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Mycotoxin Production and Animal Toxicity of Molds Isolated from Discolored Sun-Dried Red Pepper (*Capsicum annuum* L.)

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변질된 건조고추에서 분리한 곰팡이의 독소 생성 및 독성작용

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ABSTRACT – Mycotoxins produced by molds isolated from discolored sun-dried red pepper fruits were determined and the toxicity to animals was also tested by feeding mold-grown unpolished rice to rats. Among the mold species tested, only *Alternaria alternata* was toxic to experimental animals, while other mold species belonging to the genera of *Colletotrichum, Diaporthe, Diaporthopsis, Botryosphereia, Aspergillus* and Fusarium were not. Rats fed *Alternaria*-grown rice lost weight and died within two weeks of feeding period. Succumbed rats during the process of feeding study showed extreme cases of enlargements of stomach, small intestine and liver. Among the 17 Alternaria isolates, 8 species produced considerable amount of tenuazonic acid along with small amounts of other toxins including alternariol and monomethyl ether derivative of alternariol in both red pepper homogenate and unpolished rice. It is therefore advised that red pepper fruits infested by molds during the sun-drying process be discarded to avoid unnoticeable health hazards.

Key words: *Alternaria alternata*, red pepper (*Capsicum annuum* L.), mycotoxins, tenuazonic acid, cytotoxicity

Introduction

Korean people prefer sun-dried red pepper to that dehydrated by other artificial means, because of better hue and taste[16]. Natural sun-drying of whole red pepper fruits is inefficient and takes long time to achieve desired level of dehydration. Therefore pepper fruits are prone to suffer mold infection followed by mold soft-rot[14,17]. Mold-grown pepper fruits suffer soft-rot spoilage during the process of dehydration and discoloration defects on the completion of dehydration; from red color to white to off-white color, resulting in quality deterioration. This causes economic loss to producing farmers and also causes safety concern over mycotoxins[14]. Red pepper products are one of the most widely used condiments in Korea, and an average Korean consumes about 2.3 kg of red pepper every year[2].

Red pepper is not dehydrated by natural sun-drying method in any other countries in the world. It is dehydrated by artificial heat drying methods after pepper fruits are cut into small pieces to make dehydration process more efficient. Therefore all other countries except Korea do not have the mold soft rot problem. Therefore the health related research concerning mold-grown pepper fruits has not been raised in other parts of the world.

Since fresh red pepper fruits from the plant contain approximately 85% water[3] and the surfaces of the fruits are surrounded by wax layer, it is very hard to dehydrate the whole uncut pepper fruits. It takes one to two weeks depending upon the weather of drying season[15,17]. The loss due to mold infection of pepper fruits during the process of natural sun-drying is in the range of 12.9 to 36.6%[11]. If the moisture content of the atmosphere is higher, the loss due to mold growth becomes greater.

Mold species belonging to *Alternaria* are frequently found

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from dried spices including red pepper[5,12,18] and other agricultural products[1,10,12,13,18,19,21,22,28-30] and produce cytotoxic toxins. Many species of *Alternaria* including *A. alternata* (*tenuis*), *A. dauci, A. brassicae* and *A. cucumerina* are known to produce toxins[18]. *A. alternata* is known to infect red pepper fruits during storage[18,19]. Species belonging to the genera *Alternaria* are plant pathogens and thus contaminate food through infection on the field as well as through storage[25]. Mycotoxin production by other types of molds draws attention in other agricultural materials worldwide[4,7-9,20].

Nine molds representing different groups of molds previously isolated from discolored dried pepper fruits were tested for animal toxicity by feeding mold-grown unpolished brown rice to experimental animals. *Alternaria* toxins were analyzed for all 17 *Alternaria* isolates after growing them in autoclaved red pepper homogenate and on autoclaved unpolished brown rice.

Materials and Methods

Materials and molds isolated from red pepper

Dried red pepper fruits discolored due to mold growth were collected from all over the Korean peninsula. Method of mold isolation was mentioned previously[14]. The molds were grown on steamed rice at 25°C for 7 days before freeze-dried, powdered and served for animal feeding. All 17 *Alternaria* isolates were grown in autoclaved fresh red pepper homogenates and on steamed rice for toxin analysis. Fresh red pepper fruits and brown rice were purchased locally. *Alternaria* toxins including tenuazonic acid (TeA), alternariol (AOH), alternariol monomethyl ether (AME) and alternuene (ALT), were purchased from Sigma Chem. Co. (St. Louis, MO, USA).

Animal feeding test

Mold-grown brown rice was freeze-dried before ground to powder by using a Waring blender. Ninety male Sprague-Dawley rats (5-week old; Samtako Inc., Kyunggido, Korea) were purchased and each rat was housed in an individual cage and fed 5 and 10% mold-grown unpolished rice with a basal diet in powder form. Nine rats were used for the test of each mold and 9 rats received control diet without mold-grown rice. Experimental animals were weighed every week and major symptoms and death were recorded. Surviving rats were sacrificed and examined for pathological changes of body organs including stomach, small intestine and liver. One strain from each group of mold isolates was chosen for animal feeding test. They were *Colletotrichum acutatum* (C-4), *Colletotrichum acutatum* f.sp. *chromogenum* (T-1), *Colletotrichum caricae* (C-18), *Diaporthe phaseolum* var. *soyae* (AD-7), *Diaporthopsis angelicae* (L-4), *Alternaria alternata* (B-2), *Botryosphereia ribis* (J-7), *Aspergillus aryzae/flavus* (P-5) and *Fusarium incarnatum* (D-8).

Analysis of Alternaria mycotoxins

Five gram each of *Alternaria*-grown rice and red pepper homogenate was extracted with a mixture of hexane (30 mL)

Table 1. Production of *Alternaria* mycotoxins (μg/g) in homogenized red pepper and on unpolished brown rice inoculated with 17 isolates of *A. alternata* isolated from discolored sun-dried red peppers

Alternaria isolates	ALT		AOH		AME		TeA	
	Pepper	Rice	Pepper	Rice	Pepper	Rice	Pepper	Rice
AA-5	-	-	-	△	-	-	-	-
B-1	-	-	-	△	-	△	404	768
B-2	-	-	-	△	-	△	459	1572
B-3	-	-	-	△	-	△	529	947
D-9	-	-	-	△	-	-	-	-
D-10	-	-	-	-	-	-	-	-
E-1	-	-	-	-	-	-	-	-
E-2	-	-	-	-	-	-	-	-
E-4	-	-	-	△	-	△	307	865
H-4	-	-	-	△	-	-	-	-
H-13	-	-	-	-	-	△	1050	1268
H-16	-	-	-	-	-	△	871	1067
J-2	-	-	-	△	-	-	440	488
J-5	-	-	-	-	-	-	-	-
KM-4	-	-	-	△	-	△	115	522
T-2	-	-	-	-	-	△	-	-
T-4	-	-	-		-	°,	-	-

△ : Trace amount, -: Not detected, ALT: alternuene, AOH: alternariol, AME: alternariol monomethyl ether, TeA: tenuazonic acid

and methanol (15 mL), acidified with 0.5 mL concentrated HCl before the mixture was homogenized by a Waring blender and centrifuged at 17600 × g for 40 min (HMR-2001 V Centrifuge, Hanil Industrial Co., Incheon, Korea) to remove insolubles. Equal amount of water was added to 10 mL methanol layer, and *Alternaria* toxins were extracted twice with 5 mL chloroform. The combined chloroform extract was evaporated to dryness. The residue was dissolved in 1 mL of chloroform and analyzed for Alternaria toxins by HPLC[5]. *Alternaria* toxins were separated at room temperature on a reverse phase C18 column (Discovery HS F5; 15 cm × 4.6 mm, 5 μm; Sigma-Aldrich Co., St. Louis, Mo., U.S.A.) connected to a Waters 600 solvent delivery system (Waters Co., Milford, Ma., U.S.A.) and Waters 486 (Waters Co.) spectrophotometric detector. AOH and AME were monitored at 257 nm, and TeA and ALT at 280 nm[18,19].

Results and Discussion

Toxin production by Alternaria isolates

The production of toxins by 17 *Alternaria* molds isolated from discolored red pepper fruits was tested by growing them both in homogenized fresh red pepper fruits and on autoclaved brown rice. These *Alternaria* molds were among the 197 molds isolated from discolored dried red pepper fruits[14] and all were identified to be *A. alternata*. *A. alternata* comprised only 8.6% of the total isolates. *A. alternata*, however, were reported to be the frequently isolated species of molds from red pepper cultivated in Korea and produce cytotoxic mycotoxins[18,19].

Considerable amounts of tenuazonic acid (TeA) were produced both in red pepper homogenates and on brown rice by 8 isolates out of 17 strains (Table 1). The amount of TeA ranged between 520 and 1570 μg/g on brown rice and between 115 and 1050 μg/g in red pepper homogenate. The major toxin produced by *A. alternata* was TeA in another study[23]. Other toxins including alternariol (AOH) and alternariol monomethyl ether (AME) were detected at very low levels only from brown rice, but not from pepper homogenates. It was certain that *Alternaria* species produce toxins in red pepper fruits. Brown rice was a better medium for the production of toxins by *Alternaria* molds. Alternuene (ALT) was not produced by any of pepper mold isolates in both media used. Four *Alternaria* isolates (D-10, E-1, E-2, J-5) did not produce any of the 4 known toxins. Mycotoxin production patterns by *Alternaria* molds are different even in a same species[19] and almost all the Alternaria isolates from natural sources produced at least one kind of toxins[19,25].

Animal toxicity of molds isolated from red pepper fruits

Nine molds representing different groups of 197 molds previously isolated from red pepper[14] were tested for animal toxicity. The TeA most-producing strain of *A. alternata* (B-2) was included (Table 1) because of the following reason. Alternaria isolates producing TeA above certain level were all highly toxic (lethal) to experimental animals while those producing other toxins but not TeA were not[6,19]. Therefore, as long as the safety of red pepper is concerned, *Alternaria* mycotoxin to be watched on is TeA which is a protein synthesis inhibitor in vivo and in vitro[27].

Animals fed alternaria-grown rice consumed feed only about one fifth of the amount consumed by control (Table 3). Alternaria-grown rice-fed animals lost body weight by about 30% during the first week of feeding experiment (Table 2). All other animals fed with rice grown with different mold isolates were not affected.

Only *A. alternata*-grown brown rice caused death of rats

Table 2. Body weight (g) changes of rats fed a diet with 10% unpolished rice grown with molds isolated from discolored sun-dried red pepper fruits

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
Control	143.8 ± 3.2[1)ns2)]	210.3 ± 3 .4[bc 3)]	245.6 ± 3.9[abc]	281.2 ± 4.0[ab]	304.4 ± 5.1[bc]
C-4	143.7 ± 3.1	207.1 ± 2.7[bc]	238.8 ± 5.3[a]	275.4 ± 5.1[a]	295.8 ± 5.9[abc]
T-1	144.1 ± 3.2	214.2 ± 2.6[bc]	250.2 ± 2.4[abc]	284.3 ± 2.5[ab]	307.4 ± 4.1[c]
C-18	143.9 ± 3.0	207.7 ± 4.6[bc]	244.4 ± 4.6[abc]	277.7 ± 5.4[a]	295.0 ± 6.2[abc]
AD-7	144.9 ± 3.3	205.1 ± 3.9[b]	238.7 ± 3.7[a]	274.3 ± 4.3[a]	284.8 ± 4.9[a]
L-4	144.7 ± 2.5	216.2 ± 2.3[c]	253.9 ± 2.7[c]	292.0 ± 3.4[b]	311.7 ± 5.0[c]
B-2	145.6 ± 2.9	105.4 ± 3.0[a]	-[4)]	-	-
J-7	147.7 ± 2.3	209.9 ± 3.0[bc]	251.8 ± 2.7[bc]	286.9 ± 3.2[ab]	307.9 ± 4.5[c]
P-5	144.9 ± 2.4	205.0 ± 4.8[b]	242.3 ± 5.1[abc]	280.3 ± 4.8[ab]	298.4 ± 5.9[abc]
D-8	145.0 ± 2.3	206.4 ± 1.6[bc]	240.1 ± 2.2[ab]	274.1 ± 3.5[a]	290.8 ± 4.4[ab]

[1)] Values are expressed as mean ± SE

[2)] Not significant

[3)] Values within a column with different superscripts are significantly different at $p < 0.05$

[4)] No data were obtained due to death of rats in this group

Table 3. Feed intake (g/day) changes of rats fed a diet with 10% unpolished rice grown with molds isolated from discolored sun-dried red pepper fruits

Groups	Week 1	Week 2	Week 3	Week 4
Control	18.9 ± 0.3[1)bc2)]	19.6 ± 0.1[ab]	19.8 ± 0.2[b]	19.9 ± 0.1[b]
C-4	18.5 ± 0.4[bc]	19.1 ± 0.5[ab]	19.4 ± 0.3[ab]	19.4 ± 0.3[ab]
T-1	19.0 ± 0.3[bc]	19.4 ± 0.3[ab]	19.3 ± 0.4[ab]	19.4 ± 0.2[ab]
C-18	17.8 ± 0.4[b]	18.8 ± 0.4[a]	18.9 ± 0.5[a]	19.1 ± 0.3[a]
AD-7	17.9 ± 0.5[b]	18.8 ± 0.4[a]	19.6 ± 0.2[ab]	19.7 ± 0.1[ab]
L-4	19.5 ± 0.3[c]	19.9 ± 0.1[b]	19.8 ± 0.2[ab]	19.9 ± 0.1[b]
B-2	4.3 ± 0.4[a]	-[3)]	-	-
J-7	18.4 ± 0.4[bc]	19.3 ± 0.2[ab]	19.5 ± 0.2[ab]	19.7 ± 0.2[ab]
P-5	18.1 ± 0.5[b]	18.8 ± 0.3[a]	19.5 ± 0.2[ab]	19.5 ± 0.2[ab]
D-8	18.0 ± 0.3[b]	19.3 ± 0.2[ab]	19.2 ± 0.3[ab]	19.4 ± 0.2[ab]

[1)] Values are expressed as mean ± SE
[2)] Values within a column with different superscripts are significantly different at $p < 0.05$
[3)] No data were obtained due to death of rats in this group

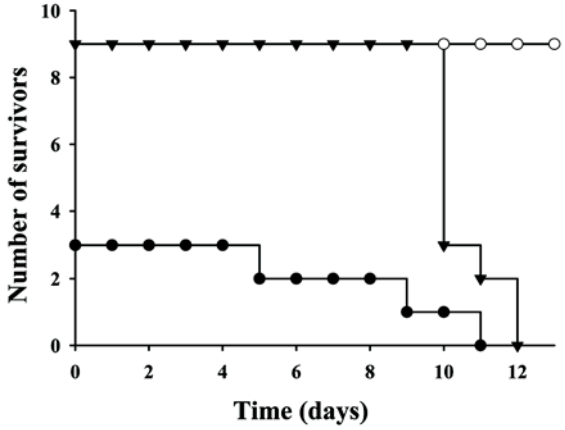

Fig. 1. Lethality of *Alternaria* B-2(*A. alternata*)-grown unpolished rice to rats.
○ : control, ▼ : Fed *Alternaria*-grown rice (10% in diet), ● : Fed *Alternaria*-grown rice (5% in diet)

within 12 days on first trial with experimental diet ontaining 10% *Alternaria*-grown rice (Fig. 1). Six out of 9 rats died on 10th day, one on 11th, and the last two on the 12th day of feeding test, when the diet contained *Alternaria*-grown rice at 10% level. When additional three rats were fed *Alternaria* rice at 5% level, death of the animals came about earlier than those fed at 10% level. One died on 5th day, another one on 9th day, and the last one on 11th day. Rats fed *Alternaria*-grown rice lost approximately 45% of their weight during the first week of the feeding experiment (Table 2). Earlier death of rats fed lower *Alternaria* rice than those fed higher *Alternaria* rice was explained that total moldy rice, and naturally total toxin(s), consumed by the animals was greater with 5% *Alternaria* rice-fed group. The amount of feed consumed by the animals was greater in the 5% *Alternaria* rice-fed group (data not shown). In the case with 10% *Alternaria* rice, the consumption of diet was extremely low (Table 3). During the first week of the feeding experiment, the rats consumed only minimal amount of diet (4.3 g/day) compared with that (18.9 g/day) of those rats of control group (Table 3). Red pepper molds other than *Alternaria* were not observed to be toxic to rats. The major clinical signs of dead rats were enlargements of stomach, small intestines, and liver.

In conclusion, mold-infested red pepper products including red pepper powder has a possibility of posing health hazard to those who consume it in large quantities. Since Koreans are the only people who like to consume sun-dried red pepper, the possible mycotoxin hazard problem is unique only to them. It is therefore advised that red pepper fruits infested by molds during the sun-drying process be discarded to avoid unnoticeable health hazards, or that Koreans discard the tradition of consuming red pepper products prepared from sun-dried whole pepper fruits.

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요 약

희나리 고추에서 분리한 곰팡이를 현미에 배양하여 실험동물(rat)에 투여하였을 때 *Colletotrichum, Diaporthe, Diaporthopsis, Botryosphereia, Aspergillus, Fusarium*의 경우 독성을 나타내지 않았으나 *Alternaria alternata*로 동정된 곰팡이는 독성을 나타내었다. *A. alternata*를 배양한 현미를 투여한 실험동물은 사료 섭취량이 상대적으로 낮았으며, 체중 감소를 보였고 2주안에 치사하였으며, 위, 소장, 간의 확대가 관찰되었다. *Alternaria*속 곰팡이는 17종

이 분리되었으며 이들 중 8종은 고추즙과 현미에 배양하였을 때 모두 상당량의 tenuazonic acid를 생산하였고, 현미 배지에서만 alternariol과 alternariol monomethyl ether 독소가 추가로 소량 검출되었다. 건조 과정에서 곰팡이가 번식된 고추는 유해한 대사물의 존재 가능성이 있으므로 식품 안전을 위해 곰팡이 번식을 최대한 억제할 필요가 있다.

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