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MYCOTOXINS PRODUCED BY *ALTERNARIA ALTERNATA* ISOLATED FROM *CERATONIA SILIQUA* LEAF SPOT

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ABSTRACT

The research work was conducted to determine the production of mycotoxins from *Alternaria alternata* isolated from carob leaves by the thin layer chromatography technique. The results showed that the production of Altertoxin I, and Alternariol monomethyl ether by *A. alternata* (A1) and of Alternariol monomethyl ether by *A. alternata* (A2), while *A. alternata* (A4) produced Altertoxin I. *A. alternata* (A3) did not produce any mycotoxins.

KEYWORDS: Mycotoxins, leaf spot, *Ceratonia siliqua*, *Alternaria alternata.*

INTRODUCTION

Mycotoxins are fungal secondary metabolites that if ingested can cause a variety of adverse effects on both humans and animals (Hampikyan *et al.*, 2010; Moreno, et al, 2012). The genera of mycotoxigenic fungi are mainly represented by *Aspergillus*, *Penicillium* and *Fusarium*, but *Trichoderma*, *Trichothecium* and *Alternaria* are also important as food contaminants or pathogens for plants, among others (Smith, 1983; Abbott, 2002; Sawane and Sawane, 2014).

Alternaria alternata is a fungal pathogen that causes Alternaria leaf spot of carob (*Ceratonia siliqua*) (El-Gali, 2014). Symptoms appear on leaves. Dark brown to black areas of dead tissue between leaf veins are caused by a fungal toxin. On the leaves, brown circular spots are often surrounded by a yellow area. Leaf spots have characteristic dark concentric rings. Some

metabolites from *Alternaria* fungi are toxic to plants and animals, and are designated as phytotoxins and mycotoxins, respectively (Strange, 2007; Mobius and Hertweck, 2009; Duke and Dayan, 2011).

Mycotoxins produced by *Alternaria* and specifically by *Alternaria alternata* are numerous. Among them the most widely studied are: tenuazonic acid (TA), alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), alter toxin I and ten toxin (Battilani et al., 2009; Lou et al., 2013). The main goal of the present study is to determine the production of mycotoxins from the fungal previously mentioned, which have been isolated from carob leaves.

MATERIALS AND METHODS

Fungal material

Associated fungi were isolated from different symptoms of carob trees leaves spot. The spots were cut into small pieces then surface-sterilized in NaOCl for 2 min and rinsed in two changes of sterile distilled water. The leaf pieces were blotted dry in between sterile Whatman No. 1 filter papers and plated on sterile potato dextrose agar (PDA) at the rate of 5 pieces per plate and incubated at room temperature of 25 ± 1^{o}C. Sub-cultures were made from emerging colonies and pure cultures obtained for subsequent studies. Four isolates of *Alternaria altenata* (A1, A2, A3 and A4) were obtained from different leaves spots on carob trees. For storage, the fungal isolates were maintained on PDA slant.

Qualitative detection of mycotoxins by using thin layer chromatography

Mycotoxin profiles were analyzed using thin-layer chromatography (TLC) as described by Singh et al. (1991). Agar plugs were cut from 14-d-old cultures on PAD and touch the agar side directly to silica gel 60 TLC plates at a baseline was drawn on the plate at a distance of 2 cm from the lower edge and allowed for one minute. The chromatoplates had previously been activated at a temperature of 105˚C for 90 min. Let the spot for 1 min to dry. The solvent system used was (benzene: methanol: glacial; 96:6:2, v:v:v) to detect the mycotoxins produced by the test isolates. The chromate-plates were removed after 90 minutes, or when the solvent reached a mark 2 cm from the upper edge of the plates. They were dried mechanically and examined under the UV/Vis lamp at short and long wave, and the specks were circled with a pencil. Reading the chromatographic spots from the discs containing the isolates fungi. The retention factors (R_f) of the compounds/toxins detected in the media were calculated. R_f = distance traveled by the compound/distance traveled by the solvent. The R_f

values were comparison with the standard reference (Centeno and Calvo, 2002) depended on standard mycotoxins (Sigma®).

RESULTS AND DISCUSSION

Based on the symptoms and morphological characterizes, the fungus was identified as *Alternaria alternata* (Ellis, 1971; Simmons, 1997; Zhang, 2003 and Woudenberg, et al., 2013). The process of isolation resulted in four isolates (referred as A1, A2, A3 and A4) of pathogen (Fig. 1).

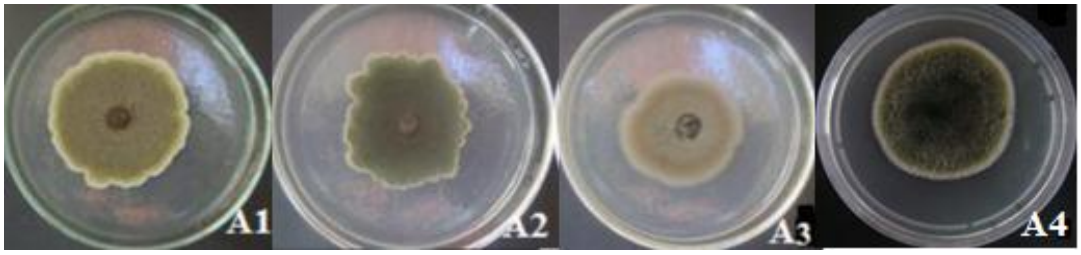

Figure 1. Deferent Colonies of *A. alternata* on PDA plate.

Fig. 2, illustrates the results obtained from the study of the production of mycotoxins by *Alternaria alternata* isolates after TLC. Mycotoxin assay revealed that two toxins were produced in this study. it can be observed that *Alternaria alternata* only produces alter toxin I and alternariol monomethyl ether. The retention factors (Rf) of two the toxins produced were determined. While *A. alternata* (A1), produced both alter toxin I (ATX) and alternariol monomethyl ether (AME), isolate *A. alternata* (A2) produced AME only and the ATX was produced by *A. alternata* (A4), the isolate *A. alternata* (A3) not produced any type of toxins.

Table 1. shows the values of the Rf corresponding to the chromatographic spots of the mycotoxins produced by *Alternaria alternata*. When comparing the four fractions of *Alternaria alternata* obtained by the thin layer chromatography with the standards of alter toxin I, and alternariol monomethyl ether, it can be observed that *Alternaria alternata* only produces alter toxin I and alternariol monomethyl ether. *Alternaria alternata* produce neither tenuazonic acid nor ten toxin under the experimental condition of this study.

The production of ten toxin is conditioned by a limited quantity of phosphate in the cultivation environment (Ramm *et al*., 1994), which may have affected the results. Tenuazonic acid is described by Stinson et al., (1980) as a mycotoxin which may be produced by numerous species of *Alternaria*, in cultures isolated from different sources. It is considered the most important *Alternaria alternata* toxic substance although its production is influenced by nitrogen concentration in a cultivation environment.

However, isolated *Alternaria alternata* did not produce tenuazonic acid in this study.

Generally, the production of mycotoxins by *Alternaria alternata* is conditioned by high water activity, by the w incubation temperature, by the pH substrate and by the type of substrate in which the microorganism grows (Burroughs *et al*., 1976; Magan *et al*., 1984).

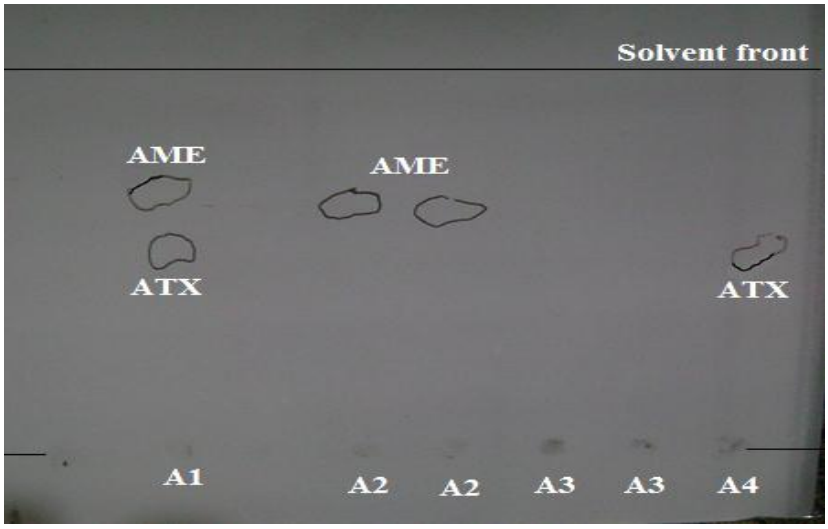

Figure 2. TLC of the mycotoxins produced by *Alternaria alternata*. ATX-I: Alter toxin I, AME: Alternariol monomethyl ether.

Table 1. Mycotoxins produced by *Alternaria alternata* isolated from carob leaves

Isolated Fungi	Mycotoxin			
	Alter toxin I (ATX-I)		Alternariol monomethyl ether (AME)	
	Retention factor (R_f)		Retention factor (R_f)	
	Standard	Detected	Standard	Detected
Alternaria alternata (A1)	0.26	+	0.38	+
Alternaria alternata (A2)	-	-	0.38	+
Alternaria alternata (A3)	-	-	-	-
Alternaria alternata (A4)	0.26	+	-	-
+ : Found, -: Not found Values of Standard depended to (Sigma®).				

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