11.7±0.78 | 18 | 38 | 29 | 11.6±0.72 | 17 | 46 | 14 |
| | | | (10) | | | | (12) | |
| | 46 | | | 57 | 35 | | | 60 |
| 10/Nov | 12.7±1.17 | 25 | 51 | 21 | 12.5±1.01 | 14 | 28 | 13 |
| | | | (2) | | | | | |
| | 25 | | | 163 | 30 | | | 157 |
| 17/Nov | 12.8±0.82 | 24 | 64 | 19 | 12.5±0.76 | 15 | 51 | 14 |
| | | | (5) | | | | (31) | |
| | 19 | | | 87 | 12 | | | 78 |
| 24/Nov | 12.6±0.90 | 28 | 63 | 13 | 12.0±0.89 | 25 | 46 | 12 |
| | | | (9) | | | | (4) | |
| | 20 | | | 113 | 34 | | | 54 |
| 01/Dec | 12.6±0.73 | 23 | 63 | 22 | 11.8±0.71 | 19 | 19 | 42 |
| | | | (22) | | | | (16) | |
| | 42 | | | 246 | 18 | | | 264 |

Table 3
Analytical data of stored maize (p-28)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | A.flavus 1 | Others(A.nig) 225 | | Germination 99% | |
| 28/Sep | M/C: 28.7+3.93(%) | | | (in 200 kernels) | | | | |
| | AFB1 (ELISA): ND(ppb) BGYF:0 | | | | | | | |
| | Store at Sample prep. Rm | | | | Store at Annex Bldg. | | | |
| | Moist | FUNGI | | Germ'tn | M/C | FUNGI | | Germ'tn |
| | AFB | A.fl | Other | BGYF | AFB | A.fl | Other | BGYF |
| 02/Oct | 26.0±2.50 | 45 | 89 | | 26.7±2.36 | 56 | 73 | |
| | 4.3 | | | 72 | ND | | | 102 |
| 06/Oct | 24.5±3.53 | 96 | 45 | | 23.5±2.30 | 59 | 76 | |
| | 62 | | | 228 | 38 | | | 116 |
| 13/Oct | 20.1±2.04 | 86 | 106 | | 20.0±1.55 | 99 | 126 | |
| | | | (6) | | | | (62) | |
| | 190 | | | 49 | 128 | | | 94 |
| 20/Oct | 20.0±1.58 | 58 | 69 | 35 | 19.5±1.34 | 64 | 71 | 37 |
| | | | (42) | | | | (51) | |
| | 344 | | | 182 | 168 | | | 313 |
| 27/Oct | 18.9±1.06 | 59 | 51 | 21 | 17.3±1.10 | 64 | 47 | 32 |
| | | | (30) | | | | (31) | |
| | 220 | | | 264 | 149 | | | 278 |
| 03/Nov | 17.7±1.36 | 74 | 74 | 34 | 15.5±1.29 | 59 | 78 | 29 |
| | | | (39) | | | | (45) | |
| | 265 | | | 181 | 178 | | | 186 |
| 10/Nov | 17.8±1.34 | 75 | 52 | 30 | 15.6±0.94 | 75 | 62 | 29 |
| | | | (32) | | | | (42) | |
| | 198 | | | 439 | 250 | | | 397 |
| 17/Nov | 16.9±1.00 | 62 | 76 | 30 | 15.3±0.78 | 60 | 85 | 35 |
| | | | (36) | | | | (43) | |
| | 274 | | | 187 | 215 | | | 181 |
| 24/Nov | 16.1±0.94 | 61 | 83 | 22 | 14.8±0.89 | 67 | 93 | 29 |
| | | | (39) | | | | (48) | |
| | 201 | | | 168 | 145 | | | 212 |
| 01/Dec | 15.8±0.73 | 90 | 86 | 23 | 13.7±0.73 | 90 | 94 | 28 |
| | | | (57) | | | | (75) | |
| | 226 | | | 330 | 261 | | | 391 |

Table 4
Analytical data of stored maize (p-29)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 29/Sep | M/C: 20.5+2.45%<br>AFB1 (ELISA): ND(ppb) BGYF: 0 | | | | A.flavus 41 | Others (A.nig) 176<br>(in 200 kernels) | | Germination 99.5% |
| | Store at sample prep. Rm | | | | Store at Annex Bldg. | | | |
| 02/Oct | 19.4+1.53 | 89 | 38 | | 20.0+1.75 | 80 | 50 | |
| | 33 | | | 6 | 65 | | | 4 |
| 06/Oct | 18.8±1.66 | 80 | 63 | | 19.4±0.84 | 74 | 60 | |
| | 232 | | | 90 | 288 | | | 25 |
| 13/Oct | 18.5±0.83 | 89 | 172 | | 18.0±1.33 | 80 | 88 | |
| | | | (100) | | | | (21) | |
| | 425 | | | 43 | 413 | | | 15 |
| 20/Oct | 18.2±1.01 | 65 | 66 | 60 | 17.6±0.94 | 67 | 74 | 68 |
| | | | (45) | | | | (47) | |
| | 450 | | | 43 | 118 | | | 43 |
| 27/Oct | 17.4±0.72 | 60 | 62 | 51 | 16.1±0.76 | 81 | 61 | 59 |
| | | | (39) | | | | (34) | |
| | 488 | | | 38 | 375 | | | 20 |
| 03/Nov | 16.8±0.57 | 95 | 67 | 46 | 14.7±0.83 | 57 | 74 | 60 |
| | | | (47) | | | | (34) | |
| | 168 | | | 34 | 275 | | | 27 |
| 10/Nov | 16.3±0.65 | 66 | 67 | 55 | 14.8±0.92 | 70 | 70 | 60 |
| | | | | | | | (43) | |
| | 281 | | | 21 | 245 | | | 26 |
| 17/Nov | 16.3±0.68 | 65 | 73 | 59 | 14.5±0.62 | 61 | 64 | 64 |
| | | | (54) | | | | (37) | |
| | 250 | | | 19 | 220 | | | 9 |
| 01/Dec | 14.9±0.71 | 90 | 67 | 50 | 13.0±0.65 | 84 | 85 | 54 |
| | | | (49) | | | | (67) | |
| | 532 | | | 161 | 228 | | | 160 |

Figure 1

Changes of Moisture Content in Maize Stored at Laboratory

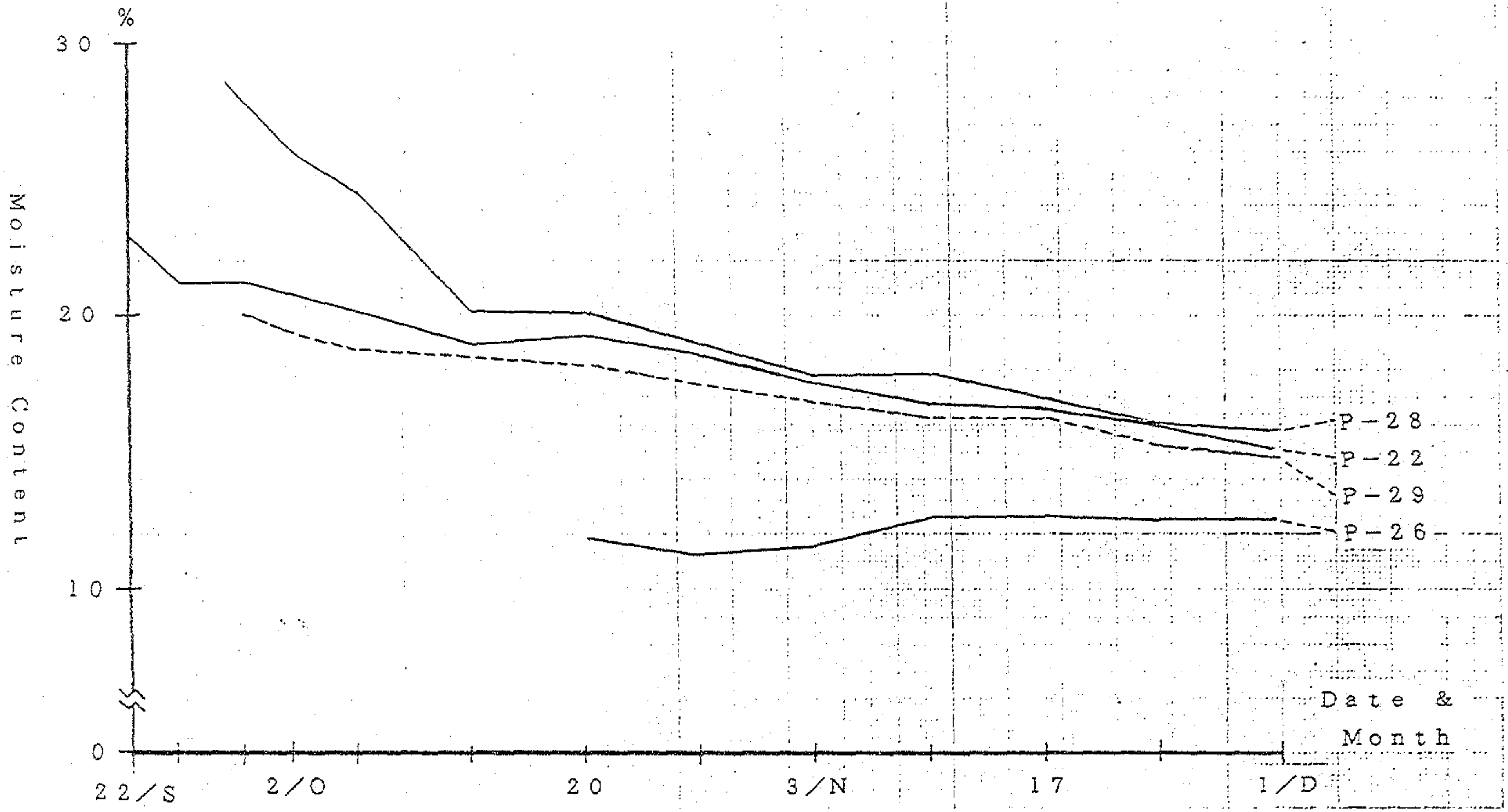


Figure 2

Changes of Moisture Content in Maize Stored at Semi-out Door Storage

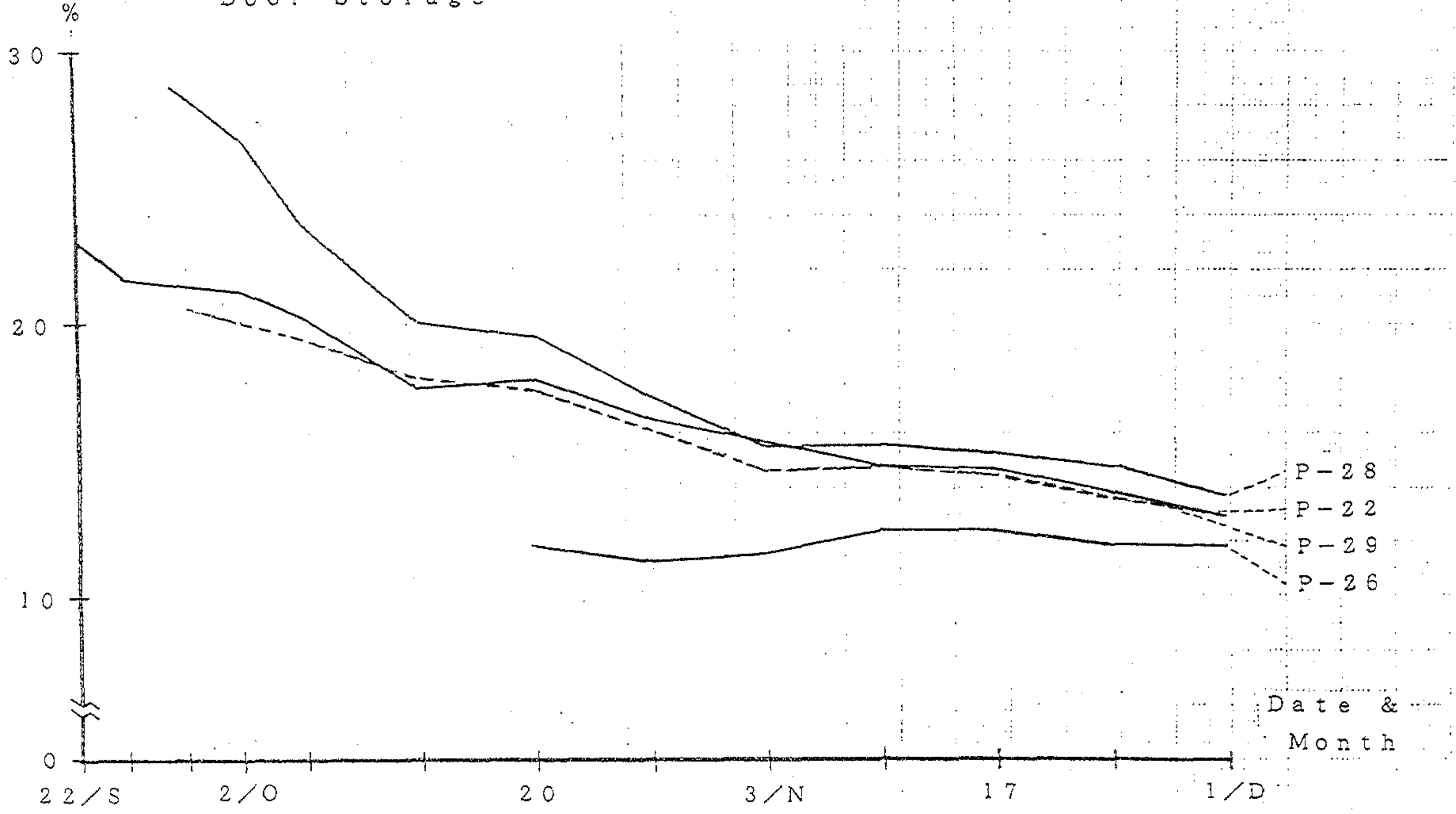


Figure 3

Changes of Aflatoxin-B1 Content
in Maize Stored at Laboratory

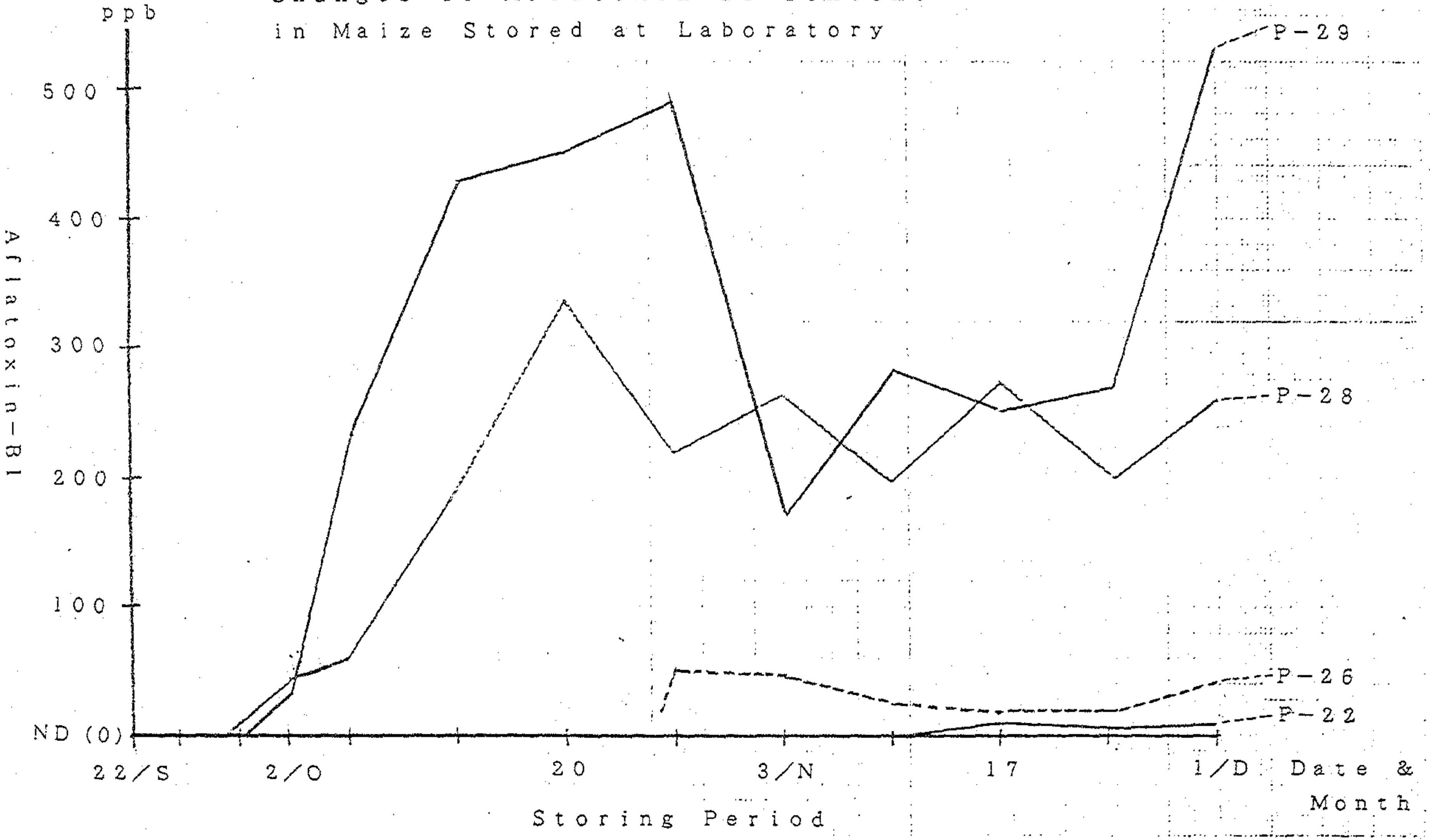
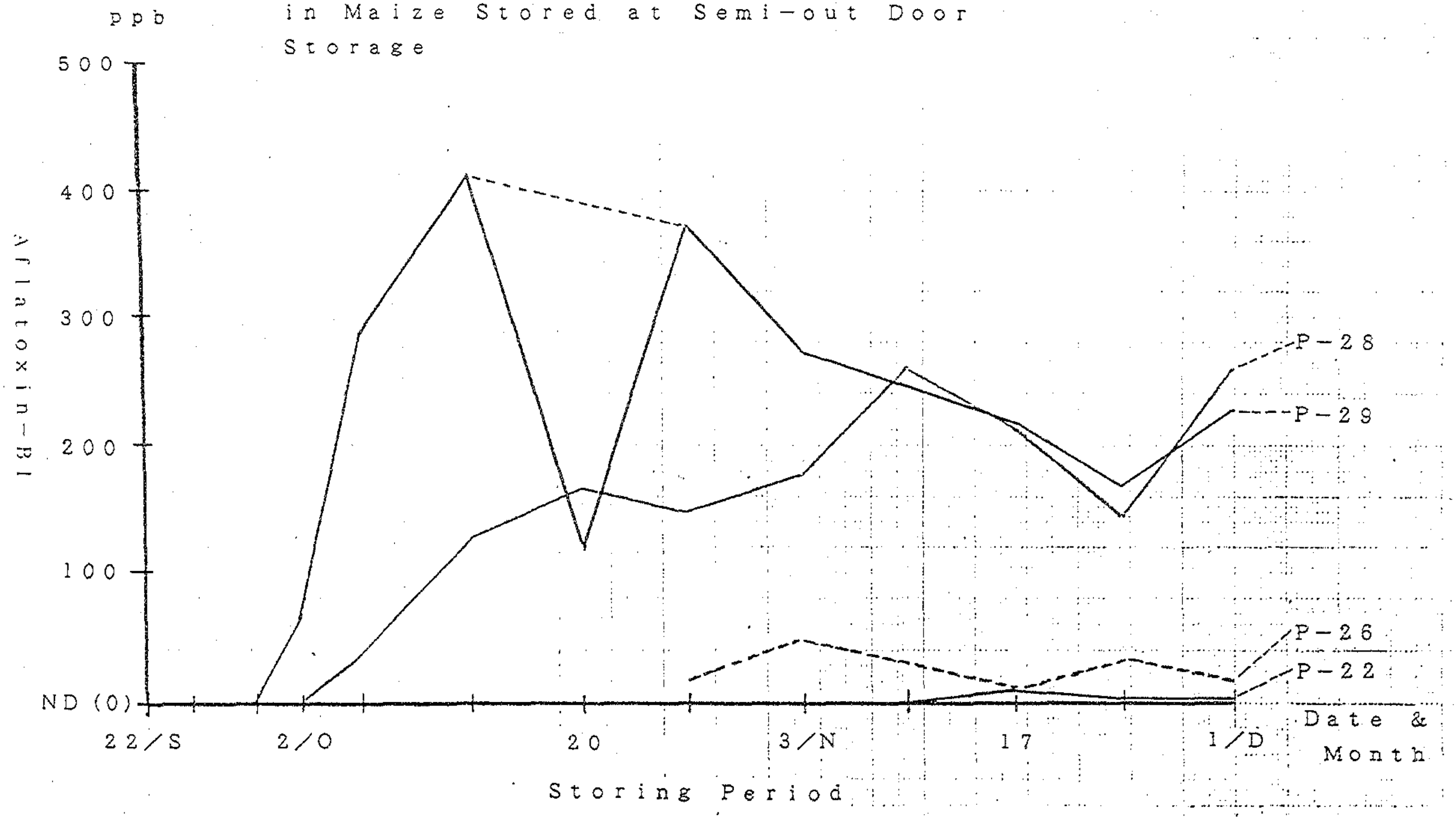


Figure 4

Changes of Aflatoxin-B1 Content in Maize Stored at Semi-out Door Storage



Water Activity of Thai Maize and Growth of *A.flavus*
Code No. III-1-(3) (MIC/V/89)

The term of water activity (Aw) is often used in the field of microbiological study of food. Moisture in food is classified into two categories, free and bond water. The free water in food is utilizable by micro-organisms but the bond water which combined with some components in food is not useful for the growth of microorganisms. Aw of food is different even in same moisture content due to the different amount of free and bond water. In the field of microbiological study or food preservation, the amount of free water, in another word Aw, is essentially important because of the growth of fungi or bacterials in food has very close relation with it.
Theoretically, Aw is expressed as P/Po in which Po is the vapor pressure of pure water and P is the vapor pressure from the material. However, Aw of the material can, generally, be measured the relative humidity occured from the material using the airtight container devised with relative humidity meter.

## Materials and methods

Firstly, a hundred samples of maize kernel. Suwan-1, freshly harvested at the Phraputtabat Field Crops Experiment Station in October, 1989, varied moisture content from 41.64 to 8.70% were prepared. To measure the Aw, Aw-Wert-Messer (Durotherm, W. Germany), a kind of the hair hygrometer, airtight container type, was used for the purpose.
The Aw meter filled with maize kernels was kept in 25 C incubator for one hour until reaching to equilibrium humidity. On the other hand, about 10 g. of same batch sample used for Aw measurement was crushed by Kett grain miller and determined total moisture content, dried at 135 C for 3 hours using aluminum container. To observe growing of *A.flavus* in maize, various Aw maize samples ranging from 1.00 to 0.43, were selected. About 100 g. of maize sample was taken to 300 ml volume of erlenmyer flask and inoculated *A.flavus* spore by a wire hook. After mixed well, the flask was kept in 28 C incubator for a week.

## Results and discussion

Figure 1 shows the relation between Aw and total moisture content in maize and Table 1 shows the growing of *A.flavus* on various Aw of maize. As can be seen from Figure 1, very clear relation between Aw and total moisture content of maize was observed. Table 1 shows the degree of infection in various Aw of maize at 7 days after inoculated *A.flavus* spores. In case of high Aw maize, 1.0 - 9.4, as total moisture content 41 - 22%, the growth of *A.flavus* was very high. Aw ranging from 0.93 to 0.85, total moisture content 20 - 18%, shows the moderate attack by *A.flavus*, but below Aw 0.85, less than 18% total moisture content, infected kernels were not observed even 7 days incubated. This results suggested that freshly harvested and shelled maize should be dried immediately to below Aw 0.85, less than 18% total moisture content so that they can be stored safely against *A.flavus* attack within 7 days or more. However, this allowance period by *A.flavus* attack was measured only at 7 days after inoculation. So, it is necessary to continue and repeat experiment from more shorter and longer to estimate minimum allowance period against *A.flavus* attack.

Figure 1 RELATION BETWEEN M/C & Aw

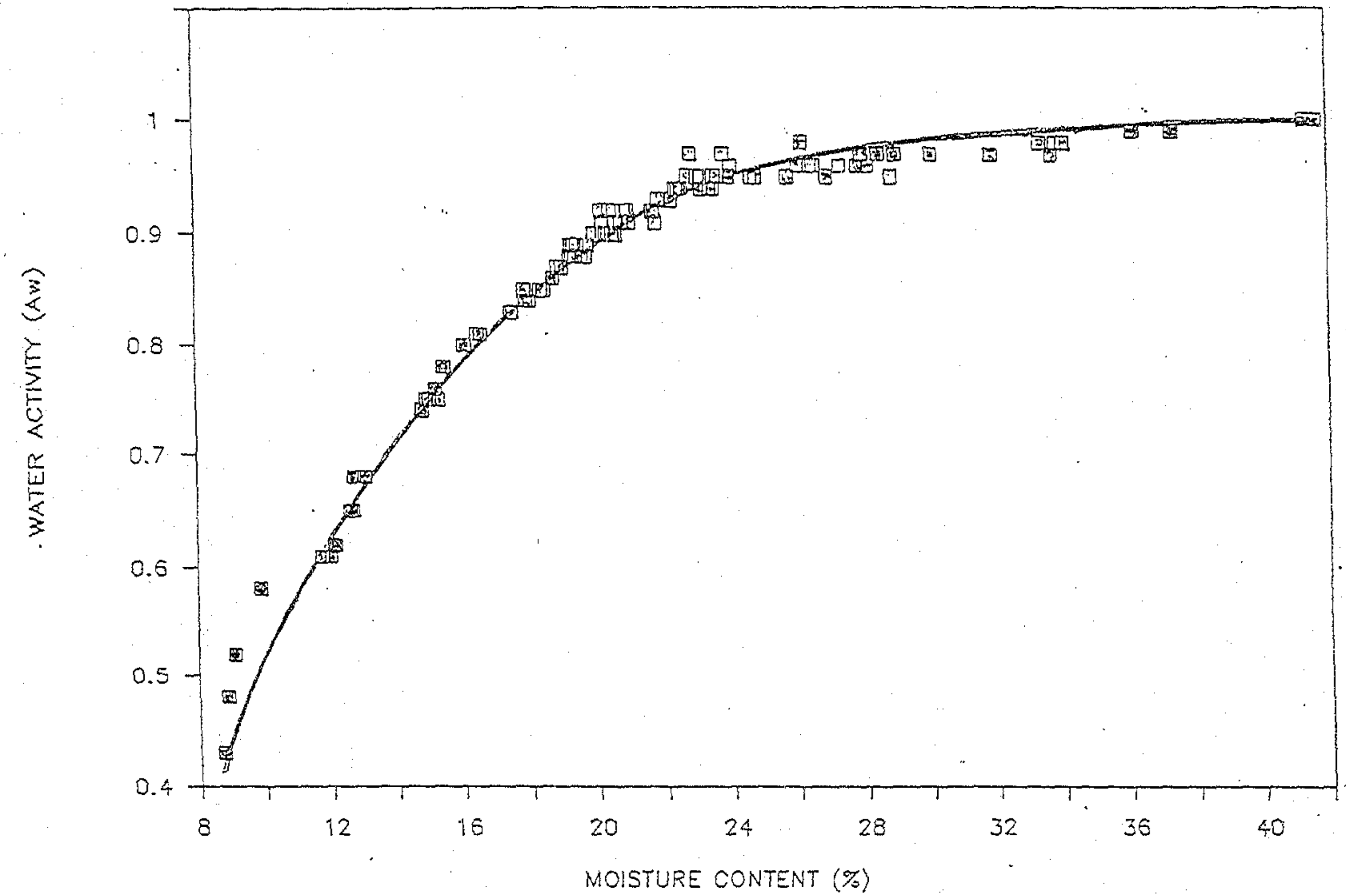


Table I

The growth of A. flavus on various Aw of kernels after inoculated for 7 days at 28 °C

| Aw | A. flavus | Aw | A. flavus | Aw | A. flavus |
|---|---|---|---|---|---|
| 1.00 | ++++ | 0.93 | ++ | 0.83 | - |
| 0.99 | ++++ | 0.93 | - | 0.82 | - |
| 0.98 | ++++ | 0.92 | ++ | 0.81 | - |
| 0.98 | +++ | 0.92 | - | 0.80 | - |
| 0.98* | - | 0.91 | + | 0.78 | - |
| 0.97 | ++++ | 0.91 | - | 0.76 | - |
| 0.97 | +++ | 0.90 | ++ | 0.75 | - |
| 0.97 | ++ | 0.90 | - | 0.74 | - |
| 0.97* | - | 0.89 | ++ | 0.68 | - |
| 0.96 | +++ | 0.89 | + | 0.65 | - |
| 0.96 | ++ | 0.89 | - | 0.62 | - |
| 0.95 | ++++ | 0.88 | - | 0.58 | - |
| 0.95 | +++ | 0.87 | + | 0.43 | - |
| 0.95 | ++ | 0.87 | - | | |
| 0.95 | + | 0.86 | - | | |
| 0.94 | +++ | 0.85 | + | | |
| 0.94 | ++ | 0.85 | - | | |
| 0.94 | - | 0.84 | - | | |

* germinated + 1~2 kernels infected +++ highly infected

\- no infect ++ few kernels infected ++++ all kernels infected

Equilibrium Moisture Content of Thai Maize and Growth of *A.flavus*
Code No. III-1-(3) (MIC/V/89)

Moisture content in maize is largely influenced by surrounding humidity during drying or storing process. Drying of wet maize will not be able to achive in high humid condition and finally molds grow on the grain, while well dried maize will absorb water when left it in high humid condition for a long time and grain might be contaminated by molds.
When maize, both wet and dry, were kept under the fixed, constant humidity for a long time, finally moisture content of maize reach to equilibrium for the humidity which defined as the equilibrium moisture contents of maize. Accordingly, it is quite necessary to pay attention on surrounding humidity for effective drying or string of maize without problems, especially contamination by *A.flavus*.

## Materials and methods

A maize variety, Suwan 1, both wet and dried, were sterilized by ethylene oxide gas by means of SEMIMEL-502 (IKIKEN Co. Ltd., Japan) for 5 hours using air-permiable bag. Initial moisture content, both wet and dried maize for the experiment, were 33.8 and 11.6%, respectively. Beside, about 2 litres of five kinds of satulated inorganic salt solution, $KNO_3$, KCl, KBr, NaCl and KI were prepared with sterilized water which can make 92.6, 84.2, 78.6, 75.5 and 70.2% of relative humidity in the closed airtight container, respectively. For a series of selected humidity, 8 glass bottles (airtight), altogether 40 bottles for 5 kinds humidity, are required in this experiment.
A satulated solution was divided equally into 8 bottles having rubber gasket, then a mini stainless perforated support was set in each bottle. About 20 sterilized maize kernels, both wet and dried, were put to two sterilized small petri dishes (ca. 4 cm ) as single layer separately. Both petri dishes containing wet and dry maize were set on the support in a bottle, closed with lid tightly, then kept in the 25 C incubator. After the scheduled period (1, 3, 5, 7, 8 and 9 weeks), one bottle in each humidity condition taken out from the incubator and measure the moisture content in maize at 135 C for 3 hours.
For the last bottle (after reached equilibrium) a wire hook of *A.flavus* spore was inoculated to maize in each petri dishes and put back to the bottles. Keep the bottles again in the incubator for a week, then observed growth of *A.flavus*.

## Results and discussion

Figure 1 shows the equilibrium moisture content curves started from both wet and dried maize. Changes of the moisture content of maize during absorb or release water were shown in Table 1 and illustrated in figure 2 and 3, absorb and release water separately. Both moisture absorbed by dried maize or released from wet maize were dramatically changed during first week in the all series, then gradually changed depending on the period prolonged. It would seems to be reached equilibrium after 9 weeks except in case of $KNO_3$ (92.6% RH) and KCl (84.2% RH). The exceptions were stopped the experiment, due to indistinct growing of fungi.

Also, same fructuations were found in the moisture content in each series but it may be due to the individual variances among the kernels of maize samples.

A.flavus inoculation test showed positive in high humidity series, such as $KNO_3$ and KCl. In $KNO_3$ (92.6% RH) series, all dried brokens and whole kernels were infected after a week, and in KCl (84.2% RH) series, 9 dried broken kernels were infected among 22 kernels.

This study is still undergoing and will be continued in this coming crop season.

Figure 1

Equlibrium Moisture Content of Maize, Suwan-1

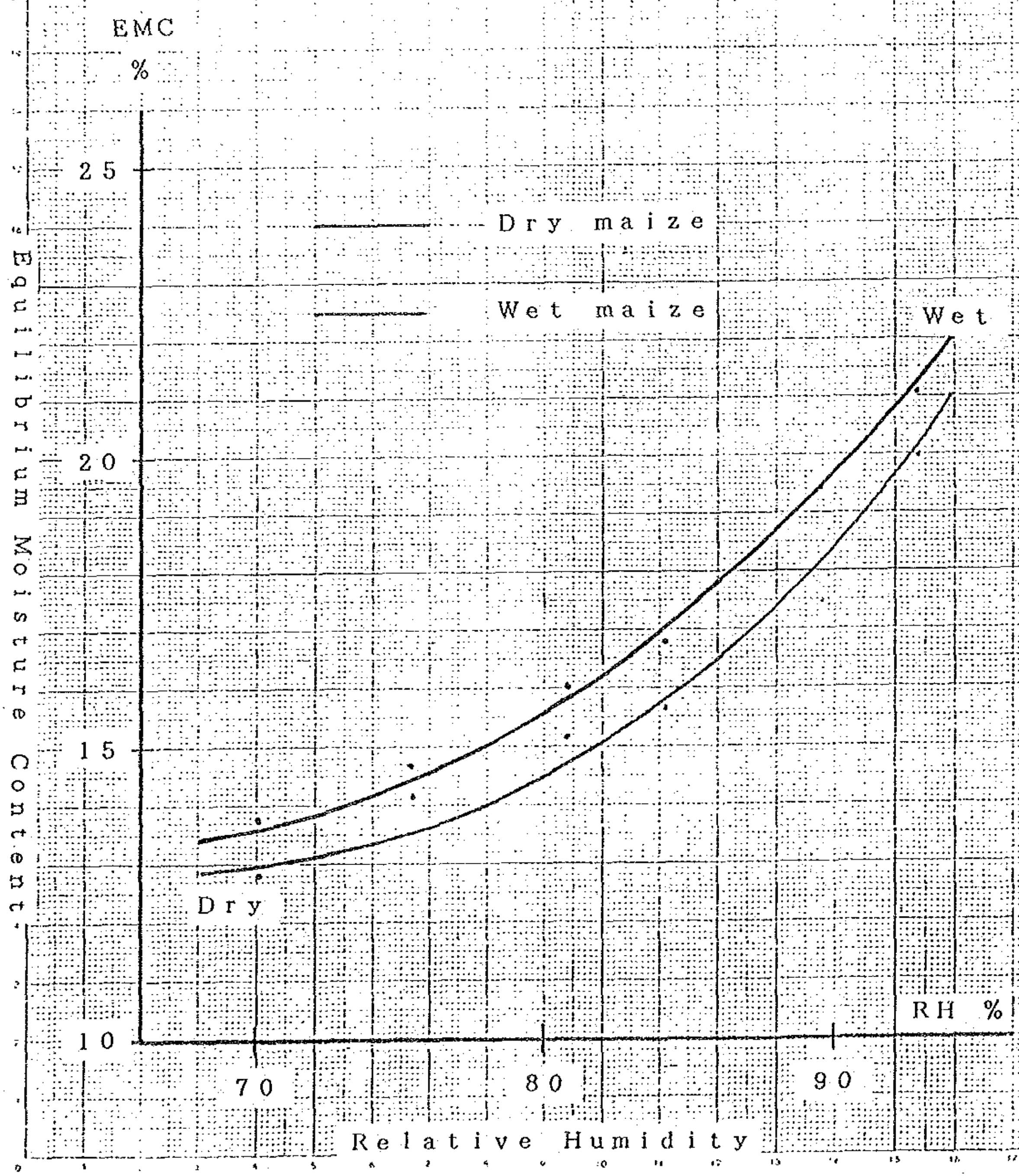


Table 1
Equilibrium Moisture Content of Maize, Suwan 1

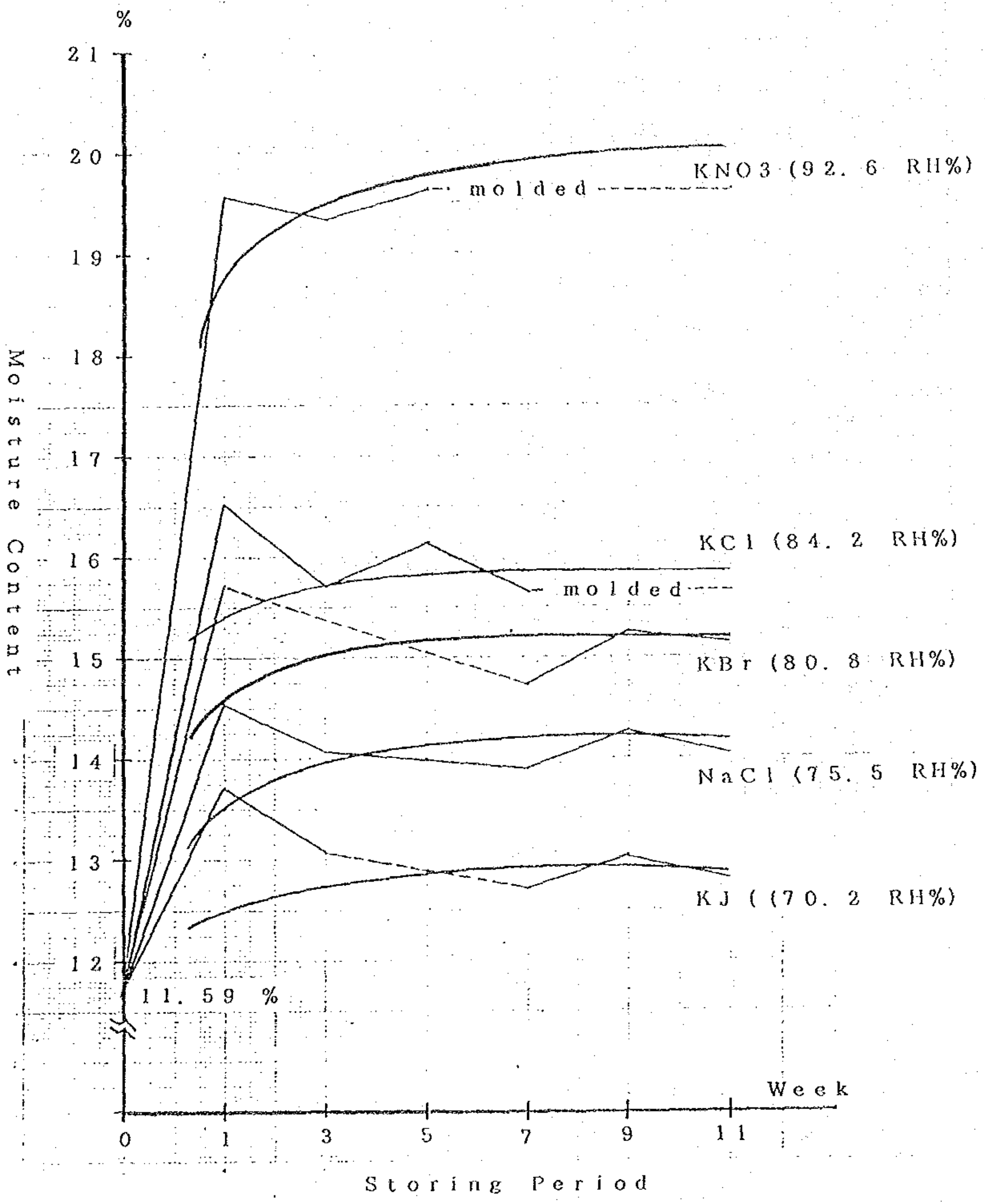
| | Week | 0 | 1 | 3 | 5 | 7 | 8 | 9 | |
|---|---|---|---|---|---|---|---|---|---|
| Satulate Salt Solution | Code | Start 25 Oct 89 | M/C 31 Oct 89 | M/C 14 Nov 89 | M/C 28 Nov 89 | M/C 12 Dec 89 | M/C 19 Dec 89 | M/C 26 Dec 89 | Mold |
| | | | | | | | | | 7day(+) |
| KNO3 | D | 11.59 | 19.52 | 19.37 | 19.62 | -* | | | DB |
| 92.6% RH | | | | | | | | | DW |
| | W | 33.75 | 22.38 | 21.41 | 21.33 | -* | | | 28/28 |
| | | | | | | | | | 7day(-) |
| KCl | D | 11.59 | 16.53 | 15.72 | 16.10 | 15.64 | -* | | 21day(+ |
| 84.2% RH | | | | | | | | | DB |
| | W | 33.75 | 18.05 | 17.34 | 17.26 | 16.97 | -* | | 9/22 |
| KBr | D | 11.59 | 15.68 | 14.03 | 12.66 | 14.68 | 15.22 | 15.13 | |
| 80.8% RH | | | | | | | | | |
| | W | 33.75 | 17.00 | 16.01 | 13.74 | 16.12 | 16.43 | 16.03 | |
| NaCl | D | 11.59 | 14.54 | 14.08 | 13.96 | 13.88 | 14.23 | 14.02 | |
| 75.5% RH | | | | | | | | | |
| | W | 33.75 | 15.93 | 15.21 | 15.05 | 14.87 | 14.59 | 14.87 | |
| KI | D | 11.59 | 13.73 | 13.05 | 14.78 | 12.72 | 12.97 | 12.80 | |
| 70.2% RH | | | | | | | | | |
| | W | 33.75 | 14.87 | 14.44 | 15.98 | 13.66 | 13.97 | 13.70 | |

D : start with dried sample
W : start with wet sample

* : Molded
DW: Dried wholes
DB: Dried brokens

Figure 2

Changes of Moisture Content in Maize Stored at Various Humidity (start from dry maize)



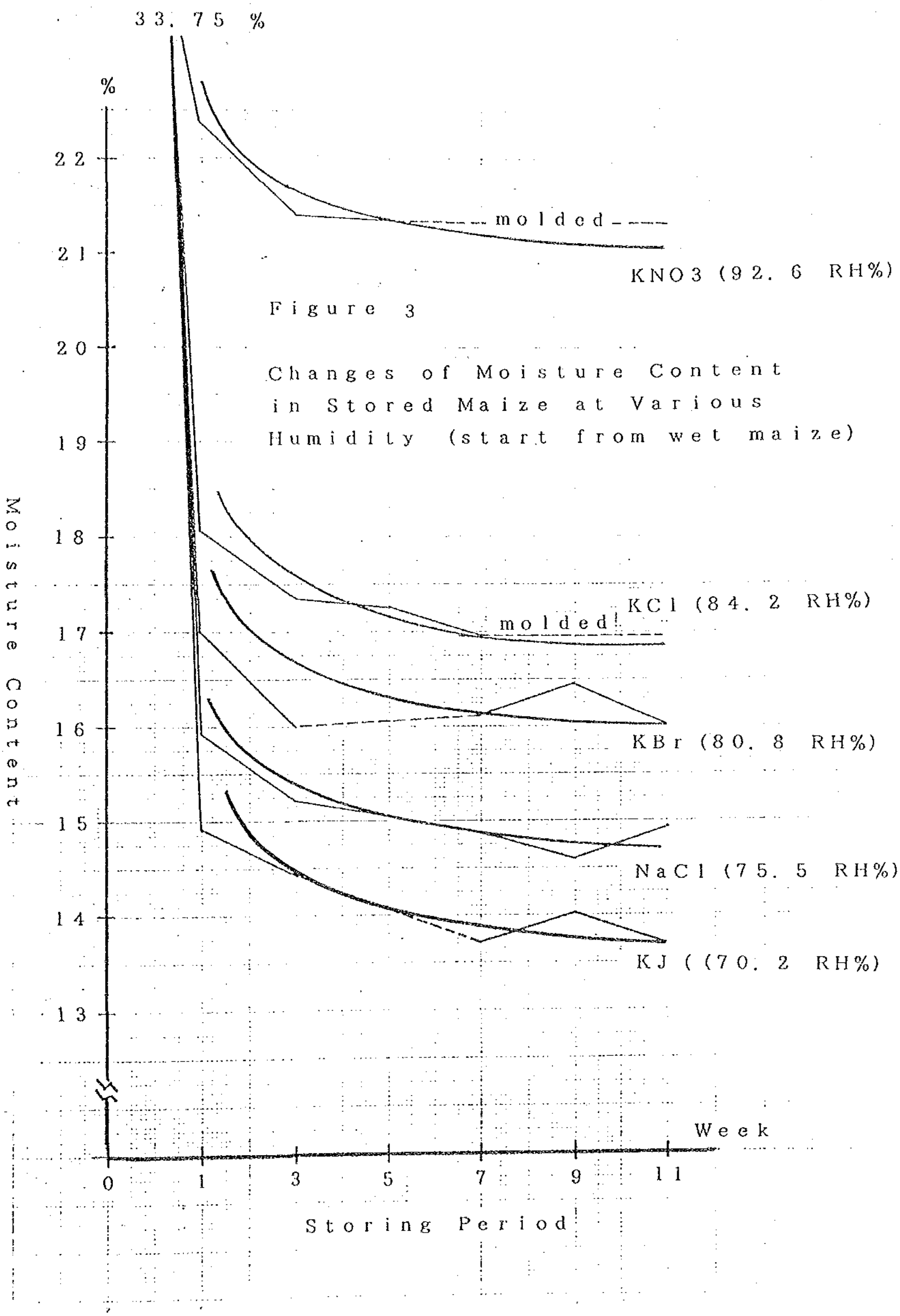



Figure 3

Changes of Moisture Content in Stored Maize at Various Humidity (start from wet maize)

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