113年台灣雜糧發展基金會補助計畫(1130101-1131231)

國產硬質玉米儲存條件與微生物毒素產生與去毒化之探討

計畫編號：113-02-005

期末報告

執行機構：國立臺灣大學農業化學系
計畫主持人：賴喜美教授
計畫執行：金禹圻、曾令偉

114.01.21

Corn (*Zea mays*), the most cultivated cereal crop

Food Applications

- Animal feed
- Sweeteners
- Thickeners

Industrial Applications

- Ethanol
- Industrial solvents
- Biofuel

sweet corn

baby corn

flint corn

Local Flint Corn: Sustainable but Scarce

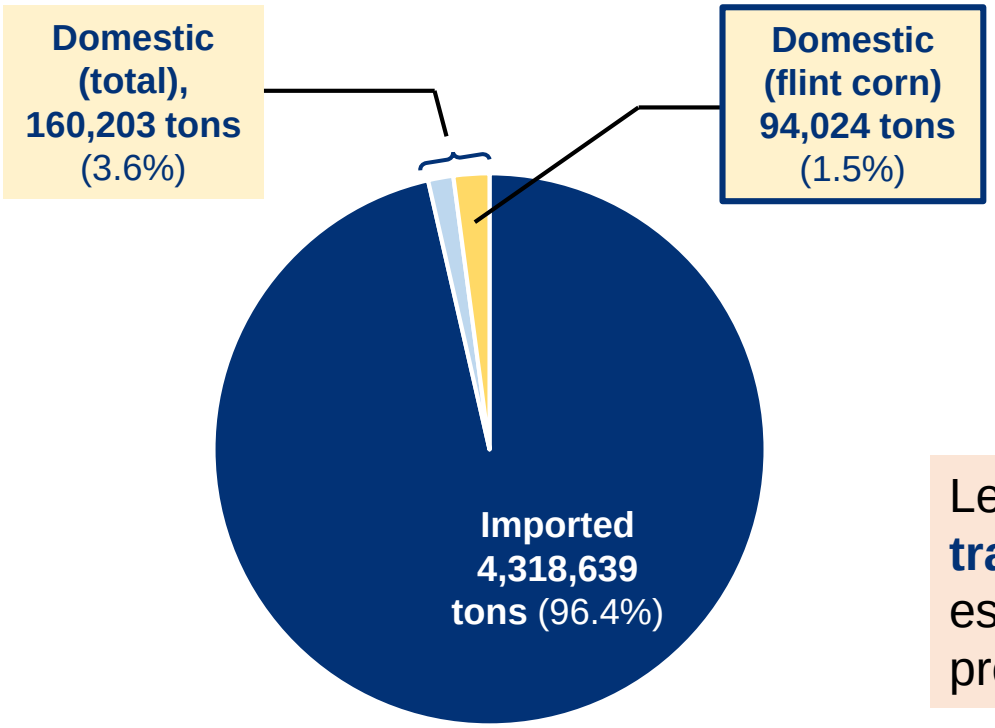


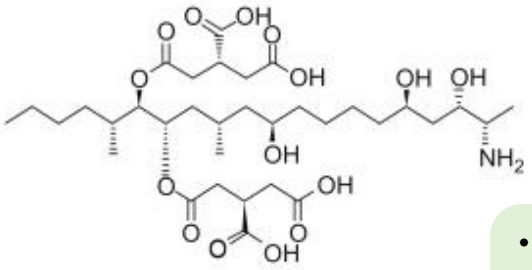
Figure 1. Proportion of locally produced flint corn in 2023.

- ✓ Freshness
- ✓ Non-genetically modified
- ✓ Low carbon footprint

Less is known about the **safety and traceability** of local flint corn, especially its microbial safety and presence of trace toxic elements.

Source: Ministry of Agriculture, R.O.C.

Fungal Infection, a prequel to mycotoxin contamination



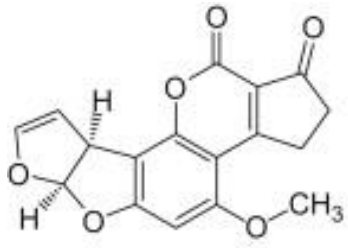
Fumonisin B1

Preharvest

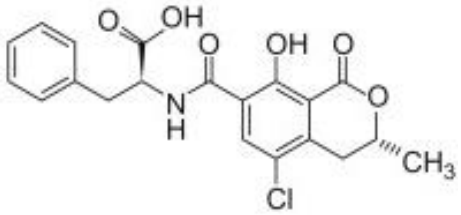
- *Alternaria spp.*
- *Fusarium spp.*
- *Cladosporium spp.*
- *Helminthosporium spp.*

Postharvest

- *Aspergillus spp.*
- *Penicilllium spp.*
- *Rhizopus spp.*
- *Mucor spp.*



Aflatoxin B1

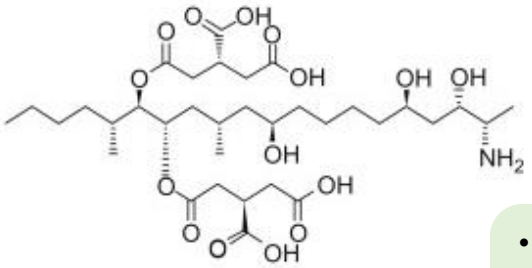


Ochratoxin A

Mycotoxin Contamination

(Pitt, 2013)

Fungal Infection, a prequel to mycotoxin contamination



Fumonisin B1

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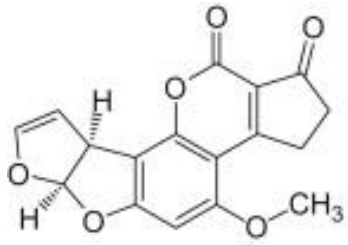
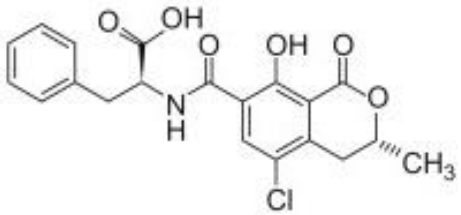


Aflatoxin B1



Ochratoxin A

Mycotoxin Contamination

(Pitt, 2013)

Introduction

Research Scheme

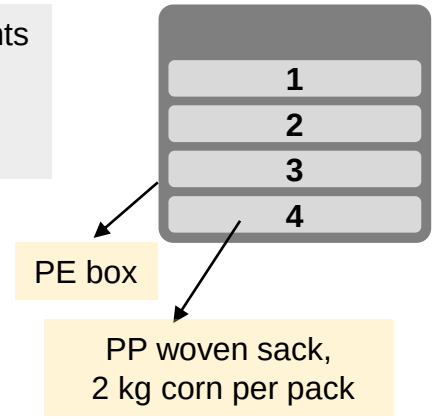
Flint Corn ‘Mingfeng No. 3’

Adjusted to 3 different moisture contents
Treatment A: 10.15% (as is)
Treatment B: 11.75% (as is)
Treatment C: 13.20% (as is)

Two batches per treatment

Storage at 15°C and 38°C for 3 months

Arrangement of samples in one batch of treatment:



Moisture standard (Food and Agriculture Organization):
15.5% m/m max

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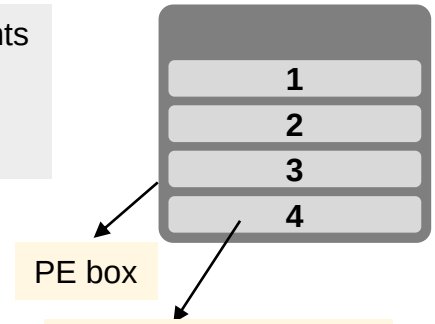


Biweekly analyses:
- ✓ Moisture content
- ✓ Water activity

Monthly analyses:
- ✓ Fungal infection rate (Tournas *et al*., 2001)
  - Non-surface disinfected (NSD) and surface disinfected (SD)
- ✓ Mycotoxins analyses (MOHWT0010.02)
  - Aflatoxin B_1, B_2, and G_1
  - Ochratoxin A
  - Fusarium toxin T-2 and HT-2
  - Deoxynivalenol (DON)
  - Zearalenone (ZEA)
  - Fumonisin B_1 and B_2

Analyses before and after 3-month storage
- ✓ Proximate analyses
  - Moisture, ash, crude lipid, crude protein

Results and discussions

Moisture and water activity 15°C

Treatment A: 10.15% M.C. | **Treatment B: 11.75% M.C.** | **Treatment C: 13.20% M.C.**

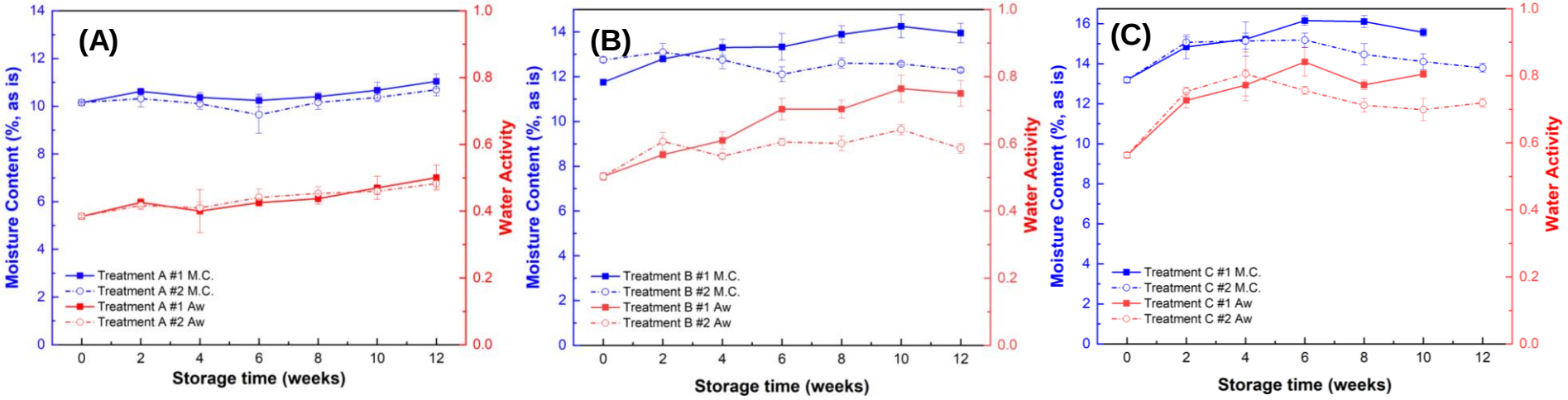


Figure 1. Moisture and water activity of flint corn at 3 different moisture contents in cold storage (A) Treatment A, 10.15% (as is); (B) Treatment B, 11.75% (as is); (C) Treatment C: 13.20% (as is), during the storage period. Molding occurred in all packages of treatment C #1 at week 10, hence the storage ended.

Results and discussions

Moisture and water activity 38°C

Treatment A: 10.94% M.C. **Treatment B: 12.33% M.C.** **Treatment C: 14.67% M.C.**

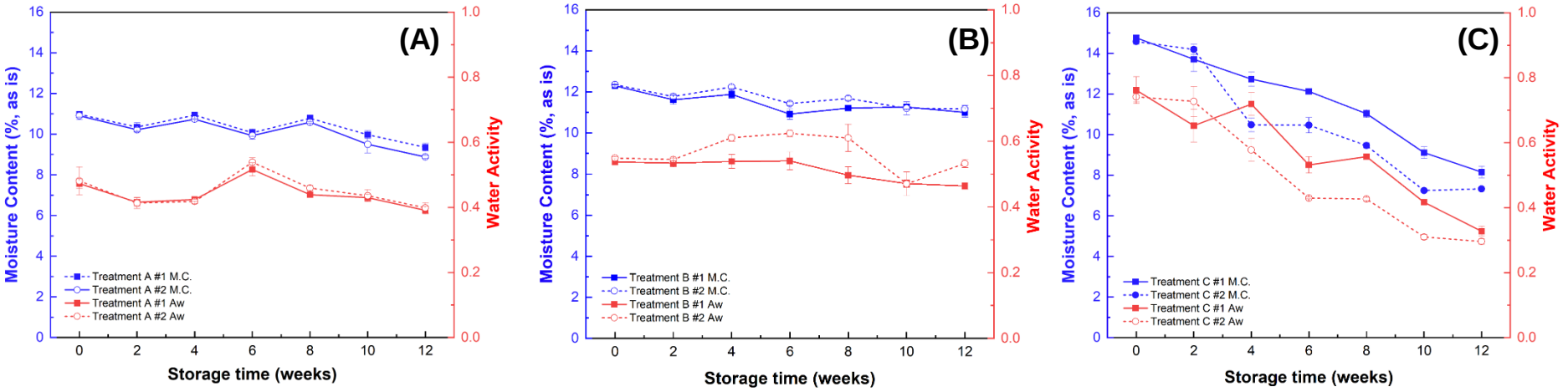
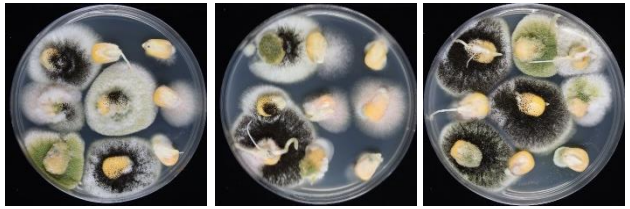


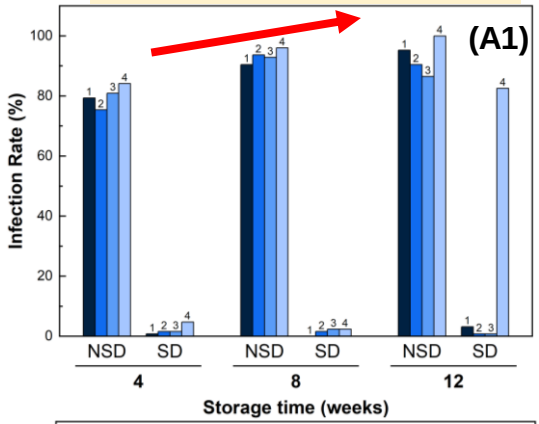
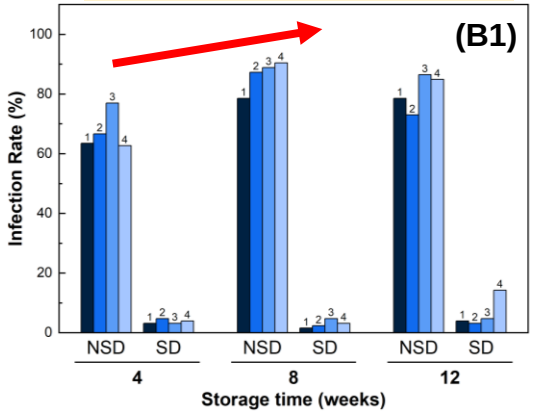
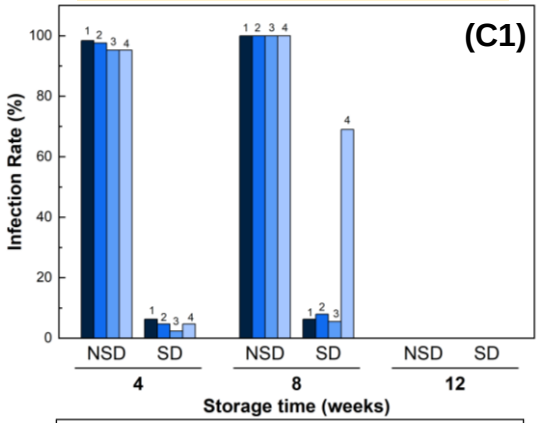
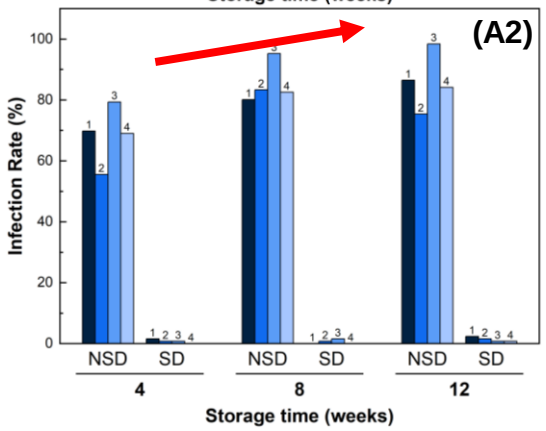
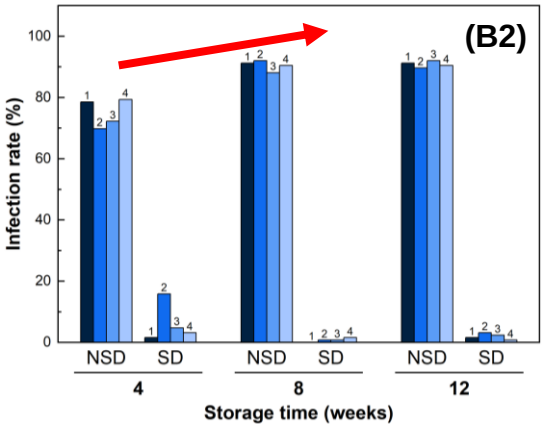
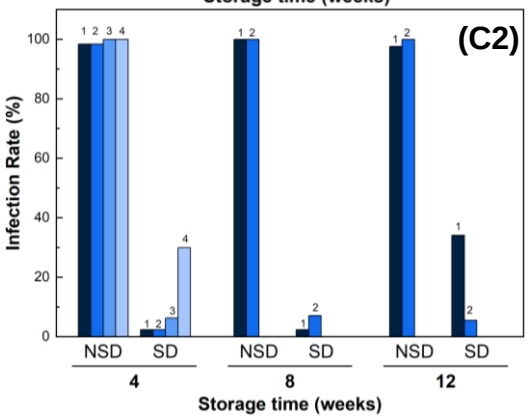
Figure 2. Moisture and water activity of flint corn at 3 different moisture contents in elevated temperature storage (A) Treatment A, 10.94% (as is); (B) Treatment B, 12.33% (as is); (C) Treatment C: 14.67% (as is), during the storage period. Molding occurred in all packages of treatment C #1 at week 10, hence the storage ended.

Infection Rate: a brief introduction

Treatment A (batch 1)		Non-surface disinfected (NSD) — Surface fungi	Surface disinfected (SD) — Endophytic fungi
Week 4 M.C. = 10.37% a_w = 0.4000	**79.96%**		**2.18%**
Week 8 M.C. = 10.40% a_w = 0.4372	**93.25%**		**1.59%**
Week 12 M.C. = 11.04% a_w = 0.5005	**93.06%**		**21.83%**

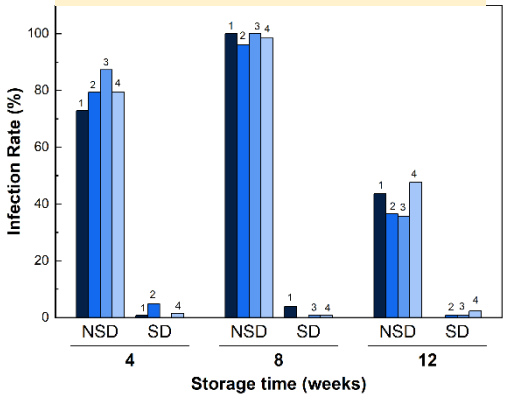
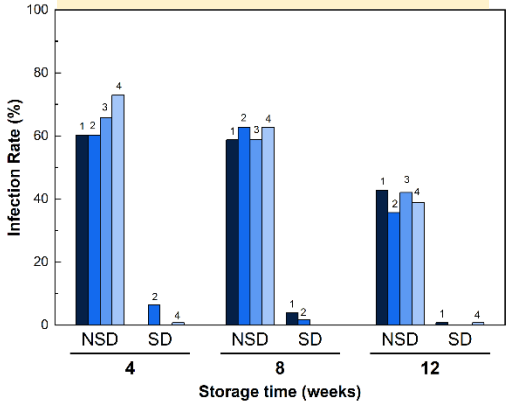
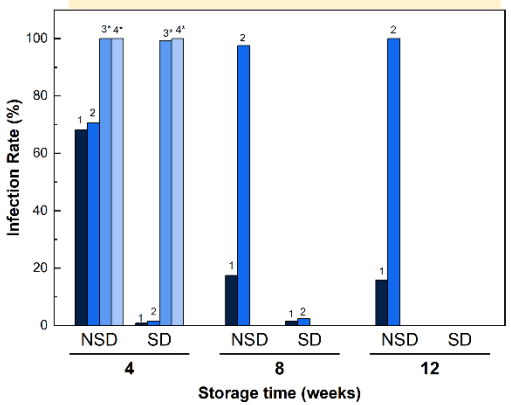
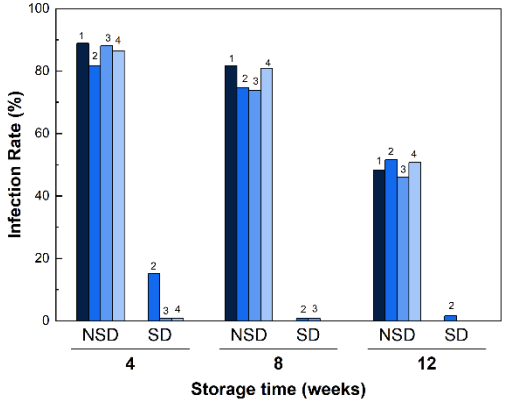
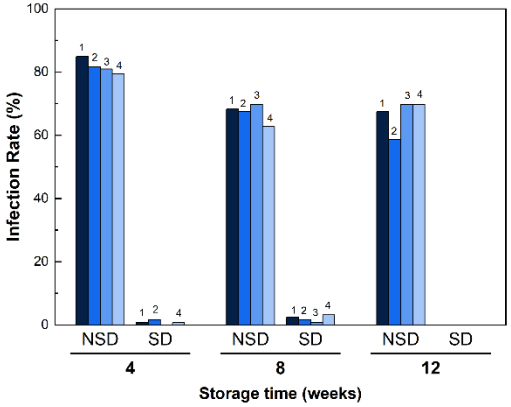
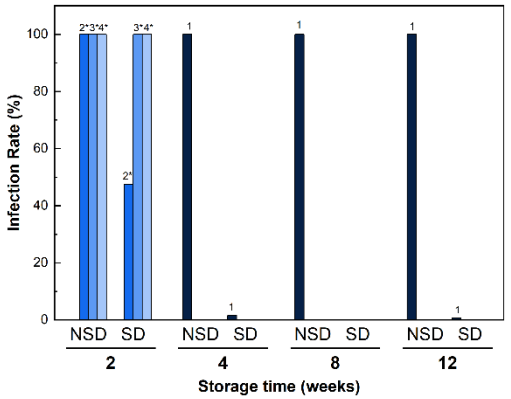
Results and discussions

Infection rate 15°C

	Treatment A: 10.15% M.C.	Treatment B: 11.75% M.C.	Treatment C: 13.20% M.C.
Batch #1			
Batch #2			

Results and discussions

Infection rate 38°C

	Treatment A: 10.94% M.C.	Treatment B: 12.33% M.C.	Treatment C: 14.67% M.C.
Batch #1			
Batch #2			

Results and discussions

Fumonisin content – Cold storage

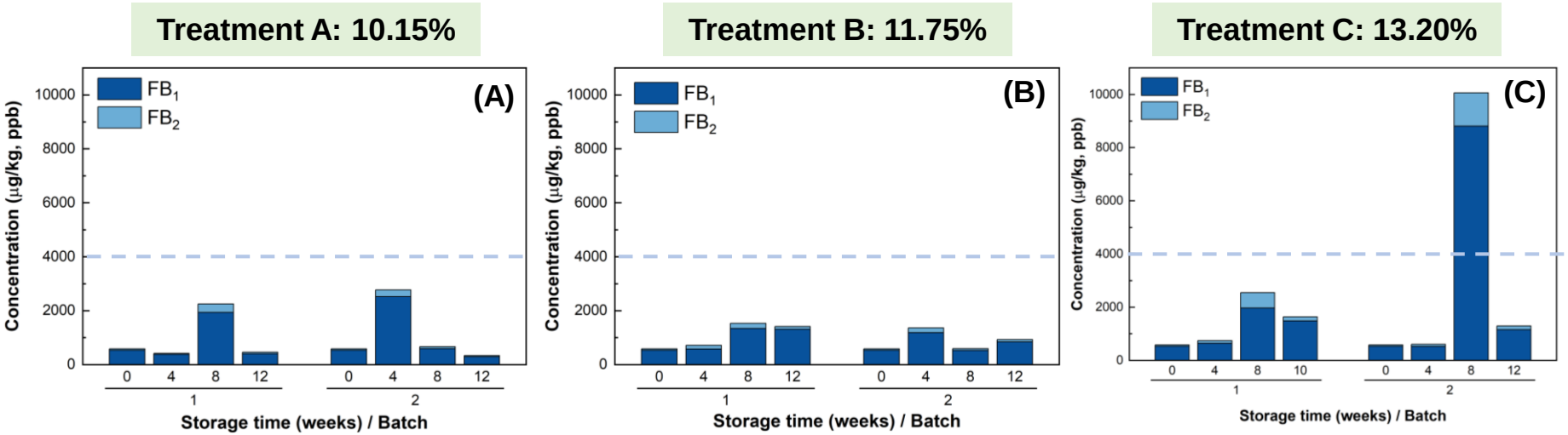


Figure 3. Mycotoxins analyses of the flint corn during storage. Moldiness was observed in all packages of batch 1 of treatment C at week 10, while in batch 2 moldiness was observed in 1 package at week 7 and another 1 package at week 8.

Limit for FB_1 + FB_2 = **4000 µg/kg**
(Ministry of Health and Welfare, Taiwan)

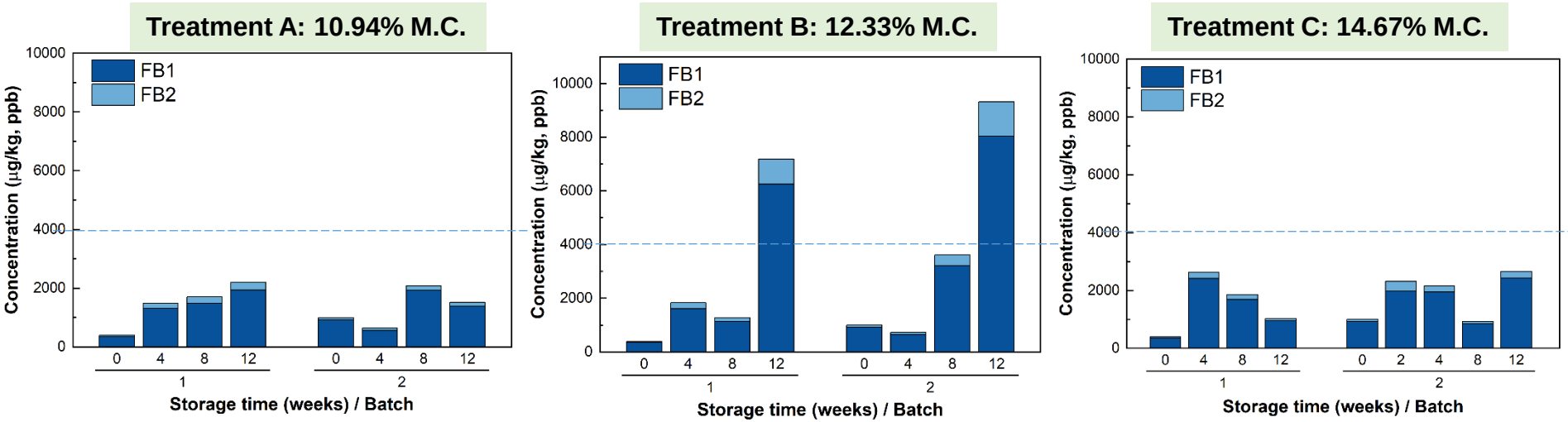
表六、玉米原料及高溫(38℃)儲藏期間(12 週)之真菌毒素含量

Treatment (MC%, wb at 0 wk)	Batch No.	Treatment: Actual moisture content (%, wb)	Treatment: Temp. (℃)	Treatment: Storage time (week)	AFG_2	AFG_1	AFB_2	AFB_1	OTA	T-2	HT-2	DON	ZEA	FB_1	FB_2	FB_1+FB_2
					μg/kg (ppb)											
MF3		12.33	-	0	ND*	ND	ND	ND	ND	ND	ND	ND	ND	346	43	389
				0	ND	ND	ND	ND	ND	ND	ND	ND	ND	925	73	998
A	1	10.94		4	ND	ND	ND	ND	ND	ND	ND	ND	ND	1313	179	1492
(10.97)	2	10.91		4	ND	ND	ND	ND	ND	ND	ND	ND	ND	556	80	636
	1	10.78	38	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	1478	221	1699
	2	10.58		8	ND	ND	ND	ND	ND	ND	ND	ND	ND	1922	161	2083
	1	9.35		12	ND	ND	ND	ND	ND	ND	ND	ND	ND	1939	259	2198
	2	8.88		12	ND	ND	ND	ND	ND	ND	ND	ND	ND	1377	140	1517
B	1	11.88		4	ND	ND	ND	ND	ND	ND	ND	ND	ND	1608	218	1826
(12.33)	2	12.24		4	ND	ND	ND	ND	ND	ND	ND	ND	ND	636	96	732
	1	11.21	38	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	1141	130	1271
	2	11.68		8	ND	ND	ND	ND	ND	ND	ND	ND	ND	3219	390	3609
	1	11.00		12	ND	ND	ND	ND	ND	ND	ND	ND	ND	6247	941	7188
	2	11.17		12	ND	ND	ND	ND	ND	ND	ND	ND	ND	8034	1283	9317
C	2	14.19		2	ND	ND	ND	ND	ND	ND	ND	ND	ND	1966	348	2314
(14.68)	1	12.73		4	ND	ND	ND	ND	ND	ND	ND	ND	ND	1941	220	2161
	2	10.48		4	ND	ND	ND	ND	ND	ND	ND	ND	ND	2416	213	2629
	1	11.03	38	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	854	66	920
	2	9.46		8	ND	ND	ND	ND	ND	ND	ND	ND	ND	1690	166	1856
	1	8.15		12	ND	ND	ND	ND	ND	ND	ND	ND	ND	2429	233	2662
	2	7.33		12	ND	ND	ND	ND	ND	ND	ND	ND	ND	956	69	1025

*Not detected, lower than LOD.

Results and discussions

Fumonisin content – elevated temperature



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Limit for FB_1 + FB_2 = **4000 µg/kg**
(Ministry of Health and Welfare, Taiwan)

Results and discussions

Correlation Analyses - 15°C storage

Table 1. Correlation analyses between moisture, water activity, infection rate (non-surface disinfected), infection rate (surface disinfected), and fumonisins (B_1 and B_2).

Parameters	Moisture	a_w	Infection rate (NSD)	Infection rate (SD)	Fumonisin B_1	Fumonisin B_2
Moisture	1.000					
a_w	**0.974*** [3]**	1.000				
Infection rate NSD	0.224 [NS]	0.294 [NS]	1.000			
Infection rate SD	**0.695** [2]**	**0.681** [1]*	0.257 [NS]	1.000		
Fumonisin B_1	**-0.082 [NS]**	**-0.048 [NS]**	**0.137 [NS]**	**0.042 [NS]**	1.000	
Fumonisin B_2	**-0.086 [NS]**	**-0.106 [NS]**	**0.192 [NS]**	**0.060 [NS]**	0.906***	1.000

[1] Indicated that there was significance at $p = 0.05$
[2] Indicated that there was significance at $p = 0.01$
[3] Indicated that there was significance at $p = 0.001$
[NS] Indicated that there were no significance

Results and discussions

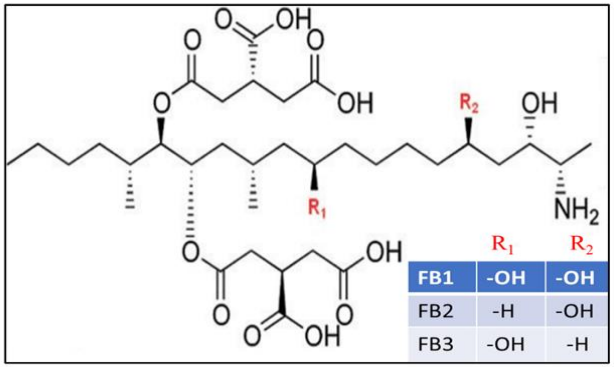
Correlation Analyses - 38°C storage

Table 2. Correlation analyses between moisture, water activity, infection rate (non-surface disinfected), infection rate (surface disinfected), and fumonisins (B_1 and B_2).

Parameters	Moisture	a_w	Infection rate (NSD)	Infection rate (SD)	Fumonisin B_1	Fumonisin B_2
Moisture	1.000					
a_w	**0.892*** [3]**	1.000				
Infection rate NSD	0.161 [NS]	0.256 [NS]	1.000			
Infection rate SD	**0.568** [2]**	**0.641** [2]**	0.415 [NS]	1.000		
Fumonisin B_1	**0.013 [NS]**	**0.066 [NS]**	**-0.287 [NS]**	**-0.033 [NS]**	1.000	
Fumonisin B_2	**0.010 [NS]**	**0.106 [NS]**	**-0.280 [NS]**	**0.024 [NS]**	0.983***	1.000

[1] Indicated that there was significance at $p = 0.05$
[2] Indicated that there was significance at $p = 0.01$
[3] Indicated that there was significance at $p = 0.001$
[NS] Indicated that there were no significance

玉米籽粒長期儲藏之建議



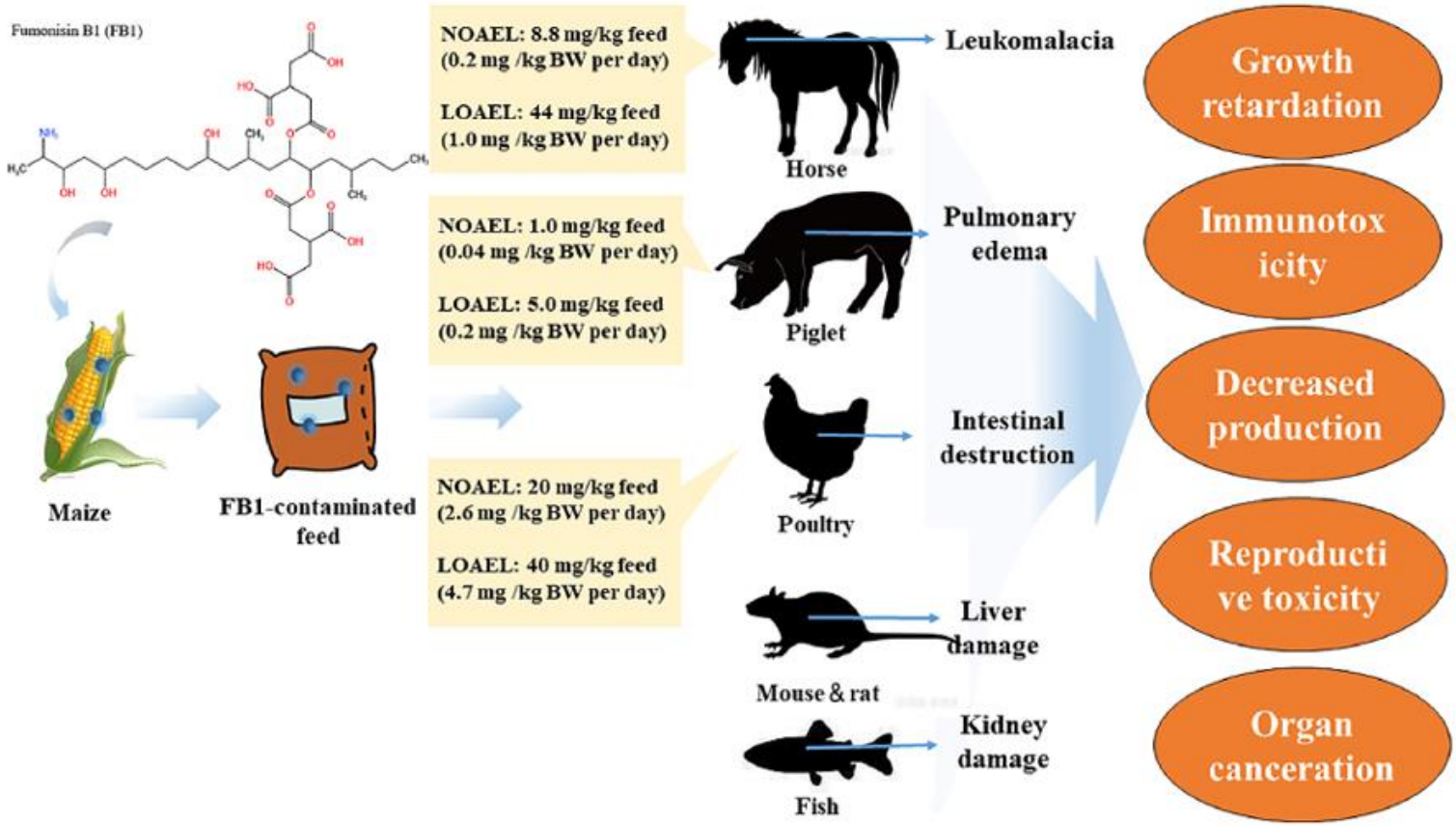
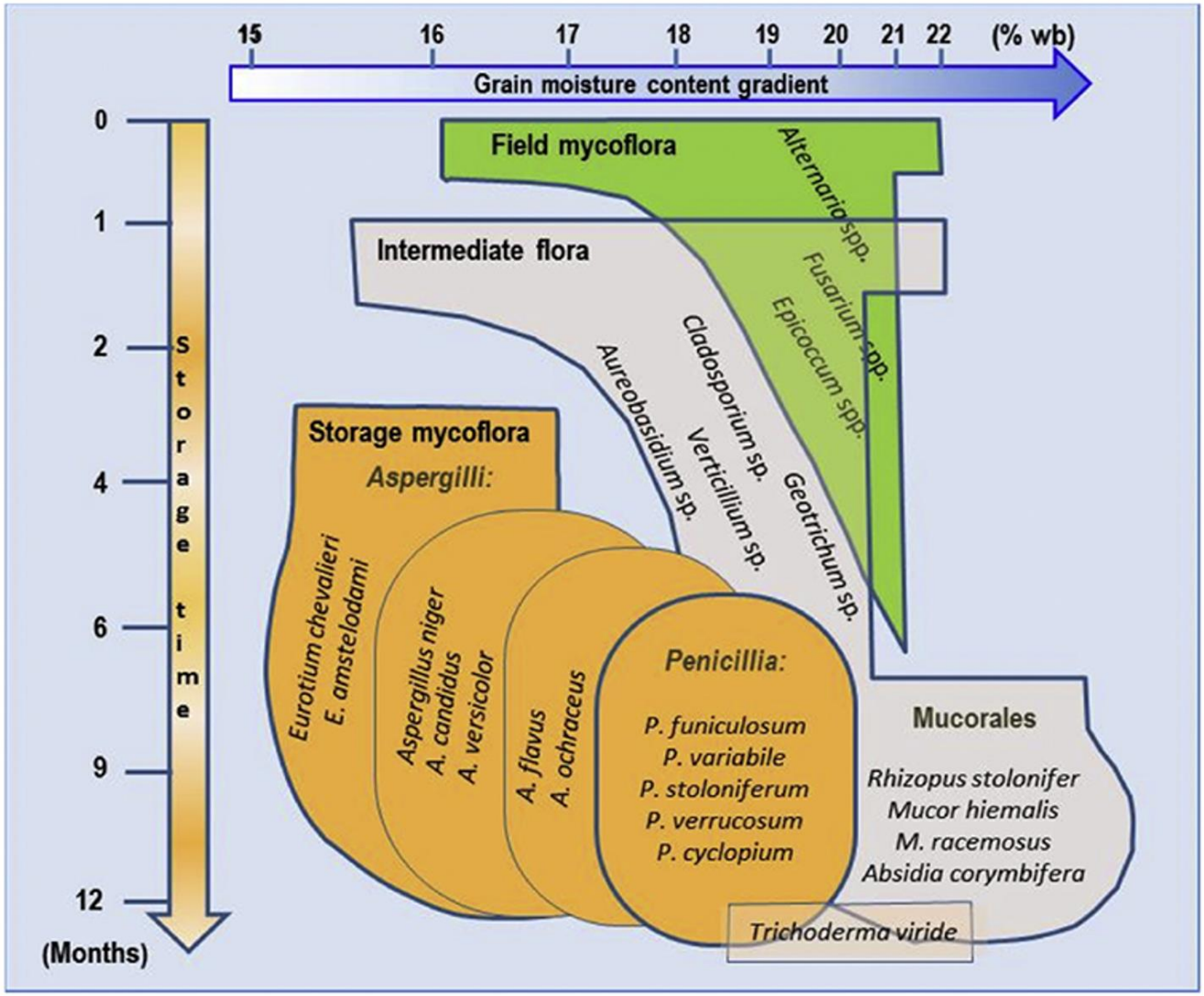


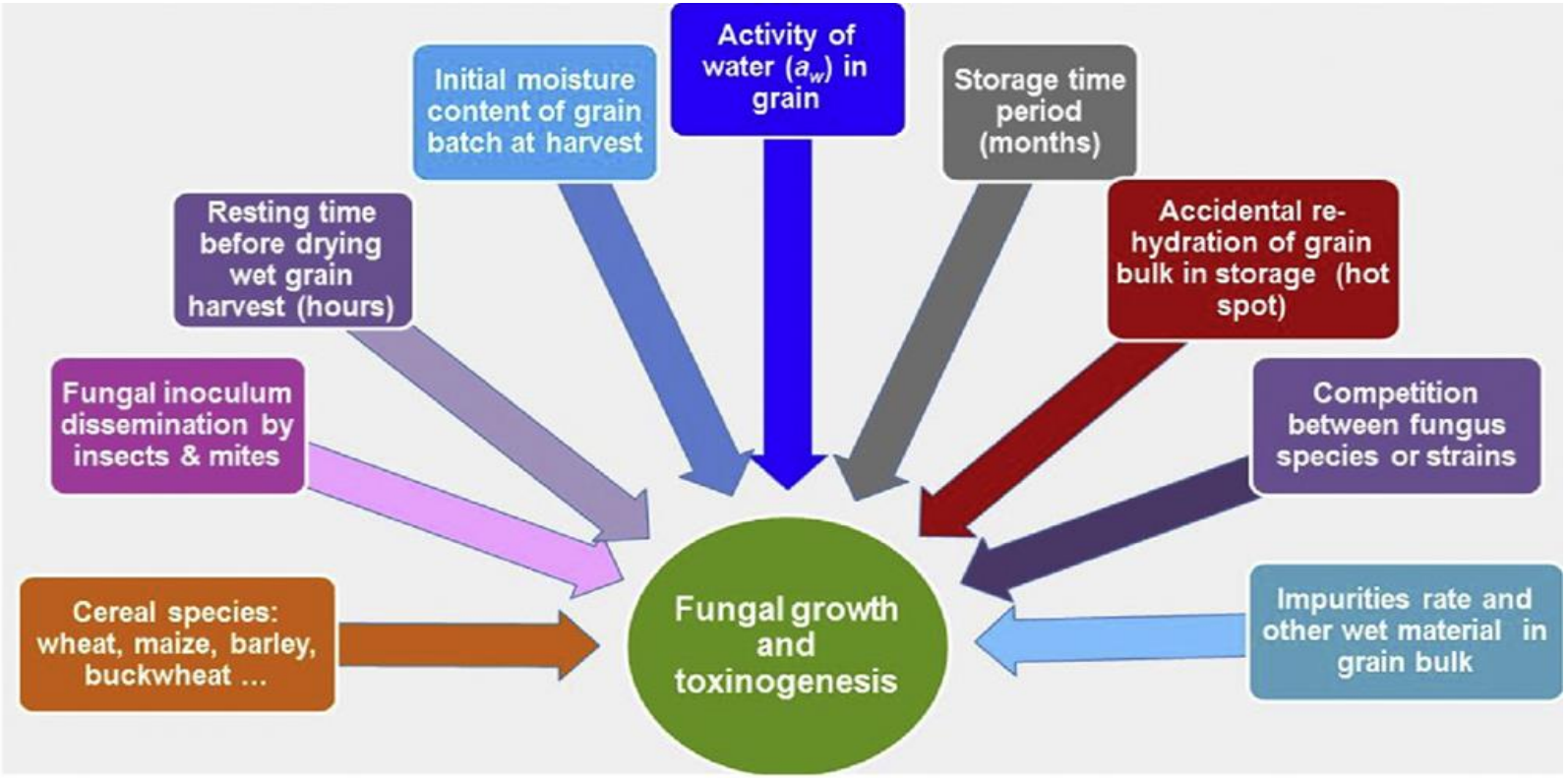
Fig. 2. The toxic effects of FB1 on various systems of animals. NOAEL: No observed adverse effect level. LOAEL: Lowest observed adverse effect level.



圖十九、玉米長期儲藏時之主要汙染真菌種類之生長演替。(Pelhate, 1988

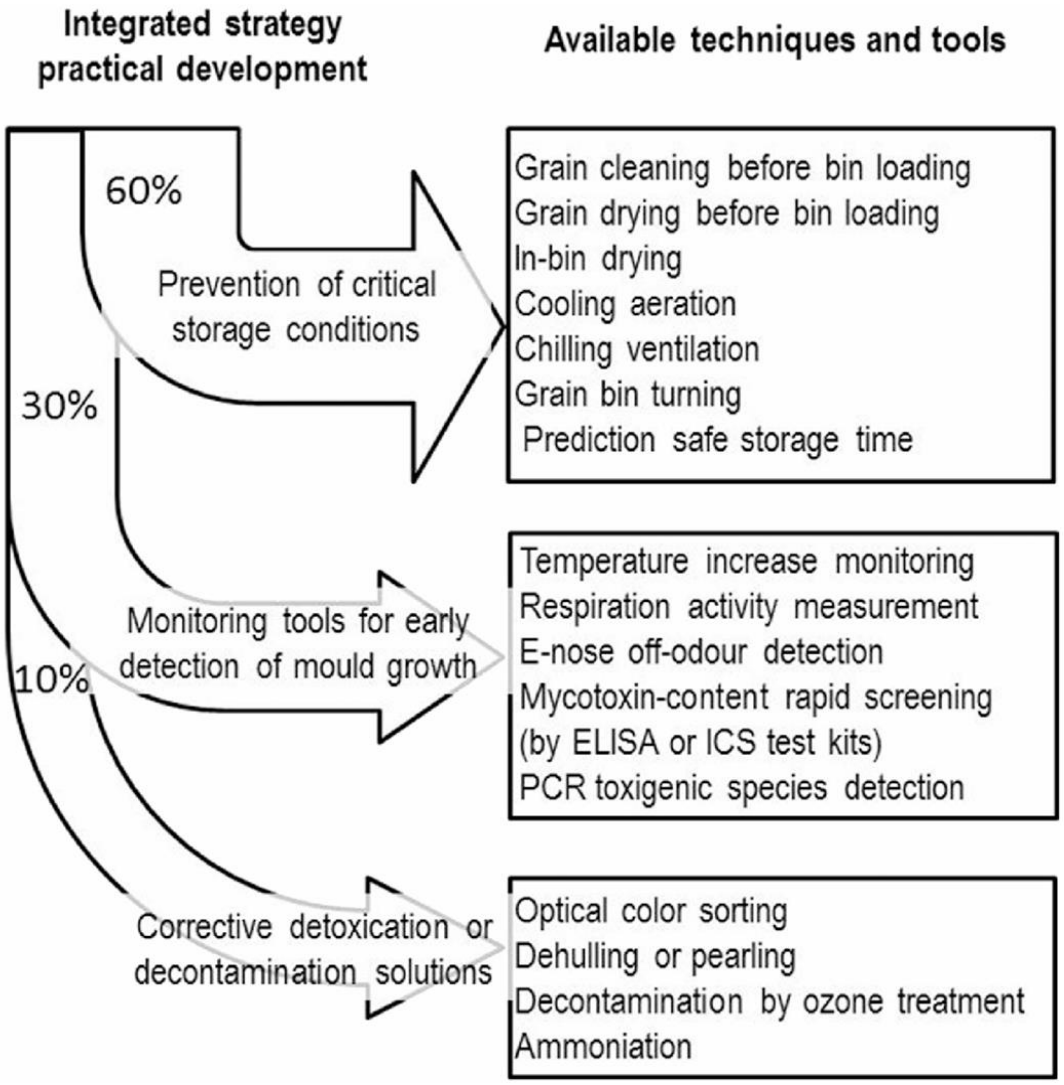
表九、穀類常見產毒素真菌之生長與產毒素之最低相對水活性及水分含量(Fleurat-Lessard, 2017)

Fungus species	Mycotoxin	a_w limit for growth	Related moisture content (%)	a_w limit for toxin production	Related moisture content (%)
Aspergillus flavus	AFB1	0.78-0.84	17.6-19.0	0.84	19.2
A. parasticus	AFB1	0.84	19.0	0.87	20.2
A. ochraceus	OTA	0.77	16.5	0.85	19.6
Penicullus patulum	Patulin	0.81	18.0	0.95	27.0
P. expansum	Patulin	0.82-0.84	18.3-19	0.99	30.0
P. aurantiogiseum	OTA	0.82-0.85	18.5-19.6	0.87-0.9	20.2-22.0
P. verrucosum	OTA	0.80-0.81	18.0	0.83-0.86	18.8-19.8
Fusarium proliferatum	FB1	0.88	20.5	0.93	25.0
F. verticilloides	FB1	0.88	20.5	0.93	25.0



圖二十、穀物長期儲藏時產毒真菌孢子萌發及生長之重要影響因子。

(Fleurat-Lessard, 2017)



圖二十二、穀物長期儲藏(至少1年)之預防性管理真菌腐敗和黴菌毒素污染風險的整合策略；百分比為每組操作(預防、監控或修正解決方案)對應於整體措施的相對影響效益。(Fleurat-Lessard, 2017)

結論

- 硬質玉米原料表面微生物感染率高，但有些玉米籽粒內部曾有微生物感染，推測為田間生長或收穫時，因微生物生長侵入玉米籽粒內。
- 不論是低溫或高溫儲藏，水分含量與水活性、伏馬毒素B_1與B_2均呈非常顯著正相關。
- 水活性與水分含量與表面殺菌之微生物感染率呈正相關性，推測此乃因受*Fusarium*感染之玉米籽粒，在水分含量及水活性提高下，促使其生長，因為其為一種嗜濕性真菌。
- 低水分(15˚C/Treatment E 與38˚C/ Treatment A)與中水分含量(15˚C/Treatment D與38˚C/Treatment B)的伏馬毒素含量以在38˚C儲藏者為高。顯示，高溫對伏馬毒素生成有促進作用，而低溫則有抑制真菌生長之作用。

穀物長期儲藏之降低真菌毒素汙染風險與確保儲藏品質之策略

- 穀物長期儲藏過程中有效控制真菌汙染變質，降低儲藏品質損失的最低風險需要藉由**整合預防措施**和**對真菌和穀物持續監測呼吸速率或產毒素真菌所釋放之"化學特徵"揮發物質**。預防手段則包括育種或選擇種植可抵抗真菌感染之穀物品種、利用生物防治方法或使用可防止穀穗(ears)及玉米芯(cobs)蟲害之殺蟲劑，使穀粒在田間收穫前及收穫時可以最大限度地減少產毒素真菌的侵入。
- 最有效預防穀物受真菌汙染之手段則是**在穀物收穫後立即進行穀物清潔和乾燥(24-48小時內完成乾燥程序)，必須將水分含量降低至微生物可生長之閾值**，也就是安全儲存的水分含量。

感謝聆聽

敬請指教