J. Turk. Phytopath., Vol. 34 No. 1-3, 1-7, 2005 ISSN 0378 - 8024

Sclerotium Production of *Aspergillus niger* Van Tieghem on Different Grains

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ABSTRACT

This study represents the determination of the fungal growth and production of sclerotia of *Aspergillus niger* on three grains. The average diameter of the colony of *A. niger* was measured on barley, wheat and sorghum grains and PDA. The best of fungal growth was determined on barley (73.60 mm), wheat (71.46 mm) and PDA (71.46 mm), although sorghum (53.60).

The highest number and weight of sclerotia obtained on sorghum and barley grains were determined. Also, the biggest measurements of sclerotia were recorded on barley grain. The form and color of sclerotia of *A. niger* are variable, most of them are in the form of globose and in pink color.

Key Words: *Aspergillus niger*, sclerotia production, barley, wheat and sorghum grains

INTRODUCTION

Aspergillus is a group of molds which is found throughout the world, especially during the autumn and winter seasons. There are over 200 different species of *Aspergillus,* and some of the more common ones include *Aspergillus niger* Van Tieghem, *A. flavus, A. utus, A. terreus, A. carbonarious,* and *A. fumigatus* (Anonymous, 2002; Porter, 2002).

Species in the genus *Aspergillus* are found in various habitats and under many different environmental conditions. *Aspergillus* is abundant in soil, air and aquatic settings. In indoors areas of high humidity such as basements and cellars, it thrives on improperly stored foods, and it can live off duct particles rich in organic matter. It has been discovered that this genus is one of the most xerotolerant groups of fungi. *Aspergillus* can withstand conditions of low moisture and extreme temperatures. This allows it to act as a "storage fungi" decaying agricultural products and dried foods (Porter, 2002).

There are a wide range of applications for *Aspergillus* species. Several *Aspergillus* fungi have been used by man for at least 3000 years to produce fermented

foods (Anonymous, 2003)**.** Some strains are used in producing antibiotics and beneficial genetic mechanisms (Zhang et al. 2003). *Aspergillus* is widely used in feed fermentation, allowing the commercial exploitation of many products. *A. niger* is also used in the production of citric acid, gluconic acid and enzymes as lipase, glucoamylase, phytase, amino peptidase, acid phosphatase, glucose oxidase (Coenen and Aughton, 1998; Ali et al., 2002; Haq et al., 2002a; Haq et al., 2002b; Mahadik et al., 2002; Mirón et al., 2002; Polakovi and Bryjak, 2002; Porter, 2002; Gargova and Sariyska, 2003; Kurbanoglu and Kurbanoglu, 2003; Liu et al., 2003)**.** Citric acid is a common preservative in soft drinks, most canned goods, and just about any type of shelf food (Porter, 2002).

Ochratoxin A is the only mycotoxin reported from *A. niger*, and is produced by 3–10% of the isolates (Schuster et al., 2002). Other metabolites including naphtho-pyrones, tetracyclic compounds, nigragillin, kotanin, orlandin and malformin A, B, and C are produced by *A. niger* (Schuster et al., 2002; Nielsen 2003). On wet building materials, nigragillin, orlandin, and more than 20 unknown metabolites including naphtho-γ-pyrones and tetracyclic compounds were detected from two examined isolates of *A. niger* (Nielsen et al., 1999; Nielsen, 2002).

Unfortunately, there are some disadvantages associated with this widespread fungal genus. The fungi (mold) producing aflatoxins can infect important food and feed crops before, during and after harvest. These fungi, especially *Aspergillus flavus* and *A. parasiticus*, growing on both living and decaying plant matter. The major products in which aflatoxins are produced include corn grain, soybeans, dry beans, cottonseed, grain sorghum, wheat, peanuts and tree seeds (Anonymous, 2002). *Aspergillus* tends to cause spoilage of foodstuffs and can decompose other materials such as wood, textiles, paint and leather.

Many of these molds can cause illness in humans and animals (Anonymous 2002), which most commonly are *A. fumigatus*, *A. niger*, *A. terreus* and *A. flavus*. If their spores are inhaled during a period when one's immune system is weakened, he will suffer increasingly serious allergies and may eventually contract the disease Aspergillosis (Porter, 2002).

There are some problems in the fungal studies to protect the culture collection for a long time without deterioration their peculiar characteristics. This can be facilitated by production of sclerotia which are resistant to extreme environmental conditions. It is also important to provide an optimum media on which culture of *A. niger* can be maintained and produce a large amount of sclerotia. Thus, it will likely be useful for scientific research on fungi and possible benefits of industrial usage of *A. niger* substances. The objective of the study was to determine an optimum media for the fungal growth and production of sclerotium of *A. niger* on barley, wheat and sorghum grains.

MATERIALS and METHODS

In the study, we used *Aspergillus niger* isolated from wheat seeds. The grains of barley (*Hordeum vulgare*, cv Şahin 91), wheat (*Tritucum durum*, cv Fırat 93), sorghum (*Sorghum bicolor*, cv. Akdarı), and PDA (Potato Dextrose Agar) were employed for fungal growth and sclerotia production of this fungus. The grains were boiled for 45 min and washed three times under tap water, then filtrated to remove excess water. The grains were sterilized at 1.0 psi and at 121^{o}C for 20 min, and inoculated by 10µl, 3.76 x 10^{6} spore/ml inoculum which was placed in the centre of Petri dishes (9 cm diameter, contained 65 g grains), and incubated at 25 ±1 °C in darkness for 30 days.

Five replicates of *A. niger* was used for each treatment. The diameter of colony of fungus was measured on the 3rd, 5th, and 7th days after inoculation, and sclerotia production were observed during 30 days. The inoculated grains were placed in a container with water and mixed for 10 minutes to separate sclerotia from grains. Then, the grains were sifted out by a fine sieve (3 mm). The spore suspension was passed through a cheese cloth, and the sclerotia were washed under tap water until the spores were separated away. The sclerotia were spread on a pepper to be dried in the laboratory conditions.

The number and weight of sclerotia from 100 g of grains, weight of 1000 sclerotia, and measurements of sclerotia were determined. For each treatment, 50 sclerotia of *A. niger* were measured under microscope.

The surface of two months old sclerotia of *A. niger* was sterilized with 1% NaOCl for 5 min, rinsed in sterile distilled water twice for 5 minutes, then planted on PDA, and incubated at 25 ± 1 °C in darkness for one week.

The statistical analyses were performed on the 3rd, 5th, 7th days for colony growth, and the number and weight of sclerotia. Variance analyses were carried out by MSTAT C and mean values were compared by using Duncan's multiple range tests ($P \leq 0.01$) in experiments.

RESULTS AND DISCUSSION

The colony growth of *A. niger* was determined to be different according to the kind of grains or medium. The sporulation and growth of fungus were observed better on the barley and wheat grains than the sorghum grains. The average diameter of the colony of *A. niger* was measured on barley, wheat and sorghum grains and PDA, and was determined as 73.60 mm, 71.46 mm, 53.60 mm and 71.46 mm, respectively (Table 1).

The mycelium was gradually condensed on the surface and then grouped on grain before forming sclerotia, and was finally converted to sclerotium. The first sclerotium production was observed on sorghum grains on the 7th day after inoculation. The production of sclerotia was increased over time. They were produced 15 days after inoculation on all sides of Petri dishes containing grains (Fig. 1a). Since sclerotia were produced in large numbers, they were not countable.

Table 1. The colony growth of *Aspergillus niger* on tested grains and PDA

Grains/Medium	Colony Diameter (mm)			
	3. Day	5. Day	7. Day	Average
Barley	47.40a*	83.40a	90.00a	73.60a
Wheat	47.20a	78.20a	89.00a	71.46a
Sorghum	27.60b	54.40b	78.80b	53.60b
PDA	47.00a	77.40a	90.00a	71.46a
Average	42.30c	73.35b	86.50a	-

*Means followed by different letters are significantly different according to Duncan's Multiple Range Test ($P \leq 0.01$)

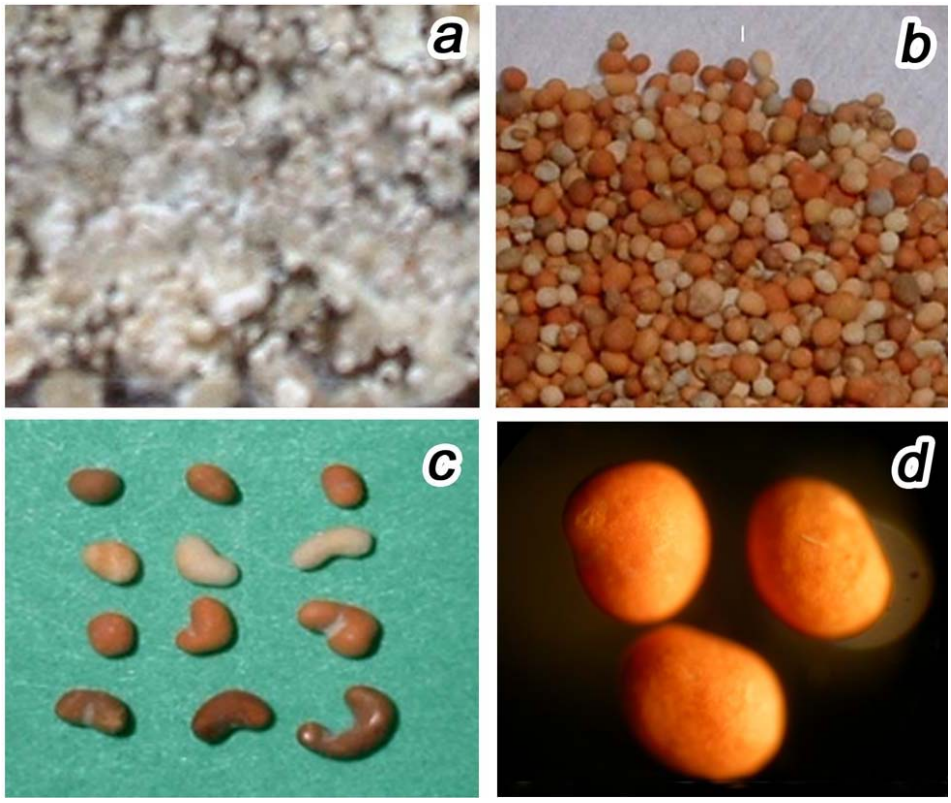

Figure 1. The sclerotia of *A. niger* **a.** Sclerotia production on sorghum grains 15 days after inoculation **b.** A general appearance of sclerotia **c.** The different forms and colors of sclerotia **d**) The appearance sclerotia under microscope (4x10)

The sclerotia were produced abundantly on all used grains, and they could be separated easily from the grains. The number and weight of sclerotia, and their measurements exhibited a wide range of variance according to the kind of grains (Table 2).

The number and weight of sclerotia from 100 g of inoculum of barley, wheat and sorghum grains, and weight of 1000 sclerotia belong to these grains were determined as 1655.96, 793.84 mg and 640.00 mg; 1003.68, 470.76 mg and 380.00 mg; 2978.67, 1202.05 mg and 540.00 mg respectively. The number and weight of sclerotia obtained from the sorghum grains were recorded to be more than those of barley and wheat grains. However, the weight of 1000 sclerotia of barley was determined to be higher with respect to those of other grains. Since, we could not locate any publications related to growth of sclerotia on grains to compare results of the present study.

Table 2. The number, weight, and measurements of sclerotia produced on some grains by *Aspergillus niger*

Grains	Number of Sclerotia from 100 g Grains	Weight of Sclerotia from 100 g Grains (mg)	Weight of 1000 Sclerotia (mg)	Measurement of Sclerotia (µ)		
				Width (Min.-Max.)	Length (Min.-Max.)	Width x Length (Average)
Barley	1655.96b*	793.84ab	640.00a	800-1375	850-1875	1010.00 ± 131.02 x 1203.00 ± 212.11
Wheat	1003.68b	470.76b	380.00b	700-1500	750-2125	1002.00 ± 141.44 x 1195.50 ± 232.73
Sorghum	2978.67a	1202.05a	540.00a	650-1400	750-1875	911.50 ± 170.67 x 1072.50 ± 257.02

* Means followed by different letters are significantly different according to Duncan's Multiple Range Test ($P \leq 0.01$)

The results suggest that the colony growth of *A. niger* was slower on sorghum grains than on barley and wheat grains, but sclerotia production was higher on this grain compared to that on the others (Table 1, 2).

The average of sclerotia obtained from barley, wheat and sorghum were measured as 1010.00 ± 131.02 x 1203.00 ± 212.11µ, 1002.00 ± 141.44 x 1195.50 ± 232.73µ and 911.50 ± 170.67 x 1072.50 ± 257.02µ, respectively. The sclerotia produced on barley grains were larger than those on wheat and sorghum grains. It is known that vegetative growth and generative formations of fungi depend on the content of media and environmental conditions.

The form and colour of obtained sclerotia of *A. niger* are variable. Most of them are in the form of globose and in pink (Fig. 1d). They may also appear in the form of ellipse, half moon, twin or kidney, and in brown, dark brown, whitish or camelhair colours (Fig. 1b, 1c). In general, whitish or camelhair colours were observed at the immature sclerotia. Also, these characters of sclerotia were observed in earlier works (Raper and Fennel, 1965).

There are some *A. niger* strains (Haq et al., 2002a; Hamari et al., 2003), widely used in feed fermentation, allowing the commercial exploitation of many products such as citric acid, gluconic acid, some enzymes, and other metabolites (Coenen and Aughton 1998; Nielsen et al., 1999; Haq et al., 2002a; Mahadik et al., 2002; Mirón et al., 2002; Gargova and Sariyska 2003; Kurbanoglu and Kurbanoglu, 2003). It is known that, when fungi are frequently transferred on media, they may lose or change some of their characteristics. However, when they are preserved as sclerotia, they can maintain their characteristics for a long time, even some of them under extreme conditions.

The fungus, *A. niger* was re-isolated from two months old sclerotia whose colony growth and other characteristics appeared similar the original isolate.

Our results suggest that *A. niger* abundantly produced sclerotia on barley, wheat, and sorghum grains, and their numbers, weight and measurements varied according to the kind of grains.

ÖZET

FARKLI TANELER ÜZERİNDE *ASPERGILLUS NIGER* VAN TIEGHEM'IN SKLEROT OLUŞUMU

Bu çalışmada, *Aspergillus niger*'ın üç farklı tahıl tanesi üzerinde fungal gelişimi ve sklerot oluşumu incelenmiştir. En iyi fungal gelişme arpa (73.60 mm), buğday (71.46 mm) ve PDA (71.46 mm)'da, en zayıf gelişme ise akdarı (53.60) üzerinde saptanmıştır.

En fazla sklerot oluşumu ve ağırlığı arpa ve akdarı danelerinde, en az ise buğday'da belirlenmiştir. Ayrıca, en büyük sklerot arpa daneleri üzerinde oluşmuştur. *A. niger* fungusunun değişik yapı ve renkte sklerotlar oluşturduğu belirlenmiştir. Sklerotların ekseriyeti yuvarlak küre şeklinde açık kahve yada koyu kahve ve pembe renklidirler.

Anahtar Kelimeler: *Aspergillus niger*, sklerot oluşumu, arpa, buğday ve darı taneleri

LITERATURE CITED

ALI, S., HAQ, I.-U., QADEER, M. A. & IQBAL, J., 2002 Production of citric acid by *Aspergillus niger* using cane molasses in a stirred fermentor. *Electronic Journal of Biotechnology.* **(**http://www.elbiotecnology.inf/content/vol5/issue3/Full/bip/index/html)

ANONYMOUS, 2002. http://www.mold-help.org/aspergillus.htm

ANONYMOUS, 2003. http://www.aspergillus.man.ac.uk/languages/faq.htm

COENEN, T. M. M. & AUGHTON, P., 1998. Safety evaluation of amino peptidase enzyme preparation derived from *Aspergillus niger*. *Food and Chemical Toxicology,* **36**, 781-789.

GARGOVA, S. and SARIYSKA, M., 2003. Effect of culture conditions on the biosynthesis of *Aspergillus niger* phytase and acid phosphatase. *Enzyme and Microbial Technology*, **32**, 231-235.

HAMARI, Z., TÓTH, B., BEER, Z., GÁCSER, A., KUCSERA, J.,PFEIFFER, I., JUHÁSZ, Á. and KEVEI, F., 2003. Interpretation of intraspecific variability in mtDNAs of *Aspergillus niger* strains and rearrangement of their mtDNAs following mitochondrial transmissions. *FEMS Microbiology Letters*, **221**, 63-71.

HAQ, I.U., ALI, S., QADEER, M.A. and IQBAL, J., 2002A. Citric acid fermentation by mutant strain of *Aspergillus niger* GCMC-7 using molasses based medium. *Electronic Journal of Biotechnology.* (http://www.elbiotecnology.inf/ content/vol5/ issue2/Full/bip/index/html)

HAQ, I. -U. ALI, S. QADEER, M. A. and IQBAL, J., 2002B. Effect of copper ions on mould morphology and citric acid productivity by *Aspergillus niger* using molasses based media. *Process Biochemistry,* **37,** 1085-1090.

LIU, J. Z., WENG, L. P., ZHANG, Q. L., XU, H. and JI, L. N., 2003. A mathematical model for gluconic acid fermentation by *Aspergillus niger*. *Biochemical Engineering Journal*, **14**, 137-141.

KURBANOGLU, E. B. and KURBANOGLU, N. I., 2003. Production of citric acid from ram horn hydrolysate by *Aspergillus niger*. *Process Biochemistry* **38**,1421-1424.

MAHADIK, N. D., PUNTAMBEKAR, U. S., BASTAWDE, K. B., KHIRE, J. M. and GOKHALE, D. V., 2002. Production of acidic lipase by *Aspergillus niger* in solid state fermentation. *Process Biochemistry,* **38**, 715-721.

MIRÓN, J., GONZÁLEZ, M. P., PASTRANA, L. & MURADO, M. A., 2002. Diauxic production of glucose oxidase by *Aspergillus niger* in submerged culture. *Enzyme and Microbial Technology*, **31**, 615-620.

NIELSEN, K. F., GRAVESEN, S., NIELSEN, P.A., ANDERSEN, B., THRANE, U. and FRISVAD, J. C.,1999. Production of mycotoxins on artificially and naturally infested building materials. *Mycopathologia,* **145**, 43–56.

NIELSEN, K.F., 2002. Mould growth on Building materials. Secondary metabolites, mycotoxins and biomarkers. Ph.D. Thesis. BioCentrum-DTU, Technical University of Denmark. Available from <http://www.biocentrum.dtu.dk/ mycology/publications/phd-kfn.pdf>

NIELSEN, K. F., 2003. Mycotoxin production by indoor molds. *Fungal Genetics and Biology,* **39**, 03-117.

POLAKOVI, M. and BRYJAK, J., 2002. Modelling of the kinetics of thermal inactivation of glucoamylase from Aspergillus niger. *Journal of Molecular Catalysis B: Enzymatic*, **19-20**, 443-450.

PORTER, C., 2002. Aspergillus. *Soil Mıcrobiology* Biol/Cses 4684 http://soils1.cses.vt.edu/ch//biol_4684/Microbes/aspergillus.html

RAPER, K. B. and FENNEL, D. I., 1965. The Genus Aspergillus. Williams & Wilkins Co., 686 pp.Schuster, E., Dunn-Coleman, N., Frisvad, J.C. & Van Dijck, P.W. 2002 On the safety of *Aspergillus niger*—a review. *Appl. Microbiol. Biot.*, **59**, 426–435.

ZHANG, L, OHTA, Y. and WANG, Y., 2003. Expression of the inulinase gene from *Aspergillus niger* in *Pichia pastoris*. *Process Biochemistry*, **38**, 1209-1212.