

## Full Length Research Paper

# Effect of Avid<sup>®</sup> on the synnema-like formation of *Aspergillus flavus* grown on Czapek medium

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The effect of Avid<sup>®</sup> on the induction of synnema-like structure of *Aspergillus flavus* (61 strains) grown on various types of agar media was investigated. Other related species of *Aspergillus* within the section *Flavi*, *A. parasiticus* (14 strains), *A. nomius* (14 strains), *A. pseudotamarii* (2 strains), *A. tamarii* (20 strains) and *A. oryzae* (5 strains), were also evaluated for comparison. When Avid<sup>®</sup> was added into five different solid media (Czapek, V8, *Aspergillus flavus* and *A. parasiticus*, corn meal and potato dextrose agar) at 0.004% (v/v), only the *A. flavus* strains (all 61 tested) were able to produce synnema-like structures, and only in the Czapek agar medium containing Avid<sup>®</sup>. The effect of carbon (sucrose, glucose and starch) and nitrogen (NaNO<sub>3</sub>, urea and peptone) sources in Czapek medium containing Avid<sup>®</sup> on the induction of a synnema-like structure was also determined, with synnema-like structure induction being found in all three carbon sources but only in NaNO<sub>3</sub> as the nitrogen source. All the three of the inert solvents in Avid<sup>®</sup> (mineral oil, butylated hydroxytoluene and n-methylpyrrolidone) did not induce synnema-like structure formation, indicating that abamectin, an active ingredient of Avid<sup>®</sup> together with NaNO<sub>3</sub> likely play important roles in the induction of synnema-like structure of *A. flavus*.

**Key words:** *Aspergillus flavus*, Avid<sup>®</sup>, synnema-like structure.

## INTRODUCTION

*Aspergillus flavus* is a fungus in *Aspergillus* subgenus *Circumdati* section *Flavi*. This species can be divided into two groups based on their aflatoxin production, the aflatoxigenic group (with the ability to produce aflatoxins) and non-aflatoxigenic group (without ability to produce aflatoxins) (Chang and Hua, 2007). Aflatoxigenic *A. flavus* strains are able to produce aflatoxins B (AFB1 and

AFB2) and some strains also produce aflatoxins G (AFG1 and AFG2) (Horn and Greene, 1995). This species is of interest since it can be found in several economically important crops including corn, cotton, peanut and tree nuts, in the field or during storage and is more frequent than other aflatoxigenic strains, such as *A. parasiticus*, *A. nomius* and *A. pseudotamaraii* (Rodriguez and Mahoney, 1994).

Avid<sup>®</sup> is a miticide or insecticide used to control mites or insects in a wide range of important crops (Van Der Geest et al., 2000). It is composed of the active ingredient, abamectin, and three inert solvents, mineral oil,

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butylated hydroxytoluene (BHT) and n-methylpyrrolidone (Syngenta Crop Protection, Inc., Greensboro, NC, USA). Avid<sup>®</sup> has been added in microbial culture media to prevent contamination caused by mites or insects associated with crop samples, and thus proved a useful practice for isolation of microorganisms from crop samples.

In our primary observation, when *Aspergillus* spp. were cultivated on Czapek agar plates containing Avid<sup>®</sup>, it was observed that *A. flavus* strains, but not other species, could form synnema-like structures. Therefore, in this research, the effect of Avid<sup>®</sup> on the induction of the synnema-like structure were evaluated in 61 strains of *A. flavus* growing on various types of solid culture media. Other related species of *Aspergillus* within the section *Flavi* (*A. flavus*, *Aspergillus parasiticus*, *Aspergillus nomius*, *Aspergillus psuedotamarii*, *Aspergillus tamarii* and *Aspergillus oryzae*) were included for comparison.

## MATERIALS AND METHODS

### Fungal cultures

Fifty-five strains of *Aspergillus flavus*, 12 strains of *A. parasiticus*, 12 strains of *A. nomius*, and 18 strains of *A. tamarii* were obtained from the Fungi Section, Professor Kasin Suvatabhandhu Herbarium, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. Six reference strains of *A. flavus* (NRRL 3357, NRRL 21882, TX 9-8, F3W4, Af 53, and Af 13) were obtained from Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA, USA. To strains of each of *Aspergillus parasiticus* (SRRC 75 and SRRC 143-A), *A. nomius* (SRRC 362 and SRRC 375), *A. tamarii* (SRRC 99 and SRRC 1088) and *A. pseudotamarii* (SRRC 2420 and SRRC 2428) plus four strains of *A. oryzae* (SRRC 302, SRRC 480, SRRC 2079, and SRRC 2085) were obtained from USDA-ARS, Southern Regional Research Center, New Orleans, LA, USA.

### Media and culture conditions

Each *Aspergillus* species / strain was grown on V8 medium (5.0% (v/v) V8 juice and 2.0% (w/v) agar) in the dark at 30°C for 7 days. Spores were harvested from these cultures using sterile water and the suspension was adjusted to 10<sup>5</sup> spores per ml. A spore suspension of each *Aspergillus* species (5  $\times$  l per plate) was inoculated onto solid media and incubated in the dark at 30°C for 7 days for morphological observation.

To investigate the effect of Avid<sup>®</sup> (0.15 EC, Novartis Crop Protection, Greensboro, NC, USA) on the synnema-like formation of *A. flavus*, and the other related species in the section *Flavi*, each fungal isolate was grown on Czapek, V8, AFPA (*Aspergillus flavus* and parasiticus agar) (Pitt et al., 1983), Corn Meal Agar (CMA, Difco, USA) and Potato dextrose Agar (PDA, Difco, USA), with or without 0.004% (v/v) Avid<sup>®</sup>, incubated in the dark at 30°C for 7 days. To determine the effect of the Avid<sup>®</sup> concentration on the synnema-like formation, Avid<sup>®</sup> at six different concentrations (0.002, 0.004, 0.008, 0.02, 0.05 and 0.01 % (v/v)) was added into the culture medium. Carbon and nitrogen sources in the culture

medium were varied so as to include one of each three carbon sources (sucrose, glucose and starch) and three nitrogen sources (NaNO<sub>3</sub>, urea and peptone). The three inert solvents in Avid<sup>®</sup>, mineral oil, butylated hydroxytoluene (BHT) and n-methylpyrrolidone (0.004% v/v), were also tested for their ability to induce a synnema-like structure by incorporating them into the Czapek medium. The colonies of fungi were observed daily for morphological changes. All experiments were performed in triplicate.

## RESULTS AND DISCUSSION

When *Aspergillus* spp. were grown on different kinds of solid culture media (Czapek, V8, AFPA, CMA and PDA) with or without Avid<sup>®</sup> at 0.004% (v/v), all cultures grew well and produced conidia regularly. However, only *A. flavus* (of all 61 strains) grown on Czapek medium containing Avid<sup>®</sup>, developed the synnema-like structures (fused white conidiophores bearing yellow to green conidia) after 3 days of incubation at 30°C in the dark (Figure 1). When this species was grown on Czapek media without Avid<sup>®</sup> or on the four other media tested with or without Avid<sup>®</sup>, the fungus did not produce a synnema-like structure (Figure 2). In this study the miticide clearly did not affect the growth of *Aspergillus* spp. However, Avid<sup>®</sup> might be able to decrease the germination, vegetative growth, and conidial number of the entomopathogenic fungus, *Beauveria bassiana*, (Oliveira and Neves, 2004). Although not commonly found, the formation of synnema by *Aspergillus* section *Flavi* was previously reported in a mutant of *A. flavus* (NRRL 29254) and some strains of *A. caelatus* which was induced by the regulation of carbon and nitrogen sources in the culture media (McAlpin, 2001; 2004).

All strains of *A. flavus* could grow and form synnema-like structures at different level concentrations of Avid<sup>®</sup>, ranging from 0.002 to 0.01% (v/v), added into Czapek media. Thus, Avid<sup>®</sup> at this concentration range, can induce the formation of a synnema-like structure of *A. flavus*. The formation of synnema-like structure of *A. flavus* was not affected by the three carbon sources tested, with all strains of *A. flavus* showing no difference in colony morphologies when they grew on Czapek media containing Avid<sup>®</sup> using either sucrose, glucose, or starch as the sole carbon source, and they all produced synnema-like structures. However, with respect to the nitrogen source, all *A. flavus* strains could form synnema-like structures only when grown on Czapek media containing Avid<sup>®</sup> with NaNO<sub>3</sub> as the sole nitrogen source. Urea and peptone were not able to induce synnema-like structure in *A. flavus*. The results support the absence of synnema-like structure of *A. flavus* strains grown on V8, AFPA, CMA, and PDA media since they contain either urea or peptone as a nitrogen source. The role of nitrogen source in morphological changes of *Aspergillus*



**Figure 1.** Colony of *A. flavus* NRRL 3357 (A) and its synnema-like structure (B). The fungus was grown on Czapek medium containing 0.004% (v/v) Avid<sup>®</sup> incubated at 30 °C in the dark for 7 days. (bar = 1 mm.)



**Figure 2.** Colony *A. flavus* NRRL 3357 (A) and its typical conidia (B) The fungus was grown on Czapek medium without Avid<sup>®</sup> incubated at 30°C in the dark for 7 days. (bar = 1 mm.)

has previously been reported. NaNO<sub>3</sub> was shown to be an excellent nitrogen source for synnema/sclerotium formation while ammonium sulfate inhibited growth, sporulation and synnema/sclerotium formation of *A. caelatus* and a mutant strain of *A. flavus* (McAlpin, 2001; 2004).

In order to clarify which ingredient in Avid<sup>®</sup> could induce the synnema-like structure of *A. flavus*, the inert solvents in Avid<sup>®</sup>, mineral oil, butylated hydroxytoluene (BHT) and n-methylpyrrolidone, were separately added in Czapek medium to the same concentration as that of 0.004% Avid<sup>®</sup>. All *A. flavus* strains failed to produce synnema-like

structures when grown on Czapek medium containing any of these three inert solvents. Thus, unless a corequirement of two or more of the inert solvents is required; the result suggests that synnema-like structure of this species was induced by abamectin, an active ingredient in Avid<sup>®</sup>.

In conclusion, the results presented here show that Avid<sup>®</sup> could induce synnema-like structure of *A. flavus* growing on Czapek medium. None of the other *Aspergillus* species tested could form this structure when grown on this medium containing Avid<sup>®</sup>. The miticide could induce synnema-like structure at a wide

concentration range (from 0.002 to 0.01% (v/v)). NaNO<sub>3</sub> in Czapek medium together with abamectin in Avid<sup>®</sup> were found to play important roles in the induction of synnema-like structure of *A. flavus*. The results open up the possibility of using Avid<sup>®</sup> in a selective culture medium to help distinguish *A. flavus* from the other *Aspergillus* in the section Flavi.

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