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Studies on the Growth and Control of Storage Fungi in Stored Paddy Rice

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米穀貯藏에 있어서 貯藏菌類의 生育 및 抑制에 관한 研究

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Abstract

Environmental conditions of fungal growth on Korean rice grain, Milyang No.23, were studied and optimum treatment conditions of the selected fumigants for controlling fungal deterioration of rice were investigated. The results are as follows.

1. Most of the fungi grew well above 80% relative humidity and 19% moisture content within 10-30 days. But at 12.5-13.0% moisture content of grains, only *Aspergillus candidus, Asperillus versicolor, Penicillium chrysogenum*, and *Trichothecium roseum* were developed within 2 months. The other fungi were only detectable for their mycelial growth under microscopic observation.
2. Among the ten fumigants tested to control the fungal growth on the rice, ethylene oxide was found to be the most effective at a level of 16mg/ℓ for 48 hrs treatment and the next was methyl bromide (32mg/ℓ), acrilonitrile and methyl iodide (64mg/ℓ). The other fumigants, such as ammonia, methyl formate, ethyl formate, carbon dioxide and propionic acid were found less effective under the tested conditions.

Introduction

According to the conservative estimate, 1-2% of the world's grain production is lost by due to the activities of microorganisms, while the adventitious effect of their activities cause even large waste.

Fungal deterioration of stored grains includes not only the loss of viability, but also reducing the quality and nutritive value by off flavor, caking, emyme activities and toxins produced.

The attempts for using chemical preservative and fumigants for inhibiting mold growth on stored grains, in the past, by various workers, was reviewed by Majumder *et al* (1) However, none of the treatments were found to be completely successful in preventing fungal growth.

Korea produces about 6,000,000M/T of rice annually and rice is maintained in several storage types at farm house level in the form of paddy itself or brown rice. In the previous work, the storage fungi were isolated and identitied from the stored Korean rice samples[2], and in this paper we will report on the result of environmental conditions of fungal growth on paddy and brown rice, and of optimium treatment conditions of fumigants for controlling fungal deterioration of brown and paddy rice.

Materials and Methods

Observation of the fungal growth in stored rice.

Growth characteristics of selected fungi at different relative humidities and moisture levels were carried out at room temperature (18-28°C).

About 5g of sterile rice and paddy samples were put into the sterile petridish pan (diameter 3.5cm) aseptically, and inoculated with each fungal spores separately, and kept in desiccators with previously adjusted relative humidities (60-100%) using different concentrations of glycerol in water.

Moisture content of test rice after sterilisation was 19.0%. The observation of fungal growth was made every five days for a period of one month. Fungal vegetative mycelia and spores were examined under the stereomicroscope.

For the observation of fungal growth at various moisture levels, 250ml Erlenmyer flasks were used. 50g of rice or paddy were put into the above flask and their moisture levels adjusted from 13% to 30% by addition of the required amount of distilled water. After sterilisation, fungal spores were inoculated aseptically and the samples were incubated at room temperature for one month.

Fumigant selection

For the selection of suitable fumigants, the method used by Ragunathan *et al.*[3] was followed.

Fungal spores grown on rice grain were fumigated for 24-120 hours and each grain was plated on Czapeck's agar after the exposure period. The growth and percentage reduction of fungal population was determined.

Fumigation was conducted in 2.4ℓ capacity vacuum desiccators, specially designed for the purpose which formed fumatoria. Gaseous fumigants were introduced into the evacuated desiccator by using glass syringe. The desiccator was restored to atmospheric pressure immediately thereafter. Liquid fumigant like ammonia water was injected by using a syringe on to a filter paper disk kept inside the desiccator. CO_2 was introduced directly to the desiccator from CO_2 gas cylinder after complete evacuation of desiccator. Propionic acid was introduced into desiccator in its gaseous form, by applying air bubbles through propionic acid bubbler and the staturated air was employed as fumigant on test grain samples.

Results and Discussion

Effect of environmental conditions on the fungal growth in rice and paddy

The relationship existing between the relative humidity of storage atmosphere and moisture content of rice and paddy grains were studied. Table 1 shows the effect of relative humidity on the fungal growth of rice.

Ten species of *Aspergillus*, five species of *Penicillium* and one species of *Trichothecium*, *Nigrospora*, *Alternaria*, *Curvalaria*, *Rhizopus*, *Mucor* and *Fusarium* were tested in this study. Sterilised rice and non-sterilised rice were kept under the same conditions as control.

As shown in Table 1, *A. candidus*, *P. chrysogenum*, and *Trichothecium roseum* were well grown at 70% relative humidity. AT 70% relative humidity, it was observed that sporulation has occurred only at germ portion of rice inoculated with, but *P. chrysogenum* and *Trichothecium roseum* sporulated on the whole surface of the grains. All the fungi tested were growing well and sporulated at 100% relative humdity. Fungi originated from field did not grow at 90% relative humdity, except some mycelial growth of *Rhizopus nigricans*.

A. fumigatus and *A. fischeri* grew showly in rice at all levels of relative humidity tested. Because these fungi commonly grow at higher temperature

Table 1. Effect of relative humidity on the fungal growth on rice

Storage fungi	Relative humidity (%)									
	M^{60}	S	M^{70}	S	M^{80}	S	M^{90}	S	M^{100}	S
A. oryzae	G+	——	W+	——	W+	G+	W+	W+	W+++	W+++
A. flavus	W+	——	W+	——	W+	——	W+	W+	W+++	W+++
A. fumigatus	——	——	——	——	——	——	W+	——	W++	W++
A. fischeri	——	——	——	——	——	——	G+	——	W++	W++
A. versicolor	——	——	——	——	W+	G+	W+	W+	W+++	W+++
A. nidulans	W+	——	W+	G+	W+	W+	W+	W+	W+++	W+++
A. chevalieri	W+	——	W+	G+	W+	W+	W+	W+	W+++	W+++
A. sydowii	——	——	G+	G+	W+	W+	W+	W+	W+++	W+++
A. candidus	W+	G++	W+	G++	W+	W+	W+	W+	W+++	W+++
A. ruber	W+	——	W+	G+	W+	W+	W+	W+	W+++	W+++
P. notatum	W+	——	W+	——	W+	G+	W+	G+	W+++	W+++
P. cyaneofurvum	W+	——	W+	G+	W+	G++	W+	W++	W+++	W+++
P. chrysogenum	W+	——	W+	W+	W+	W+	W+	W+	W+++	W+++
P. steckii	W+	——	W+	——	W+	G+	W+	G+	W++	W++
P. atramentosum	W+	——	W+	——	W+	G+	W+	G+	W++	W+
Trichothecium roseum	G+	——	W+	W+	W+	W+	W+	W+	W++	W+++
Nigrospora sphaerica	——	——	——	——	——	——	——	——	W+++	W+
Altermaria grisea	——	——	——	——	——	——	——	——	W+++	W++
Curvalaria interseminata	——	——	——	——	——	——	——	——	W+++	W++
Rhizopus nigricans	——	——	——	——	——	——	W+	——	W+++	W++
Mucor sp.	——	——	——	——	——	——	——	——	W+++	W++
Fusarium sp.	——	——	——	——	——	——	——	——	W+++	W+
Sterilised rice	——	——	——	——	——	——	——	——	——	——
Non sterilised rice	——	——	W+	——	W+	W+	W+	W+	W+++	W+++

After 1 month at room temperature, moisture of rice 19.0%, non sterilised rice 13.5%
M: Mycelia ; S: Spore; G: Germ portion only; W: Whole grain surface; +: Scant growth
++: Moderate growth; +++: Heavy growth

than the tested conditions. The most species of *Pencillium* tested in this study showed well developed mycelia and good sporulation above 70% relative humidity.

It was found that in non-sterilised rice mycelia has developed above 70% relative humdity and spore formation has occured above 80% relative humidity. However, mycelial growth and sporulation were not detected at 60% relative humidity.

Under the conditions of 60% relative humdity, we could store rice grain safely without any treatment, but further studies may be necessary to examine whether fungal deterioration at these conditions occurs.

Table 2 shows the fungal sporulation on rice at various relative humidities.

A. candidus was the only spore forming organism at all levels of relative humidities within 15 days as found in this study.

Most of the strains sporulated within 5 days at 100% relative humidity but some of the species have delayed in sporulation.

Table 3 shows the growth patterns of storage fungi on paddy and rice at 12.5% and 13% moisture

Table 2. Effect of relative humidity on the fungal sporulation on rice

Storage fungi	Relative humidity (%)				
	60	70	80	90	100
		Number of days			
A. oryzae	--	--	15*	10	5
A. flavus	--	--	15	10	5
A. fumigatus	--	--	--	--	30
A. fischeri	--	--	--	--	30
A. versicolor	--	--	15	10	5
A. nidulans	--	30	15	15	5
A. chevalieri	--	15	15	15	10
A. sydowii	--	20	15	15	10
A. candidus	15	10	10	5	5
A. ruber	--	20	15	15	10
P. notatum	--	--	15	15	5
P. chrysogenum	--	15	10	5	5
P. cyaneofurvum	--	15	10	10	5
P. steckii	--	30	20	20	10
P. atramentosum	--	--	30	20	10
Trichothecium roseum	--	20	20	15	5
Non-sterilised rice	--	--	20	15	5

* The number indicate the days after inoculation

contents. As shown in Table 3 the most well growing fungi on paddy were *A. candidus, A. versicolor,* and *Trichothecium roseum.* On rice, most of the fingal growth were not seen with naked eye at these conditions, but mycelial growth and sporulation were detectable when observed under the stereomicroscope.

The effect of moisture content on the fungal growth in rice is shown in Table 4. At 13 and 17% moisture levels in grains all the test fungi mycelial growth and sporulation were not detected until 30 days, however, at 30% moisture cotent, heavy mycelial growth were detected within 3-6 day and moderate sporulation was observed within 4-10 days at 22% moisture content.

Ghosh (4) has already mentioned that *A. glaucus* and *A. candidus* can grow on rice at 70% relative humidity or below, whereas *Mucor* spp. and

Table 4. Effect of moisture content on the fungal deterioration of rice

Storage fungi	Moisture content (%)			
	13	17	22	30
A. fumigatus	--(30)	--(30)	+(4)	+++(3)
A. candidus	--	--	++(5)	+++(5)
P. notatum	--	--	+(10)	+++(4)
P. cyaneofurvum	--	--	+(10)	++(4)
Trichothecium roseum	--	--	+(10)	++(6)

-- : No visible sporulation
\+ : Scent sporulation
++ : Moderate sporulation
+++ : Heavy sporulation
Number indicate days observed

Fusarium spp. required about 85% of relative humidity for their growths.

Shown (5) also studied microscopically the moldy growing at fixed moisture content in vaious feedstuffs held at 60-70°F. He noted that certain species of *A. glaucus* group and *A candidus* grew at relative humidities ranging from 100% to as low as 65%. Other *Penicillia, Aspergilli,* and *Mucor* species grew only at higher relative humdity than 70%. At 100% relative humidity, members of the Mucorales developed rapidly within 2 to 3 days, and were accompanied within a week by members of the *A. glaucus* group and *Penicillium* species, within another week by *A. niger,* within third week by *A. candidus* and finally by members of Fungi Imperfecti, such as *Sporotricum.*

Majumder *et al* (1) have reported that at 80% relative humidity and at a temperature of 28-31°C can cause visible mold growth in rice and other grains, but there was no visible mold growth at the treatment of rice samples stored at 58% relative humidity. Also, *A. flavus* was the predominant species found to grow at 70% relative humidity, and *Mucor* and *Fusarium* at higher humidities (85%).

Thus the present study has cleared the limiting relative humidity which could be utilised by ecosystem of the molds grown on stored rice.

Table 3. Growth pattern of storage fungi on paddy and rice

Storage fungi	Paddy			Rice		
	NE	SM		NE	SM	
		Mycelia	Spore		Mycelia	Spore
A. oryzae	+	+	+	–	+	+
A. flavus	+	+	+	–	+	+
A. fumigatus	–	–	–	–	+	+
A. fischeri	–	–	–	–	+	–
A. versicolor	++	+	+++	–	+	+
A. nidulans	–	–	–	–	+	–
A. chevalieri	–	–	–	–	+	+
A. sydowii	+	+	+	–	+	+
A. candidus	+++	+++	+++	–	+	+
A. ruber	–	+	+	–	+	+
P. notatum	+	–	+	–	+	–
P. chrysogenum	++	+	++	–	+	–
P. cyaneofurvum	+	–	++	–	+	–
P. skeckii	–	–	–	–	+	–
P. atramentosum	+	+	–	–	+	–
Trichothecium roseum	+++	+++	+++	–	+	+
Nigrospora sphaerica	–	–	–	*	*	*
Alternaria grisea	–	–	–	*	*	*
Cutvalaria interseminata	–	–	–	*	*	*
Rhizopus nigricans	–	+	–	*	*	*
Mucor sp.	+	+	–	*	*	*
Fusarium sp.	+	+	–	*	*	*

After 2 months at room temperature (18-28 °C), relative humidity 100%

Initial moisture content: Paddy 13.0%, Rice 12.5%

NE: Naked eye observation; -: No detectable growth; +: Growth present, ++: Moderate growth; +++: Heavy growth;

SM: Stereomicrospic observation; -: Not detectable; +: Growth and spore formation observed

* : Not tested

Selection of Fumigants and optimum conditions for the control of fungal growth on rice

In order to select the suitable fumigants for the control of storage fungi growing on rice, ten fumigants were tested against 23 fungi belonging to *Aspergilli, Penicillia,* and other field fungi at selected concentrations. As recorded in Table 5, the effective dosages of fumigants in controlling most of the fungal growths in rice were acrilontrile (64mg/1), ethylene oxide (16mg/1), methyl bromide (32mg/1) and methyl iodide (64mg/1). However, some other fumigants were also effective on a few species at dosage level given in this study.

It was found that the most resistant species against fumigants were *A. flavus* and *A. fumigatus.* The next resistant species were *A. fischeri, A. nidulans,* and *A. versicolor.* When compared to the species of storage fungi such as *Aspergillus* and *Penicillium,* those fumigants were effective to the field fungi.

Table 5. Effect fumigants on storage fungi of rice

Storage fungi	Fumigants									
	1	2	3	4	5	6	7	8	9	10
A. oryzae	+	+	+	+	+	–	+	+	–	+
A. flavus	+	+	+	+	+	–	+	+	+	+
A. fumigatus	+	+	+	+	+	–	+	+	+	+
A. fischeri	+	+	+	+	+	–	+	+	80	+
A. versicolor	–	+	+	+	+	–	–	+	50	+
A. nidulans	+	+	+	+	+	–	+	+	20	+
A. chevalier	–	+	+	+	+	–	–	+	–	+
A. sydowii	–	+	+	+	+	–	–	+	–	+
A. candidus	–	+	+	+	+	–	–	+	–	+
A. ruber	–	+	+	+	+	–	–	+	–	+
P. notatum	–	+	+	+	+	–	–	+	–	+
P. cyaneofurvum	–	+	+	+	+	–	–	+	–	+
P. steckii	–	+	+	+	+	–	–	+	–	+
P. chrysogenum	–	–	+	+	+	–	–	+	–	+
P. atramentosum	–	+	+	+	+	–	–	+	–	+
Trichothecium roseum	–	80	+	+	+	–	–	+	–	–
Nigrosphora sphaerica	–	–	+	+	–	–	–	+	–	60
Alternaria grisea	–	–	+	–	+	–	–	40	10	–
Curvalaria interseminata	–	–	+	+	+	–	–	+	20	+
Rhizopus nigricans	–	+	+	+	+	–	–	+	–	+
Mucor sp.	–	+	+	+	+	–	–	+	+	+
Fusarium sp.	–	–	+	+	+	–	–	+	10	+
Helminthosporium sp.	–	–	+	+	+	–	–	+	–	–

Fumigants: 48 hours treatment
Numericals represents the percentage growth

1. Acrilonitrile, 64mg/l
2. Ammonia, 64mg/l
3. Carbon dioxide, 100% (gas)
4. Ethyl formate, 96mg/l
5. Ethylene dibromide, 64mg/l
6. Ethylene oxide, 16mg/l
7. Methyl bromide, 32mg/l
8. Methyl formate, 96mg/l
9. Methyl iodide, 64mg/l
10. Propionic acid, 100% (as gas)

+ : 100% growth; - : No growth

Exposure to carbon dioxide for 48 hours was not effective in controlling fungal growth. However, amonia (64mg/l) and propinonic acid (gas form) were efffective on certain species.

Ethyl formate, ethylene dibromide, and methyl formate were not effective in controlling the fungal growth at the level of dosage tested in this study. Table 6 shows the effective dosage for controlling fungal growth on rice at various concentration of fumigants against selected fungi. The optimum dosage of each fumigant tested were acrilonitrile 64mg/l, ethylene oxide 16mg/l, methyl bromide 32mg/l and methyl iodide 64mg/l.

A. flavus and *P. notatum* were used as test organism to confirm the minimum dosage requirement and exposure time of selected fumigants. As

Table 6. Effect of selected fumigant concentration on the growth of fungi in rice

Dosage (mg/1)	Fungi				
	A. flavus	*A. candidus*	*P. notatum*	*P. cyaneofurvum*	*Trich. roseum*
Acrilonitrile					
32	+	+	+	+	+
64	–	–	–	–	–
96	–	–	–	–	–
128	–	–	–	–	–
Ethylene oxide					
4	+	+	+	+	+
8	10	–	–	–	–
16	–	–	–	–	–
32	–	–	–	–	–
Methyl bromide					
8	+	+	+	+	+
16	40	40	–	20	30
32	–	–	–	–	–
64	–	–	–	–	–
Methyl iodide					
32	+	+	+	+	40
64	–	–	–	–	–
96	–	–	–	–	–
128	–	–	–	–	–

Fumigants: 48 hours treatment
Numerical represents the percentage growth
+ : 100% growth
- : No growth

shown in Table 7, exposure time of fumigants were prolonged depending on the concentration of fumigants.

At a level of 4mg/1 dosage, minimum exposure time of ethylene oxide was 96 hours, but at 8mg/1 concentration, 72 hours treatment was enough to control both *A. flavus* and *P. notatum.*

Five days exposure of 8mg/ 1 of methyl bromide was not enough to control both *A. flavus* and *P. notatum,* but at 16mg/1, and 32mg/1 of methyl bromide was effective in controlling both fungi with 96 hours and 48 hours treatment respectively. For controlling the mould growth on rice,higher dosage and short period of exposure was more effective than lower dosage and long period treatment. But it is necessary to study the economics of such fumigation.

Schroeder (6) reported that sodium propionate was able to control only *A. candidus* but not *A. glaucus.*

Rangaswamy *et al* (7) concluded that both acetic and propionic acids at 0.1% level are effective in controlling the fungal infection for more than four months in sorghum with moisture content up to

Table 7. Effect of dosage and exposure time of the fumigants against *A. flavus* and *P. notatum*

Fumigants	Dosage (mg/1)	Exposure time (hrs)	Control of growth(%) *A. flavus*	*P. notatum*
Ethylene oxide	4	24	00	00
		48	00	00
		72	70	80
		96	100	100
	8	24	00	00
		48	90	100
		72	100	100
Methyl bromide	8	72	00	00
		96	00	00
		120	00	90
	16	48	60	90
		72	70	100
		96	100	100
	32	24	75	100
		48	100	100

16%. But later they found that both acetic and propionic acids were found to be ineffective in controlling the fungal infection at 0.2% level when the moisture content of sorghum was 20% and above.[1] In this study propionic acid incorporated as gaseous state was not effective against both *Aspergilli* and *Penicillia* tested. This might be due to the concentration of propionic acid falling below the required threshold levels. Future studies are required in this line.

Jay and Pearman [8] have used carbon dioxide for control of insect infestation in stored corn. Cardern and Carmi [9] assumed that the carbon dioxide acted as a carrier and facilitated the penetration of the methyl bromide in their studies on fumigation trials with a mixture of methyl bromide and carbon dioxide in vertical bins. In this study, it is found that the treatment of carbon dioxide alone for a period of 48 hours is not effective for the control of storage fungi.

The fungicidal action of methyl bromide and ethylene oxide was reported by Srinivasan and Majumder. [10]

Ragunathan *et al* [2] also studied on the control of internal fungi of sorghum by fumigation. Out of eight fumigants tested, ethylene oxide (32mg/1), methyl bromide (64mg/1) were effective in controlling the internal fungi. In an experiment on controlling toxigenic fungi, using fumigants, Majumder *et al* [11] confirmed that ammonia (96mg/1) could control all the four species of *A. fumigatus, A. flavus, A. candidus* and *P. islandicum* completely. But methyl bromide (96mg/1) did not help control *A. fumigatus* and ethylene oxide (32mg/1) was not effective on controlling *A. candidus*. In this study, it is found that ammonia (64mg/1) was not effective in controlling all the species of *Aspergillus* tested but a few species of *Penicillium* and field fungi were controlled by this fumigant. In this study, the effective optimum dosage of methyl bromide and ethylene oxide were slightly lower than those of the above results.

Recently Rajendran and Muthu [12] have studied the minimum dosage requirements of methyl bromide for effective fumigation of food commodities. They found that the minimum effective dosage were 8-48mg/1 depending on food commodities. Methyl iodide, methyl formate, ethyl formate, and acrilonitrile were tested by earlier workers but their effect was not promising when compared with ethylene oxide at the same level of dosage and exposure times.

These results have thrown light on a following-up study which should include the design of storage bin or Tongari in terms of gaseous disinfection and humidity control inside the grain bulk.

要 約

韓國産 米穀 밀양 25號의 貯藏中에 出現한 菌類의 生育環境條件을 調査하고, 이들 菌類에의한 米穀의 變敗를 抑制하기 위하여 選定된 燻蒸劑의 最適處理條件을 檢討하여 다음과 같은 結果를 얻었다.

1. 大部分의 菌類는 水分含量 19%, 相對濕度 80% 以上의 米穀에서는 10～30日 以內에 잘 生育하였다. 그러나 水分含量 12.5～13.0 %의 米穀에서는 *Aspergillus candidus, Asperigillus versicolor, Penicillium* chrysogenum 및 *Trichothecium roseum*만 2個月 以內에 肉眼으로 生育이 確認되었고, 其他의 菌類는 顯微鏡下에서 菌糸의 生育만 觀察할 수 있었다.

2. 10種類의·供試燻蒸劑中에서 貯藏菌類의 生育을 抑制하는데 가장 効果가 있었던 것은 ethylene oxide였으며 그 最低處理濃度 및 時間은 16mg/ℓ와 48時間이었다. 同一處理時間에서 methylbromid는 32mg/ℓ, acrilonitrile과 methyl iodide는 64mg/l가 最低處理濃度였으며, ammonia, methyl formate, ethyl formate, carbon dioxide 및 propionic acid는 주어진 處理條件에서 거의 効果가 없었다.

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