

# Inhibitory Effect of Green Onion on the Growth of *Aspergillus parasiticus*

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This study was performed to investigate the inhibitory effect of green onion produced in Korea on the growth of *A. parasiticus*, a toxigenic strain. The effect was studied using different concentrations of freeze-dried green onion in solid culture with potato-dextrose agar (PDA) or in liquid culture with yeast-extract sucrose (YES) broth at 25°C for 15 days. The addition of green onion to the media showed inhibiting the fungal growth after three days of cultivation. The 1.0% concentration of green onion significantly reduced fungal diameter on PDA on the 3rd day, 6th day and 9th day, while 0.1%, 0.5% and 1.0% concentrations of green onion significantly reduced the mycelial weight on YES broth on the 9th day, 12th day and 15th day ( $p < 0.05$ ). When inhibition of the fungal growth due to increasing the concentration of green onion was observed over time, remarkable effect was observed on the 9th day in the solid culture and on the 12th day in the liquid culture. As a result of addition of green onion, the fungal diameter on PDA was reduced by 4.6% ~ 11.5%, and mycelial weight on YES was reduced by 16.9% ~ 40.8% at those times. Dose-response relationships were observed between the concentration of green onion and inhibition of growth both in solid culture and in liquid culture during the incubation periods. This study indicates that a human consumption level of green onion produced in Korea could be an effective inhibitor of the growth of *A. parasiticus*.

**Keywords** green onion; *A. parasiticus*; PDA; YES broth

## 1. Introduction

Green onion is a perennial herb which belongs to *Allium* of the Alliaceae and has been used as vegetables or spices worldwide for a long time. It is also called as scallion, Welsh onion, spring onion, or Japanese bunching onion. Green onion is typically used in cooking in Asian countries such as Korea, Japan and China. As an important crop, especially in Korea, approximately 500 thousand tons are produced per year [1]. The production of green onion was 542,981 tons, and annual supply per capita was 8.05 kg in Korea in 2006 [2-3]. According to the National Health and Nutrition Examination Survey in Korea in 2007, the intake of green onion per person per day was 10.9 g, equivalent to 0.9% of total food intake of the year [4].

As interests in the bioactive compounds of the plants of *Allium* are increased recently, studies on their antimicrobial activity and antioxidant activity have been also increased [5]. The *Allium* plants are usually known to have inhibiting activity against pathogenic bacteria, fungi, mycotoxins, and putretive bacteria [6]. For example, antimicrobial activity of garlic, scallion, chives, onions, shallots, and onion extract has been studied, and inhibitory effect of *Allium* plants on the growth of *Aspergillus species* had been reported [7].

Among the many useful ingredients of green onion, especially methyl methanethiosulfinate and 10-dialk(en)yl thiosulfonates are volatile substance containing sulfur [8]. It has been reported that sulphur-containing compounds can suppress the growth of fungi [9]. There was research indicated that the monoglyceride and tianshic acid isolated from the seeds of green onion showed antifungal activity and tianshic acid presented higher activity than monoglyceride [10].

*Aspergillus* sp. commonly presents in soil or in the environment, and has been used for human life in a wide range. Some species of *Aspergillus* are useful in the production of traditional Korean foods such as fermented soy sauce and soybean paste. However, *A. flavus*, *A. parasiticus*, and *A. nomius* are highly toxic and are known to produce aflatoxin, a human carcinogen, in certain conditions [11].

For years, protection of food from harmful fungi has been drew great attention and various suggestions, such as physical, chemical and biological methods were tried to control harmful fungi. These methods may be improper to apply to food, or some methods would make food inedible even though applied. Therefore, some scholars have made efforts to find proper ways. Among the numerous and abundant components that can be easily obtained, plant extracts have been considered as natural inhibitors and can be used as an alternative controller of pathogens. Inhibitory effects of *Allium* plants on the toxic fungi have been reported [12-14], however, a study on green onion produced in Korea is still rare, to the author's knowledge.

Green onion is an important ingredient in Korean cuisine. It is one of the most frequently used spices in Korean foods. The purpose of this study is to investigate the effect of green onion (*Allium fistulosum* L.) on the growth of *A. parasiticus*, an aflatoxigenic strain, which is consumed on a daily basis in Korea. We believe that this study on daily food will further contribute to reducing harmful effects of the mold.

## 2. Materials and methods

### 2.1. Green onion

Green onion, *Allium fistulosum* L., produced in Korea (Gyeongsangbuk-do) was purchased from a local market. The green onion was freeze-dried and used for this study. The recovery of freeze-dried green onion was 7.8% of raw material.

### 2.2. Fungal strain and inoculum preparation

Fungal inoculum was prepared from single-spore cultures of *A. parasiticus* ATCC 15517. Commercial potato-dextrose agar (PDA) (Difco Lab., Detroit, Mich., U.S.A.) slant was used to support the production of spores of *A. parasiticus*. The strain was subcultured onto a PDA slant and incubated for 10 days at 25 °C. The fungus was grown on PDA in Petri plates for 8 days at 25 °C to ensure maximum conidial formation.

Spores were washed from the plates with sterile distilled water containing 0.1% Tween 80. The concentration of dislodged spores was determined with a hemacytometer and diluted to 10<sup>6</sup> conidia/ml. Spore suspensions were prepared one day before inoculation and stored at 4 °C.

### 2.3. Growth media and condition

Commercial PDA medium was used for growth of *A. parasiticus* ATCC 15517 in solid culture. Yeast-extract sucrose (YES) broth medium was used for growth of *A. parasiticus* ATCC 15517 in liquid culture according to the method of Kim et al. [15]. A volume of one liter of this liquid medium contains 20 g of yeast-extract and 200 of sucrose. The pH of the PDA and YES broth was adjusted to 5.6. The media were sterilized at 121 °C for 15 min and cooled to room temperature. Concentrations of the green onion in the media were 0.1%, 0.5% and 1.0% (w/v).

Culture tubes and PDA plates, each containing the same volume of the prepared medium, were inoculated with spore suspension of *A. parasiticus*, and then incubated aerobically at 25 °C for 15 days. An *A. parasiticus* culture grown in the absence of green onion was used as control.

### 2.4. Determination of fungal growth

Diameters of the growing fungal colonies in solid culture were measured and used to plot growth curves. Mycelial mats from liquid culture were collected on dried, preweighed Whatman No. 1 filter paper. They were then washed with distilled water and dried at 55~60 °C overnight. The dry weight of mycelial mats was used as the measurement of fungal growth in liquid culture. Fungal growth in both cultures was measured at every three days.

### 2.5. Statistical analysis

The data obtained from samples were compared by the analysis of variance. Significant differences among means were determined by using Duncan's multiple range test. Differences were considered significant at  $p < 0.05$ .

## 3. Results

### 3.1. Effects of green onion on the growth of *A. parasiticus* in solid culture

Diameters of the growing fungal colonies on PDA with green onion were measured at every 3 days during the 15-day incubation period and used to determine growth in solid culture. The effects of green onion on the growth of *A. parasiticus* on PDA are shown in Table 1 and Fig. 1.

Green onion at each level ranging from 0.1% to 1.0% reduced fungal growth to varying degrees. Overall, the increase of the concentration rate of green onion more inhibited the growth of the strain. The growth of *A. parasiticus* was significantly reduced by the concentration of green onion at 0.5% and 1.0% on the 3rd day and also on the 6th day ( $p < 0.05$ ).

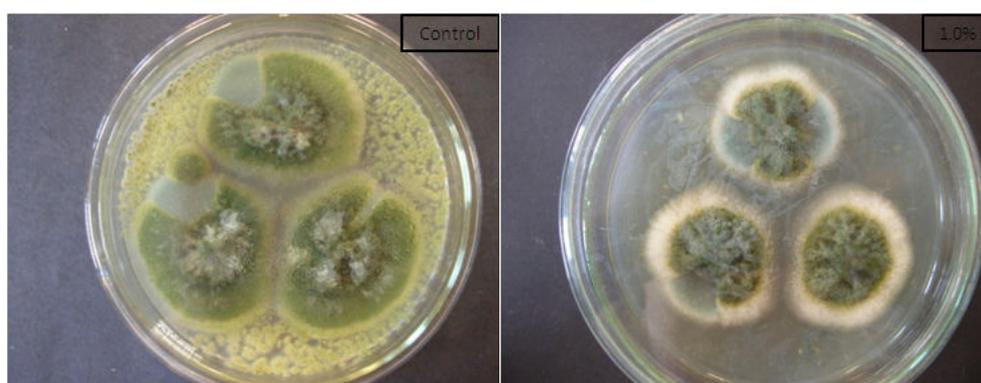
Maximum inhibition of the growth of *A. parasiticus* on PDA was observed on the 9th day when 1.0% of green onion concentration was added to PDA (Fig. 1). At this time the percentage of inhibition of diameter was 4.6%, 6.9%, and 11.5%, respectively, and fungal growth was significantly affected by the concentration of green onion at 1.0% ( $p < 0.05$ ). No more growth was observed on the PDA plates over 9 days.

**Table 1.** Inhibitory effect of green onion on the growth of *A. parasiticus* on potato-dextrose agar

Concentration of green onion	Diameter (mm)				
	3 days	6 days	9 days	12 days	15 days
Control	20.2±0.4 <sup>a</sup>	37.2±2.8 <sup>a</sup>	39.3±3.9 <sup>a</sup>	39.3±3.9 <sup>a</sup>	39.3±3.9 <sup>a</sup>
0.1%	20.1±0.1 <sup>ab</sup>	35.2±0.8 <sup>ab</sup>	37.5±1.0 <sup>ab</sup>	37.5±1.0 <sup>ab</sup>	37.5±1.0 <sup>ab</sup>
0.5%	19.6±0.3 <sup>b</sup>	34.7±0.5 <sup>b</sup>	36.6±0.4 <sup>ab</sup>	36.6±0.4 <sup>ab</sup>	36.6±0.4 <sup>ab</sup>
1.0%	18.8±0.7 <sup>c</sup>	33.6±2.1 <sup>b</sup>	34.8±2.3 <sup>b</sup>	34.8±2.3 <sup>b</sup>	34.8±2.3 <sup>b</sup>

All values represent the mean ± S.D. of six samples.

Values with different superscript letters in columns are significantly different ( $p < 0.05$ ).



**Fig. 1.** Growth of *A. parasiticus* on potato-dextrose agar containing green onion incubated at 25 °C for 9 days.

Left: control, right: 1.0% concentration of green onion.

### 3.2. Effects of green onion on the growth of *A. parasiticus* in liquid culture

The growth of *A. parasiticus* ATCC 15517 on YES broth with green onion was monitored by mycelial mat at every 3 days during the 15-day incubation period. The effects of green onion on the growth of *A. parasiticus* on YES broth are shown in Table 2 and Fig. 2.

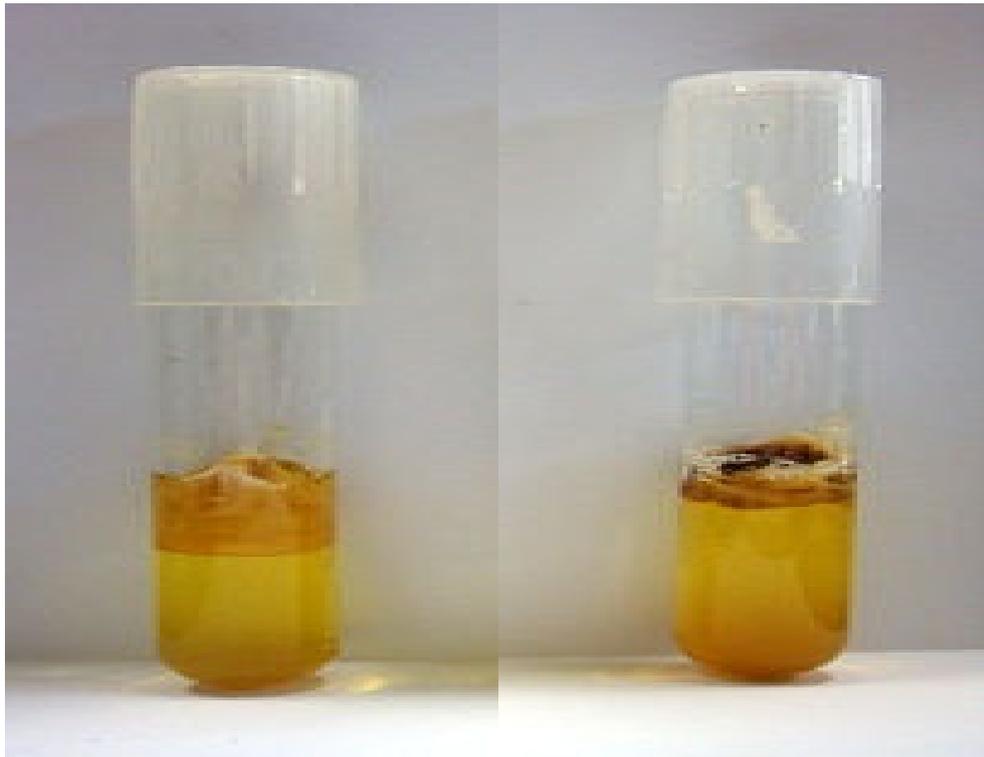
The growth of *A. parasiticus* was affected by the addition of green onion to YES broth during the incubation period although the growth of the strain increased over time. With increasing rates of green onion concentration, the degree of fungal growth inhibition was increased. The mycelial growth of *A. parasiticus* was significantly reduced by the concentration of green onion at 0.5% and 1.0% on the 3rd day and also on the 6th day ( $p < 0.05$ ). After 9 days of cultivation, 0.1%, 0.5% and 1.0% concentration showed significant differences compared with the control group ( $p < 0.05$ ). This tendency was maintained until the end of incubation. The most inhibition of mycelial growth was exhibited on the 12th day when 1.0% of green onion concentration was added to the YES broth (Fig. 2). At this time the percentage of inhibition of mycelial weight was 16.9%, 32.4%, and 40.8%, respectively.

**Table 2.** Inhibitory effect of green onion on the growth of *A. parasiticus* on yeast-extract sucrose broth

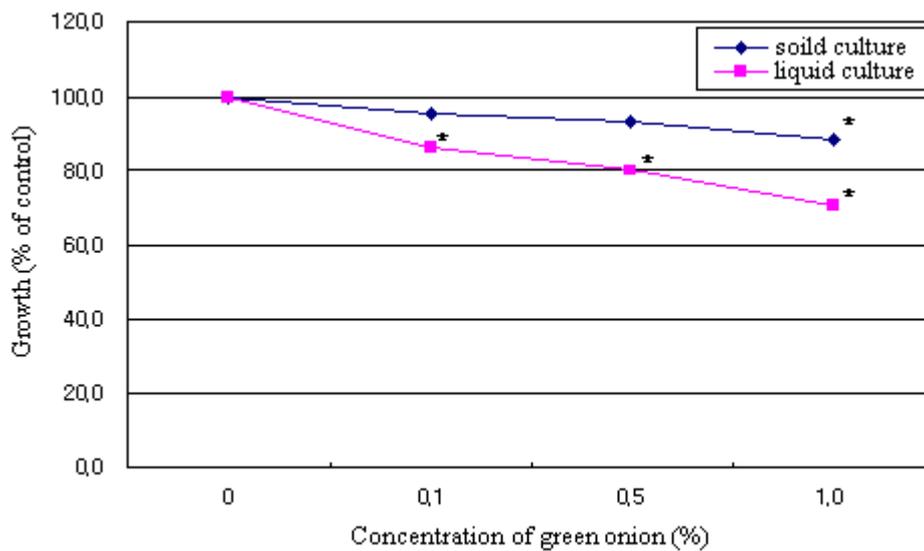
Concentration of green onion	Mycelial weight (mg)				
	3 days	6 days	9 days	12 days	15 days
Control	24.0±1.6 <sup>a</sup>	58.9±4.0 <sup>a</sup>	93.1±8.4 <sup>a</sup>	125.8±12.3 <sup>a</sup>	158.0±4.4 <sup>a</sup>
0.1%	23.5±1.5 <sup>a</sup>	55.3±5.3 <sup>a</sup>	80.5±0.7 <sup>b</sup>	104.6±3.2 <sup>b</sup>	139.0±1.5 <sup>b</sup>
0.5%	15.9±2.7 <sup>b</sup>	47.3±0.9 <sup>b</sup>	74.4±1.6 <sup>b</sup>	85.1±9.3 <sup>c</sup>	110.0±2.0 <sup>c</sup>
1.0%	12.8±1.5 <sup>b</sup>	42.0±1.7 <sup>b</sup>	65.6±2.1 <sup>c</sup>	74.5±1.1 <sup>c</sup>	95.3±2.2 <sup>d</sup>

All values represent the mean ± S.D. of six samples.

Values with different superscript letters in columns are significantly different ( $p < 0.05$ ).



**Fig. 2.** Growth of *A. parasiticus* on yeast-extract sucrose broth containing green onion incubated at 25 °C for 12 days. Left: control, right: 1.0% concentration of green onion.



**Fig. 3.** Comparison of the inhibitory effect of green onion on the growth of *A. parasiticus* in solid culture (potato-dextrose agar) and in liquid culture (yeast-extract sucrose broth) for a 9-day incubation period.

Each point represents the mean  $\pm$  S.D. of six samples. \* $p < 0.05$  compared to control.

#### 4. Discussion

In this study, the antifungal activity of green onion on a toxic fungal strain *A. parasiticus*, which can produce aflatoxin, a human carcinogen. In practice, both a solid culture and a liquid culture were attempted because green onion is

consumed with various forms of daily food intake. In liquid culture, mycelial growth of experimental group was less than that of control group during the incubation period of 15 days. In solid culture, however, the addition of green onion inhibited the fungal growth till the 9th day. After the time periods, further growth of the fungal strain in both experimental and control groups did not happen; therefore this is assumed due to exhaustion of nutrients in the medium.

We observed that green onion at each concentration ranging from 0.1% to 1.0% reduced fungal growth to varying degrees in our experiments. The highest inhibition was observed on the 9th day in PDA (Fig. 3) and on the 12th day in YES broth. At those times, the percentages of inhibition of fungal growth ranged from 4.6% to 11.5% in solid culture and from 16.9% to 40.8% in liquid culture. Maximum inhibition was observed when 1.0% concentration of green onion was added to PDA and YES broth. When increasing concentrations of green onion were added to PDA and YES broth, an increasing tendency of inhibitory effect was noted on the growth of the fungal strain.

The inhibitory effects of other plants of *Allium* on the growth of fungi were explored by several researchers. In particular, garlic, onions, hot peppers, ginger, Chinese parsley and basil extracts have been studied in relation to the growth inhibition of *A. niger* and *A. flavus* [16]. Ethanol extracts of Welsh onion inhibited the growth of *A. flavus* and *A. parasiticus* in liquid culture [17]. The inhibition of the growth of *A. parasiticus* due to Korean fermented vegetables (kimchi), where green onion, garlic, and red pepper are basically used as spice ingredients, has been reported [18]. The degree of inhibitory effect and inhibition rates varied in these reports. In this study, green onion itself was tested rather than its extracted components and the growth of *A. parasiticus* was inhibited by green onion at daily intake levels from 0.1 to 1.0%. Moreover, significant inhibition was observed at the 1.0% level of green onion, both in the solid culture and liquid culture. The intake of green onion of Koreans (over a population of 1 year old or older) is 10.9 g per person per day, and this amount occupies 0.9% of their total food intakes, according to the Fourth National Health & Nutrition Examination Survey 2007, Korea [4]. Our results therefore, show that green onion at a human consumption level was able to inhibit *A. parasiticus*, an aflatoxigenic fungus. Although *Aspergillus* species are known to be the most resistant to the antifungal activity and the most difficult to inhibit, this study indicates some inhibiting effect of green onion to the growth of *A. parasiticus*. Unlike other studies of similar attempts, the results of this work showed the inhibitory effect both in solid culture and liquid culture. In particular, the inhibition rate was significant ( $p < 0.05$ ) after 9 days of cultivation; at 1.0% concentration in solid culture and all experimented concentrations in liquid culture. This suggests that we can assume the effectiveness regardless of the intake forms of green onion. To confirm this assumption, further study is needed with a human population. Unfortunately, recent data of the Koreans indicate that the intake of green onion tends to decrease; it was included in the 30 most consumed Korean food in 2007 [4] but not in 2008 [19].

Although *Allium* plants have the potential to inhibit fungal growth, they have limitations in their use as food additives because of the instability and strong smell of their main components such as allicin, thiosulfonates, and their related compounds [20]. If we could solve these problems, they would be used to control harmful fungi in food chain in any form of natural food. We also need to study on the inhibitory effect of green onion against the production of aflatoxins.

## 5. Conclusions

The work was carried out to investigate the inhibitory effect of green onion on the growth of *A. parasiticus*. We experimented three concentrations of green onion both in solid and liquid cultures incubated at 25 °C for 15 days. Then we observed inhibition of the growth of *A. parasiticus* in both cultures. Due to the addition of green onion to the cultures, the inhibitory effect was significant after 9 days of cultivation; for 1% concentration in solid culture and all experimented concentrations in liquid culture. In our experiments, the maximum inhibition of fungal growth was observed by the concentration of 1.0% on the 9th day in solid culture and 12th day in liquid culture.

Overall, the higher the concentration of green onion was, the more inhibition of the fungal growth was observed both in solid culture and liquid culture. Therefore, dose-response relationships were found between the concentration of green onion and inhibitory effect. The results of our work suggest that the health hazard associated with aflatoxigenic fungi could be reduced through daily consumption of beneficial foods such as green onion. However, long-term and controlled clinical trials are needed to evaluate the effect of green onion in human population.

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