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**In vitro effect of some fungicides on growth and aflatoxins production by *Aspergillus flavus* isolated from Capsicum powder**

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*In vitro* effect of some fungicides on growth and aflatoxins production by *Aspergillus flavus* isolated from *Capsicum* powder

Running title: Effect of some fungicides on *Aspergillus flavus*

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13 **Abstract**

14 The aim of this study was to determine the effect of some pre-harvest fungicides on growth  
15 and aflatoxin (AF) production of three *A. flavus* strains found in *Capsicum* powder. Each  
16 isolate, previously isolated from paprika, chilli and smoked paprika, was inoculated on Yeast  
17 Extract Sucrose agar and on a 3% paprika extract agar medium supplemented with different  
18 fungicides and incubated at 20 and 30 °C during 7 days. Growth measurements were obtained  
19 on days 3, 5 and 7, and the AF production was determined on day 7. The significance of the  
20 effects of the factors (strain, medium, temperature, time and fungicides) and their interaction  
21 over colony diameter and AF production was determined. Temperature constrained the  
22 effectiveness of fungicides in reducing growth, the fungicides being most effective at 20 °C.  
23 The efficacy of the fungicides over AF production depended on the medium used and  
24 temperature. The most effective fungicides in inhibiting growth and AF production,  
25 regardless of the strain tested or applied conditions, were tebuconazole 25% and mancozeb

80% applied at a concentration of 0.75 and 3.5 g L<sup>-1</sup>, respectively. Care should thus be taken in the choice of a suitable fungicide, because their effectiveness may depend on intra-specific variation and temperature. Moreover, it is necessary to take into account that the most efficient fungicide in reducing growth is not always the best choice for pre-harvest treatments, because it may promote AF production. Thus, the best fungicide is the one which can simultaneously prevent growth and AF production.

**Introduction**

The possible presence in food and feed of aflatoxins (AF), fungal toxic compounds to animals and man, has led to increased research aiming to prevent and detect such compounds, mainly due to their carcinogenic, mutagenic and teratogenic properties. The *Aspergillus* section *Flavi* is well known for its AF production. The more common AF-producers species are *Aspergillus flavus* and *A. parasiticus*, though *A. arachidicola*, *A. bombycis*, *A. minisclerotium*, *A. nomius*, *A. parvisclerotigenus*, *A. pseudotamarii* and *A. toxicarius* also have the ability to produce AF (Samson et al. 2006). The AF production may happen either in the field, during harvest and/or transportation or storage. The AFB1, AFB2, AFG1 and AFG2 are the most studied AF, being the AFB1 the most toxic.

Some species of the genus *Capsicum* are grown for their fruits, which can be consumed as dried powder, usually named paprika and chilli. *A. flavus*, which have the ability to produce AF, can be found in this kind of commodities (Christensen et al., 1967; Mandeel, 2005; Hashem and Alamri, 2010). A review about mycotoxin contamination of *Capsicum* powder indicated that out of 1027 samples, 61.4% were positive for AF (Santos et al., 2008), the contamination being in the range from limit of detection to 969 µg kg<sup>-1</sup> (Santos et al., 2008; Bircan et al. 2008; Hierro et al. 2008; O’Riordan and Wilkinson 2008; Santos et al. 2010b). The mycotoxin contamination of spices should not be underestimated despite the small

amounts consumed, since the hazard due to mycotoxins is the repeated ingestion of small quantities (Ehlers et al. 2006). The best way to prevent mycotoxin contamination in *Capsicum* fruits to control the conditions of harvesting and processing methods, the storage conditions and post-harvest treatments (Reddy et al. 2001, Erdogan 2004, Heperkan and Ermis 2004, Bircan 2005, Colak et al. 2006, Almela et al. 2007).

The use of fungicides has become an essential part of modern agriculture (Matheron and Porchas, 2008). The fungicides as pre-harvest treatment can be an efficient, cost-effective way to prevent mould growth, and consequently mycotoxins production. In the last few years, intensive studies have been carried out on the search of several chemical compounds (e.g. antioxidants, carotenoids, flavonoids, surfactants, herbicides...) which could inhibit the fungal growth. Some studies have proven that fungicides can affect growth (Olajire and Oluyemisi 2009) and AF production of *A. flavus* (Wheeler 1991, Reddy et al. 2009) isolated from different matrices.

In this study we have attempted to understand the effect of some pre-harvest fungicides on the growth and AF production of some *A. flavus* found in *Capsicum* powder.

## Material and Methods

### *Strains*

Three AF-producing strains of *A. flavus* (UdLTA 3.193, UdLTA 3.215, and UdLTA 3.217) were used in the study. These moulds were previously isolated from paprika (UdLTA 3.217), chilli (UdLTA 3.193) and smoked paprika (UdLTA 3.215) and belong to the Food Technology Department Collection of the University of Lleida, Spain. In order to confirm the accuracy of morphological identification, the 3 strains were subjected to molecular analysis with specific primers for these species (Santos et al., 2010a). In order to confirm the accuracy

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of morphological identification, the 3 isolates were subjected to molecular analysis with specific primers for this species. All genomic DNAs were tested for suitability for PCR amplification using universal primers ITS1 and ITS4. Specific PCR assays were carried out using primers FLA1 and FLA2 based on ITS1-5.8SITS2 region and the protocol described elsewhere (González-Salgado et al., 2008).

***Fungicides***

The fungicides used in this work are those which are commonly used for the treatment of peppers and are listed in Table 1.

***Medium***

Yeast Extract Sucrose agar (YES) (yeast extract 20 g; Czapek concentrate 7.5 mL; sucrose 150 g; MgSO<sub>4</sub>·7 H<sub>2</sub>O 0.5 g; agar 20 g; H<sub>2</sub>O 885 mL to 1 L) and a 3% paprika extract agar (3% paprika) were used for mould cultures. The 3% paprika medium was prepared by boiling 30 g of chilli powder in 1 L distilled water for 30 min. The resulting mixture was filtered through a double layer of muslin and the volume was made up to 1 L (Marín et al. 2009). The fungicides concentrations used were the recommended by manufacturers and are listed in Table 1.

***Inoculation***

For inocula preparation, one plate of Malt Extract Agar (MEA) (Malt Extract 15 g; peptone 0.75 g; glucose 15 g; agar 9 g; H<sub>2</sub>O 750 mL to 1 L) per strain was inoculated and incubated for 7 days at 25 °C. On the seventh day, suspensions of 1-5·10<sup>6</sup> spores mL<sup>-1</sup> (in sterile water plus Tween 80) for each strain were prepared and adjusted using a Thoma counting chamber. YES and 3% paprika plates were inoculated once in the centre, with an inoculation needle.

***Growth Conditions***

Three replicate plates per fungicide and strain plus controls (media without fungicides) were incubated in the dark at 20 and 30 °C.

### ***Growth Measurements***

Petri plates were examined on days 3, 5 and 7 and the diameters of the growing colonies were measured in two directions at right angles to each other.

### ***Determination of AF production***

On day 7 of incubation, a high performance liquid chromatography (HPLC) method was used to determine the AF production. The extraction method was a modification of the Bragulat et al. (2001) protocol. Three plugs (diameter 5 mm) were aseptically removed from the inner, middle, and outer area of each colony. The plugs were placed into 4 mL glass vials for extraction. Extraction was done adding 1 mL of acetonitrile and vortex-mixing for 5 seconds. After 1 hour, the vials were shaken again and the solvent was filtered (Millex HV filter 0.45 mm, Millipore, Bedford, MA, USA) on a HPLC glass vial. After filtration, 300 µL of acetonitrile extract and 700 µL water were mixed and 100 µL of this solution was injected in the HPLC system (Waters, Milford, MA, USA) with a reverse-phase C18 silica gel column (Waters Spherisorb® 3 µm ODS2 4.6 × 150 mm, Milford, MA, USA) kept in a column oven at 40 °C. Detection was achieved by fluorescence (Waters 2475 fluorescence detector, Waters, Milford, MA, USA); excitation and emission wavelengths were set at 362 and 440 nm, respectively. An isocratic mobile phase of water-acetonitrile-methanol (60:25:15, v/v/v) was used at a flow rate of 1.0 mL min<sup>-1</sup>. Post-column derivatization was achieved by using a photochemical reactor for enhanced detection (PHRED) (LCTech UVE, Dorfen, Germany). For confirmation of the presence of AF, the PHRED was switched off and the decrease of the AFB1 and AFG1 peaks was registered. For total AF (AFt), limit of detection (LD) was 0.6 ng



g<sup>-1</sup> and limit of quantification 2.5 ng g<sup>-1</sup>. Retention time for the different mycotoxins was 7.3 min for AFG<sub>2</sub>, 8.5 min for AFG<sub>1</sub>, 9.7 min for AFB<sub>2</sub> and 11.6 min for AFB<sub>1</sub>.

**Statistical analysis**

A multifactor analysis of variance (ANOVA) was used to test the significance of the effects of the factors (strain, medium, temperature, time and fungicides) and their interactions on the colony diameter using JMP® 8.0.1 (SAS Institute Inc, Cary, NC, USA). The Tukey HSD test was used to differentiate among levels of factors. AF concentrations detected under each condition were also evaluated with ANOVA taking strain, medium, temperature and fungicides as factors. ANOVA was also used to evaluate the effects of factors on YES and 3% paprika media (controls) over colony diameter and AF production.

**Results and Discussion**

This study attempts to understand the effect of some pre-harvest fungicides on *A. flavus* found in *Capsicum* powder. Currently, in Europe there is no registered fungicide for the specific post-harvest treatment of *Capsicum* fruits. Although the fungicides chosen for this study are usually used for the pre-harvest treatment of *Capsicum* spp. diseases caused by moulds like *Botrytis*, *Fusarium* or *Alternaria*, it could be possible that they may also be effective in the growth and AF production control of moulds usually found in soil or during storage, such as *A. flavus*.

**Growth and mycotoxin production in the controls**

AF production is usually affected by many abiotic parameters like temperature, water activity, pH, osmotic pressure, substrate nature and also biotic factors (Martins et al. 2000) in our case, the AF production was affected by the medium, temperature and isolate used. Many of these

parameters have been investigated separately but it must be noted that they all interact under natural conditions (Martins et al. 2000). Some strains of *A. flavus* are known to be non-toxicogenic or to produce small quantities of AF, as the isolate UdLTA 3.215. The ability to produce AF relies on the metabolic system of each individual (Martins et al., 2000). Those *A. flavus* that produce AF, usually produce only B-type AF, however there are some strains also capable to produce G-type AF. All the isolates used in this study produced B-type and G-type AF, depending on the conditions. Like us there are other authors who also found *A. flavus* G-type producers (Gabal et al., 1994; Ehrlich et al., 2007). The toxigenicity of *A. flavus* also varies with strain, substrate and geographic origin (Klich 2007). As mentioned before, each strain derived from different a substrate.

Usually, *A. flavus* growth is possible in the range of 10 °C or a little higher to about 43°, being the optimal growth at temperatures slightly exceeding 30 °C (ICMSF 1996). The usual optimal temperature for AF production by *A. flavus* is 20-35°C and AF production decreases as temperature goes down or above these levels (Schroeder and Hein 1967, Northolt et al. 1977, Gqaleni et al. 1997, Ogundero 2007). There are some authors who have observed that *A. flavus* can also have an optimal temperature for AF production at 13-16 °C or 16-24 °C, depending on the strain (Northolt et al. 1977). In our study, although only two temperature levels were assayed, it was observed that *A. flavus* grew better and produced more AF at 30 °C.

In the controls where no fungicide was added, growth and Aft, AFB1 and AFB2 production were significantly higher in YES than in 3% paprika medium. In general, the three strains grew significantly faster and produced significantly more AF at 30°C than at 20°C in both media. Regarding the AFB2 production, the temperature only influenced the strain UdLTA

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168 3.193, on YES. Moreover, AFG1 and AFG2 production was not influenced by either medium  
169 or temperature. As expected, increasing colony diameters were observed with time, so all the  
170 results described *a posteriori* were based on the results of the day 7.

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172 In the controls when comparing strains and regardless of the medium used, it was possible to  
173 observe that the UdLTA 3.217 strain grew significantly faster ( $P<0.05$ ). The UdLTA 3.193  
174 produced significantly more AFt, AFB1 and AFB2 than the other strains, and no significant  
175 differences were found among the strains for the production of AFG1 and AFG2 ( $P<0.05$ ).  
176 The ability to produce AF relies on the metabolic system of each individual strain (Martins et  
177 al. 2000). Thus, some strains of *A. flavus* are non-toxigenic or produce less quantity of AF, as  
178 the strains UdLTA 3.215 and UdLTA 3.217.

179 ***Growth response to fungicides***

180 Regarding fungicide effects on growth of all strains, the fungicides were more efficient at 20  
181 °C and on 3% paprika ( $P<0.05$ ). The data from the effects of fungicides treatment over growth  
182 are showed in the Table 2. Almost all fungicides reduced significantly the growth of all  
183 strains, except F3 (copper oxychloride 50%) which never showed good results on YES, F4  
184 (triadimenol 31.2%) and F5 (sulphur 80%) which showed a light effect, in particular for  
185 isolates UdLTA 3.215 and UdLTA 3.217, and F9 (trifloxystrobin 50%), which was not  
186 effective at 30 °C. The fungicides F6 (tebuconazole 25%) and F7 (mancozeb 80%) totally  
187 prevented growth of all isolates, regardless of medium or temperature. The fungicide F1  
188 (cypronidil 37.5% + fludioxonil 25%) reduced growth of all strains in 95 to 100%, regardless  
189 of the temperature and medium used. The fungicide F2 (dodine 40%) reduced growth in YES  
190 over 99%, but in 3% paprika it had lower efficiency (over 20%), being lower at 30 °C (20-

191 29%). The fungicide F8 (cyproconazole 10%) inhibited growth in rates of 31 to 83%,  
192 depending on medium and temperature.

193 Copper oxychloride 50% (F3) and triadimenol 31.2% (F4) were the worst fungicides for  
194 growth controlling of *A. flavus* and the best ones were tebuconazole 25% (F6) and mancozeb  
195 80% (F7), regardless of isolate, medium and temperature.

196 It is important to note that temperature constrained the effectiveness of fungicides in reducing  
197 growth, being the fungicides more effective at 20 °C. For a proper election of the fungicides it  
198 is also necessary to discern their effect on the AF production.

#### 199 *AF production response to fungicides*

200 Since fungicides are widely used to control crop diseases, it is pertinent to consider the effects  
201 of these agents on mycotoxin production. In both laboratory studies with pure cultures of  
202 moulds and field trials with crop plants the resulting evidence concerning the effectiveness of  
203 fungicides is contradictory and in certain cases unexpected (D'Mello et al. 1998), as it is  
204 possible to observe through the results of this study.

206 For statistical purposes, an AF production of 0.0 ng AF/g of agar was assigned to no-growth  
207 observations. Moreover, aflatoxin results for <5 mm growth observations were omitted. Thus  
208 it was not possible to analyse the effect of the fungicides F1 (cypronidil 37.5% + fludioxonil  
209 25%) over the AF production capacity of all strains at 30 °C, and also the effect of the  
210 fungicide F2 (dodine 40%) over the strain UdLTA 3.215 at 20 °C in 3% paprika and over the  
211 strain UdLTA 3.217 at 30 °C in YES. The data from the effects of fungicides treatment over  
212 AF production are shown in the Tables 3, 4, 5 and 6.

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3 213 The efficacy of the fungicides over AF production depended on the medium used and  
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5 214 temperature ( $P<0.05$ ). Regarding AFt production in 3% paprika the only effect observed was  
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7 215 a significant increase of the AFt production for the strain UdLTA 3.217 at 30 °C and for the  
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9 216 strain UdLTA 3.215 at 20 °C when using triadimenol 31.2% (F4) ( $P<0.05$ ). The opposite was  
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11 217 only observed in YES. The fungicides had a significantly inhibitory effect over the AFt  
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13 218 production of 51 to near 100%, regardless of the strains and temperature ( $P<0.05$ ), except for  
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15 219 strain UdLTA 3.193 at 20 °C where fungicides had no significant effect. The fungicides effect  
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17 220 over AFB1 production was similar to the effect over AFt production. There was an absence of  
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19 221 any significant effect in 3% paprika and a significant inhibitory effect between 51 and near  
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21 222 100%, on YES, regardless of strain and temperature, except at 20 °C for the UdLTA 3.193  
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23 223 strain in which no effect was observed ( $P<0.05$ ). Regarding fungicide effect in AFB2  
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25 224 production, there was no significant effect in 3% paprika, while in YES the fungicides  
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27 225 reduced significantly the AFB2 production in the range 39-100% for the strain UdLTA 3.217,  
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29 226 regardless of the temperature level and for the strain UdLTA 3.193 at 30 °C. For AFG1 and  
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31 227 AFG2 production, there was no significant effect, except in YES at 30 °C for the strains  
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33 228 UdLTA 3.193 and UdLTA 3.215. However, there were some cases the presence of fungicides  
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35 229 F1 (cypronidil 37.5% + fludioxonil 25%), F2 (dodine 40%) and F9 (trifloxystrobin 50%)  
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37 230 promoted AF production.  
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50 232 In the present study, the most effective fungicides in inhibiting growth and AF production,  
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52 233 regardless of the strain, mycotoxin or applied conditions, were tebuconazole 25% (F6) and  
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54 234 mancozeb 80% (F7). Tebuconazole and mancozeb fungicides belong to the triazole and  
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56 235 dithiocarbamates groups, respectively. The dithiocarbamates action has direct effect on  
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58 236 moulds by interfering with sporulation, inhibiting some enzymatic process and disrupting the  
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60 237 lipids metabolism (Yoneyama et al. 1978), whereas tebuconazole inhibits the biosynthesis of

ergosterol which had an effect on mycelia growth (Godwin et al. 1992). The same active ingredients of these fungicides (mancozeb and tebuconazole) proved to be effective in reducing growth and sometime in inhibiting mycotoxin production: the use of mancozeb was shown to be effective in controlling the *Alternaria*, *Fusarium* and *Botrytis* growth (Boughalleb et al. 2006, Nallathambi et al. 2009). Mancozeb was also capable to inhibit effectively the growth of *A. niger*, *A. carbonarius*, *A. flavus*, *A. parasiticus* and *A. ochraceus*, but also AFB1 and ochratoxin A production (Bellí et al. 2006, Reddy et al. 2009). The use of tebuconazole was also effective in reducing the growth of some *Fusarium* species (Müllenborn et al. 2008). There are also other fungicides which are capable of inhibiting growth of *A. flavus*, as well as the AFB1 production, such as bitertanol 25%, carbendazim 50%, carpropamid, hexaconazole 5%, propiconazole, propineb 70%, tricyclazole 75% and carbendazin + mancozeb (Reddy et al., 2009). Diodine is also effective in blocking the growth of *A. flavus* (Luz et al. 2007).

Some authors suggested that production of some mycotoxins is not associated with rapid growth of the mould; rather, higher growth rates seem to restrict mycotoxin production (Häggblom 1982). Although this was observed in paprika medium, in YES the strain UdLTA 3.193 grew well and also produced high levels of AF. Moreover, in presence of sulphur 80% (F5) it grew well and AF production was further promoted.

The use of fungicides can be very attractive in reducing mycotoxigenic fungal load, but we must be aware that in some cases it may reduce fungal growth and also stimulate the mycotoxin production (Medina et al. 2007). In our study this fact occurred in the presence of

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3 261 dodine 40% (F2) in 3% paprika at 20 °C for the isolate UdLTA 3.193, which reduced 79% of  
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6 262 growth but promoted a high level of AF production.  
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11 264 Finally, care should be taken in the choice of a suitable fungicide, because its effectiveness  
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13 265 depends on the strain, temperature and substrate used. It is also necessary to note that results  
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15 266 obtained *in vitro* are unlikely to be similar to those obtained in the field trials due to the  
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17 267 influence of several factors. When choosing a fungicide it is necessary to take into account  
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19 268 that not always the most efficient fungicides in reducing fungal growth is the best choice  
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21 269 because it can also promote the production of AF. Also it should be remembered that even if  
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23 270 fungal growth is not reduced, the AF production could still be promoted. Thus, the best  
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25 271 fungicide is the one which can prevent fungal growth and AF production at the same time.  
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Table 1 – List of fungicides used and their concentrations.

Fungicide code	Commercial name	Active substance	Recommended/Used dose
F1	Switch	Cypronidil 37.5% + Fludioxonil 25%	1 g L <sup>-1</sup>
F2	Syllit®flow	Dodine 40%	1.6 g L <sup>-1</sup>
F3	Copper fungicide	Copper oxychloride 50%	3 g L <sup>-1</sup>
F4	Bayfidan SC312	Triadimenol 31.2%	0.4 mL L <sup>-1</sup>
F5	Sufrevit	Sulfur 80%	5 mL L <sup>-1</sup>
F6	Folicur 25WG	Tebuconazole 25%	0.75 g L <sup>-1</sup>
F7	Micene ® WP	Mancozeb 80%	3.5 g L <sup>-1</sup>
F8	Caddy 10	Cyproconazole 10%	0.20 g L <sup>-1</sup>
F9	Flint	Trifloxystrobin 50%	0.25 g L <sup>-1</sup>

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Table 2 - Effects of fungicide treatments (Cypronidil 37.5% + Fludioxonil 25% (F1), Dodine 40% (F2), Copper oxychloride 50% (F3), Triadimenol 31.2% (F4), Sulfur 80% (F5), Tebuconazole 25% (F6), Mancozeb 80% (F7), Cyproconazole 10% (F8), and Trifloxystrobin 50% (F9)) over growth (mm) of *A. flavus* isolates (n=3, means followed by different letters are significantly different,  $P<0.05$ , according toTukey HSD test).

Fungicides	3 % paprika					
	20 °C			30 °C		
	UdLTA 3.193	UdLTA 3.215	UdLTA 3.217	UdLTA 3.193	UdLTA 3.215	UdLTA 3.217
Control	30.8 A	30.5 A	32.0 AB	74.7 A	71.2 A	76.2 A
F1	0.0 E	0.0 E	0.0 E	2.8 F	2.2 DE	3.2 D
F2	6.3 D	2.7 DE	0.0 E	58.0 C	56.8 AB	54.0 B
F3	13.3 C	9.3 CDE	13.8 D	19.0 E	15.2 CDE	26.3 C
F4	22.0 B	20.2 AB	30.0 B	49.5 D	31.8 BCD	50.2 B
F5	23.8 B	11.2 BCD	34.7 A	66.3 B	38.7 BC	70.8 A
F6	0.0 E	0.0 E	0.0 E	0.0 F	0.0 E	0.0 D
F7	0.0 E	0.0 E	0.0 E	0.0 F	0.0 E	0.0 D
F8	15.3 C	13.7 BC	19.2 C	47.0 D	37.3 BC	52.5 B
F9	3.0 DE	6.3 CDE	11.7 D	47.3 D	39.3 BC	49.8 B
Fungicides	YES					
	20 °C			30 °C		
	UdLTA 3.193	UdLTA 3.215	UdLTA 3.217	UdLTA 3.193	UdLTA 3.215	UdLTA 3.217
Control	57.0 A	52.3 A	53.3 A	84.0 A	85.0 A	85.0 A
F1	0.0 C	0.0 D	0.0 E	2.7 E	4.0 E	3.0 C
F2	00.0 C	0.0 D	0.0 E	0.0 E	0.0 E	0.7 C
F3	49.3 A	46.7 B	50.0 A	85.0 A	85.0 A	85.0 A
F4	28.5 B	11.5 C	21.8 C	50.7 C	30.7 D	48.5 B
F5	32.5 B	0.0 D	41.3 B	69.5 B	0.0 E	83.7 A
F6	0.0 C	0.0 D	0.0 E	0.0 E	0.0 E	0.0 C
F7	0.0 C	0.0 D	0.0 E	0.0 F	0.0 E	0.0 C
F8	9.7 C	11.2 C	14.2 D	43.2 D	37.2 C	48.0 B
F9	5.2 C	1.3 D	13.2 D	81.2 A	70.8 B	85.0 A

Table 3 - Effects of fungicide treatments (Cypronidil 37.5% + Fludioxonil 25% (F1), Dodine 40% (F2), Copper oxychloride 50% (F3), Triadimenol 31.2% (F4), Sulfur 80% (F5), Tebuconazole 25% (F6), Mancozeb 80% (F7), Cyproconazole 10% (F8), and Trifloxystrobin 50% (F9)) over AF production (ng AF g of agar<sup>-1</sup>) of *A. flavus* (UdLTA 3.193) (n=3, means followed by different letters are significantly different,  $P<0.05$ , according to Tukey HSD test).

Fungicides	3 % paprika															
	20 °C								30 °C							
	AFB1		AFB2		AFG1		AFG2		AFB1		AFB2		AFG1		AFG2	
Control	0.8	A	<LD	A	<LD	B	<LD	A	43.4	AB	45.7	A	<LD	B	<LD	BC
F1	0.0	A	0.0	A	0.0	B	0.0	B								
F2	240.2	A	68.2	A	128.1	A	<LD	A	16.8	AB	24.8	A	1.0	B	<LD	BC
F3	13.6	A	19.3	A	0.5	B	<LD	A	5.4	AB	4.8	A	<LD	B	<LD	BC
F4	1.7	A	2.5	A	<LD	B	<LD	A	3.0	AB	1.0	A	<LD	B	<LD	BC
F5	1,427.1	A	1,491.0	A	3.0	B	<LD	A	10.9	AB	5.9	A	9.6	A	0.8	B
F6	0.0	A	0.0	A	0.0	B	0.0	B	0.0	B	0.0	A	0.0	B	0.0	C
F7	0.0	A	0.0	A	0.0	B	0.0	B	0.0	B	0.0	A	0.0	B	0.0	C
F8	38.7	A	5.1	A	<LD	B	<LD	A	4.8	AB	1.7	A	<LD	B	<LD	BC
F9	113.6	A	92.6	A	<LD	B	<LD	A	65.3	A	25.2	A	0.4	B	2.5	A
Fungicides	YES															
	20 °C								30 °C							
	AFB1		AFB2		AFG1		AFG2		AFB1		AFB2		AFG1		AFG2	
Control	389,405.4	AB	430,105.8	AB	<LD	A	<LD	A	1,363,823.0	A	1,067,610.0	A	276,190.9	A	<LD	A
F1	0.0	B	0.0	B	0.0	A	0.0	B								
F2	0.0	B	0.0	B	0.0	A	0.0	B	0.0	D	0.0	D	0.0	B	0.0	B
F3	2.0	B	<LD	B	<LD	A	<LD	A	0.5	D	<LD	D	38.8	B	<LD	A
F4	548,931.5	A	578,392.6	A	<LD	A	<LD	A	356,676.0	BC	341,592.0	BC	<LD	B	<LD	A
F5	12,333.5	B	8,251.3	B	<LD	A	<LD	A	136,420.0	CD	132,917.0	CD	<LD	B	<LD	A
F6	0.0	B	0.0	B	0.0	A	0.0	B	0.0	D	0.0	D	0.0	B	0.0	B
F7	0.0	B	0.0	B	0.0	A	0.0	B	0.0	D	0.0	D	0.0	B	0.0	B
F8	50.8	B	53.5	B	6.2	A	<LD	A	667,932.0	B	652,526.0	B	<LD	B	<LD	A
F9	17.3	B	11.1	B	<LD	A	<LD	A	1,104.0	D	1,129.0	D	<LD	B	<LD	A

Note: LD (AFB1, AFB2, AFG1 and AFG2) = 0.2 ng g<sup>-1</sup>; Absence of values indicates analysis not done (growth < 5mm).



Table 4 - Effects of fungicide treatments (Cypronidil 37.5% + Fludioxonil 25% (F1), Dodine 40% (F2), Copper oxychloride 50% (F3), Triadimenol 31.2% (F4), Sulfur 80% (F5), Tebuconazole 25% (F6), Mancozeb 80% (F7), Cyproconazole 10% (F8), and Trifloxystrobin 50% (F9)) over AF production (ng AF g of agar<sup>-1</sup>) of *A. flavus* (UdLTA 3.215) (n=3, means followed by different letters are significantly different, *P*<0.05, according to Tukey HSD test).

Fungicides	3 % paprika															
	20 °C								30 °C							
	AFB1		AFB2		AFG1		AFG2		AFB1		AFB2		AFG1		AFG2	
Control	1.4	A	2.1	AB	<LD	A	<LD	A	3.7	AB	<LD	A	<LD	A	<LD	A
F1	0.0	A	0.0	B	0.0	A	0.0	B								
F2									11.4	A	<LD	A	3.2	A	<LD	A
F3	<LD	A	<LD	B	<LD	A	<LD	A	<LD	AB	<LD	A	<LD	A	<LD	A
F4	36.5	A	<LD	B	21.6	A	<LD	A	5.4	AB	<LD	A	<LD	A	<LD	A
F5	10.0	A	11.8	A	<LD	A	<LD	A	3.1	AB	0.5	A	<LD	A	<LD	A
F6	0.0	A	0.0	B	0.0	A	0.0	B	0.0	B	0.0	A	0.0	A	0.0	B
F7	0.0	A	0.0	B	0.0	A	0.0	B	0.0	B	0.0	A	0.0	A	0.0	B
F8	0.6	A	<LD	B	<LD	A	<LD	A	6.3	AB	<LD	A	<LD	A	<LD	A
F9	2.9	A	<LD	B	<LD	A	<LD	A	4.3	AB	<LD	A	<LD	A	<LD	A
Fungicides	YES															
	20 °C								30 °C							
	AFB1		AFB2		AFG1		AFG2		AFB1		AFB2		AFG1		AFG2	
Control	219.5	A	1.4	A	<LD	A	<LD	A	623.1	A	<LD	A	93.5	A	0.6	A
F1	0.0	B	0.0	A	0.0	A	0.0	B								
F2	0.0	B	0.0	A	0.0	A	0.0	B	0.0	B	0.0	A	0.0	B	0.0	A
F3	<LD	B	<LD	A	<LD	A	<LD	A	0.4	B	<LD	A	<LD	B	<LD	A
F4	8.7	B	5.3	A	<LD	A	<LD	A	176.8	B	0.9	A	<LD	B	<LD	A
F5	0.0	B	0.0	A	0.0	A	0.0	B	0.0	B	0.0	A	0.0	B	0.0	A
F6	0.0	B	0.0	A	0.0	A	0.0	B	0.0	B	0.0	A	0.0	B	0.0	A
F7	0.0	B	0.0	A	0.0	A	0.0	B	0.0	B	0.0	A	0.0	B	0.0	A
F8	3.6	B	<LD	A	<LD	A	<LD	A	161.1	B	<LD	A	<LD	B	<LD	A
F9									181.0	B	<LD	A	11.0	B	<LD	A

Note: LD (AFB1, AFB2, AFG1 and AFG2) = 0.2 ng g<sup>-1</sup>; Absence of values indicates analysis not done (growth < 5mm).

Table 5 - Effects of fungicide treatments (Cypronidil 37.5% + Fludioxonil 25% (F1), Dodine 40% (F2), Copper oxychloride 50% (F3), Triadimenol 31.2% (F4), Sulfur 80% (F5), Tebuconazole 25% (F6), Mancozeb 80% (F7), Cyproconazole 10% (F8), and Trifloxystrobin 50% (F9)) over AF production (ng AF g of agar<sup>-1</sup>) of *A. flavus* (UdLTA 3.217) (n=3, means followed by different letters are significantly different,  $P < 0.05$ , according to Tukey HSD test).

Fungicides	3 % paprika															
	20 °C								30 °C							
	AFB1		AFB2		AFG1		AFG2		AFB1		AFB2		AFG1		AFG2	
Control	2.6	A	0.4	A	<LD	A	<LD	A	4.5	AB	0.3	A	<LD	B	<LD	B
F1	0.0	A	0.0	A	0.0	A	0.0	B								
F2	0.0	A	0.0	A	0.0	A	0.0	B	0.8	B	<LD	A	24.5	A	2.3	A
F3	0.5	A	<LD	A	<LD	A	<LD	A	0.5	B	<LD	A	<LD	B	<LD	B
F4	10.0	A	14.0	A	<LD	A	<LD	A	8.0	AB	5.3	A	<LD	B	<LD	B
F5	4.2	A	2.4	A	<LD	A	<LD	A	2.7	B	<LD	A	<LD	B	<LD	B
F6	0.0	A	0.0	A	0.0	A	0.0	B	0.0	B	0.0	A	0.0	B	0.0	B
F7	0.0	A	0.0	A	0.0	A	0.0	B	0.0	B	0.0	A	0.0	B	0.0	B
F8	49.7	A	63.8	A	0.3	A	<LD	A	16.8	A	3.1	A	<LD	B	<LD	B
F9	2.5	A	1.3	A	0.0	A	<LD	A	5.1	AB	1.1	A	<LD	B	<LD	B
Fungicides	YES															
	20 °C								30 °C							
	AFB1		AFB2		AFG1		AFG2		AFB1		AFB2		AFG1		AFG2	
Control	6,264.2	A	5,529.2	A	0.1	A	<LD	A	4,175.3	A	4,714.4	A	<LD	B	<LD	A
F1	0.0	B	0.0	D	0.0	A	0.0	B								
F2	0.0	B	0.0	D	0.0	A	0.0	B								
F3	21.1	B	1.0	D	<LD	A	<LD	A	<LD	B	<LD	B	<LD	B	<LD	A
F4	1,252.8	B	1,761.5	B	<LD	A	<LD	A	24.3	B	16.3	B	3.9	B	<LD	A
F5	580.1	B	926.8	BC	<LD	A	<LD	A	5.7	B	<LD	B	<LD	B	<LD	A
F6	0.0	B	0.0	D	0.0	A	0.0	B	0.0	B	0.0	B	0.0	B	0.0	B
F7	0.0	B	0.0	D	0.0	A	0.0	B	0.0	B	0.0	B	0.0	B	0.0	B
F8	14.9	B	17.3	D	0.3	A	<LD	A	19.1	B	2.6	B	<LD	B	<LD	A
F9	79.2	B	181.1	CD	0.0	A	<LD	A	<LD	B	<LD	B	1,472.2	A	<LD	A

Note: LD (AFB1, AFB2, AFG1 and AFG2) = 0.2 ng g<sup>-1</sup>

Table 6- Effects of fungicide treatments (Cypronidil 37.5% + Fludioxonil 25% (F1), Dodine 40% (F2), Copper oxychloride 50% (F3), Triadimenol 31.2% (F4), Sulfur 80% (F5), Tebuconazole 25% (F6), Mancozeb 80% (F7), Cyproconazole 10% (F8), and Trifloxystrobin 50% (F9)) over Aft production (ng AF g of agar<sup>-1</sup>) of *A. flavus* isolates (n=3, means followed by different letters are significantly different, *P*<0.05, according to Tukey HSD test).

Fungicides	3 % paprika											
	20 °C						30 °C					
	UdLTA 3.193		UdLTA 3.215		UdLTA 3.217		UdLTA 3.193		UdLTA 3.215		UdLTA 3.217	
Control	1.1	A	3.7	B	3.2	A	89.3	A	4.0	A	5.0	BC
F1	0.0	A	0.0	B	0.0	A						
F2	436.6	A			0.0	A	42.6	A	14.8	A	28.0	A
F3	33.5	A	<LD	B	0.7	A	10.4	A	<LD	A	08	BC
F4	4.3	A	58.3	A	24.1	A	4.1	A	5.6	A	13.5	ABC
F5	2,921.2	A	21.9	AB	6.8	A	27.2	A	3.8	A	2.9	BC
F6	0.0	A	0.0	B	0.0	A	0.0	A	0.0	A	0.0	C
F7	0.0	A	0.0	B	0.0	A	0.0	A	0.0	A	0.0	C
F8	44.0	A	0.9	B	113.9	A	6.6	A	6.6	A	20.1	AB
F9	206.2	A	3.1	B	4.0	A	93.5	A	4.6	A	6.3	BC
Fungicides	YES											
	20 °C						30 °C					
	UdLTA 3.193		UdLTA 3.215		UdLTA 3.217		UdLTA 3.193		UdLTA 3.215		UdLTA 3.217	
Control	819,511.0	AB	221.1	A	11,793.5	A	2,707,624.0	A	717.3	A	8,889.9	A
F1	0.0	B	0.0	B	0.0	C						
F2	0.0	B	0.0	B	0.0	C	0.0	D	0.0	B		
F3	2.3	B	<LD	B	22.3	C	39.0	D	1.4	B	<LD	B
F4	1,127,324.0	A	14.2	B	3,014.4	B	698,268.0	C	177.9	B	44.6	B
F5	20,585.0	B	0.0	B	1,507.1	BC	269,337.0	CD	0.0	B	6.0	B
F6	0.0	B	0.0	B	0.0	C	0.0	D	0.0	B	0.0	B
F7	0.0	B	0.0	B	0.0	C	0.0	D	0.0	B	0.0	B
F8	111.0	B	3.9	B	32.7	C	1,320,458.0	B	161.4	B	21.8	B
F9	28.0	B			260.5	BC	2,233.0	D	192.2	B	1,472.5	B

Note: LD (AF) = 0.6 ng g<sup>-1</sup>; Absence of values indicates analysis not done (growth < 5mm).