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Antifungal activity of *Euphorbia* species against moulds responsible of cereal ear rots

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ABSTRACT

Aims

This work aimed to identify secondary metabolites from aerial parts of *Euphorbia* species functional for control of toxigenic *Fusarium* species responsible of cereal grain rots.

Methods and Results

Aerial parts of *Euphorbia serpens*, *E. schickendantzii* and *E. collina* were sequentially extracted with hexane, ethyl acetate and methanol. The extracts were tested against strains of *F. verticillioides* and *F. graminearum* by microdilution tests.The hexane extract of *E. collina* provided the lowest IC_{50}s on both fungal species.Further fractionation showed that cycloartenol (CA) and 24-methylenecycloartanol are associated to the moderate inhibitory effect of the hexane extract on fungal growth.Sublethal concentrations of CA and 24MCA blocked deoxynivalenol (DON) and fumonisins production.CA and 24MCA co-applied with potassium sorbate, a food preservative used for *Fusarium* control, synergized the growth inhibition of fungi. The mixtures reduced mycotoxins accumulation when applied at sublethal concentrations.

Conclusions

CA and 24MCA inhibited both fungal growth and mycotoxins production. This fact is an advantage respect to potassium sorbate which increased the mycotoxins accumulation at sublethal concentrations.

Significance and Impact of the Study

CA and 24MCA synergized potassium sorbate and their mixtures offer a lower mycotoxigenic risk than potassium sorbate for control of the *Fusarium* species.

Keywords: Agriculture, biotechnology, fungi, food safety, preservatives.

INTRODUCTION

Fusarium verticillioides and *F. graminearum* are important etiological agents of cereal ear rots (Belizán et al., 2019). They contaminate the grains with mycotoxins which have adverse health effects on humans and animals. Fumonisins and deoxynivalenol (DON) are among the most common mycotoxins found in grains. *F. verticillioides* is the main species associated with fumonisin production. Chronic consumption of fumonisins can lead to liver and kidney dysfunctions (Kamle et al., 2019). Fumonisins deplete sphingolipid biosynthesis which is believed

responsible of esophageal cancer in humans (Myburg et al., 2002). They disrupt the cardiovascular system leading to leukaencephalamalacia in equines and pulmonary edema in pigs (Kamle et al., 2019). In the case of DON, it is often produced by *F. graminearum*. DON intake inhibits protein synthesis and induces strong oxidative stress (Pestka, 2008). It has been associated with diarrhea, emesis, anorexia, feed refusal and growth retardation observed in farm animals (Belizán et al., 2019). Prevention of *Fusarium* growth and mycotoxin contamination are mainly based on the application of azole fungicides at cereal flowering and food preservatives such as potassium sorbate during grain storage. Nevetheless, the uncontrolled use of fungicides has favoured the appearance of resistance in both *F. verticillioides* and *F. graminearum* (Kumle et al., 2019). It is noteworthy that azoles used in medicine and agriculture have a very similar structure. This fact does raise the possibility of the appearance of clinical *Fusarium* isolates less susceptible to azoles (Brauer et al., 2019). Additionally, current food preservatives can modify grain organoleptic properties at doses that completely suppress mold growth and sometimes trigger the accumulation of mycotoxins at subinhibitory concentrations (Jiménez et al., 2014). Hence, chemical control of *Fusarium* species requires the incorporation in the market of new antifungals or additives of existing antifungals. *Euphorbia* species might provide these compounds. In La Pampa province (Argentina), several *Euphorbia* species are widely used in folk medicine for wound healing and as antiseptic (Barboza et al., 2009). However, they have been scarcely investigated for their antimicrobial compounds. In this work, we tested the antifungal activity of extracts from *E. serpens, E. schickendatzii* and *E. collina* against *Fusarium* strains and identified the bioactive compounds involved.

RESULTS

Impact of the plant extracts on fungal growth

The methanol, ethyl acetate and hexane extracts obtained from aerial parts of the *Euphorbia* species were tested against the *Fusarium* strains. The extracts of *E. serpens* and *E. schikendantzii* and the methanolic extract of *E. collina* did not exhibit antifungal activity in the microdilution tests. For this reason, Table 1 displays IC_{50}s only for the hexane and ethyl acetate extracts of *E. collina*. Both fungal species showed the lowest values when exposed to the hexane extract. They were equally sensitive to the ethyl acetate extract, with IC_{50}s that were similar to the values recorded for potassium sorbate and two to four fold higher than the IC_{50}s of the hexane extract.

The hexane and ethyl acetate extracts of *E. collina* showed an antifungal effect several orders of magnitude weaker than that of tebuconazole.

Isolation of antifungals from the methanolic extract of *E. collina*

The hexane extract of *E. collina* was subjected to a bioassay guided isolation. Pools and fractions recovered during the separation steps were tested by microdilution. However, antifungal activity only was found for the pool G10 recovered from the column chromatography of the hexane extract and the fractions F1 and F2 collected after HPLC of pool G10 (Table 2). Strains of *F. verticillioides* were similarly inhibited by pool G10, F1 and F2. The same was observed for strains of *F. graminearum*. GC-MS analysis displayed in Figure 1 and Table 3 indicated that G10 was a mixture of 51% cycloartenol (CA) and 49% 24-methylenecycloartanol (24MCA). These compounds were recovered from HPLC of G10 in fractions F1 and F2. GC-MS analysis of F1 and F2 indicated that they comprised 98% of CA and 97% of 24MCA, respectively.

Joint action between the isolated antifungals and potassium sorbate

CA and 24MCA synergized the growth suppression exerted by potassium sorbate (PS) on the *Fusarium* strains. This is indicated by the FICI values below 0.5 obtained for these pentacyclic triterpenes when combined with PS (Table 4). The combinations corresponding to the FICI values were F1+PS (375+188 µg/ml) and F2+PS (94+188 µg/ml) on *F. graminearum*, and F1+PS (188+94 µg/ml) and F2+PS (94+188 µg/ml) on *F. verticillioides*. The partial concentrations at which PS participates in these blends mean a lowering of 1/8x MIC to 1/16x MIC when compared to the effect of PS alone.

Antimycotoxigenic activity

Sublethal concentrations of CA, 24MCA and their blends with PS were tested against the strains of both *Fusarium* species to know whether these compounds affect mycotoxin production (Table 5). F1 and F2 completely blocked the DON biosynthesis at concentrations higher than 94 µg/ml and at 188 µg/ml the biosynthesis of fumonisins. The mixtures strongly reduced the accumulation of mycotoxins although complete suppresion was not observed in the range of sublethal concentrations tested. The lowest DON and fumonisins accumulations recorded for F1+PS (2:1, w/w) were at concentrations greater than 47+23 µg/ml and 94+47 µg/ml, respectively. The mycotoxin contents registered at these concentrations indicate an average fall of 50% DON and

80% fumonisins respect to controls. F2+PS (1:2, w/w) showed the smallest contents of DON at concentrations equal and higher than 23+47 µg/ml, while the lowest levels of fumonisins were achieved at 47+94 µg/ml. Compared to the controls, they showed average reductions in mycotoxin accumulations of 70% and 85%, respectively.

DISCUSSION

The highest antifungal activity was observed for the hexane extract of *E. collina* while the remaining extracts were inactive or exhibited less inhibition on strains of both *F. verticillioides* and *F. graminearum*. Previous reports indicated antimicrobial and antihelmintic activities for extracts of some of the *Euphorbia* species investigated in this work. The methanolic extract of aerial parts of *E. serpens*, which was inactive on the *Fusarium* species at the concentrations and doses investigated here, suppressed the growth of *Streptococcus lutea*, *S. aureus* and *E. coli* at a dose of 10 mg in disc diffusion tests and *Vibrio cholerae* at concentrations comprissed between 3.9 and 12.3 mg/ml in macrodilution assays (Bakhuni et al., 1974; Payne et al., 2015). Methanolic extracts of *E. schickendantzii* and *E. collina* were previously tested for antihelmintic activity which was moderate on *Ancylostoma caninum* at concentrations equal and higher than 7.5 mg/ml (Alvarez et al., 2018). The methanolic extract of *E. schickendantzii* also moderately inhibit the motility of *Haemonchus* spp at concentrations of 1 to 4 mg/ml (Lamberti et al., 2009). As far as we know, the current work reports for the first time antifungal activity associated to *E. schickendantzii* and *E. collina.* The search for the antifungal agents responsible of the antifungal activity of the hexane extract of *E. collina* led to the isolation and identification of GA and 24MGA. These pentacyclic triterpenoids are intermediates in plant biosynthesis of phytosterols and were reported in high contents into the aerial parts of several *Euphorbia* species (De Pascual et al., 1987; Zare et al., 2015;). They are naturally present in several plant foods and edible plant oils. CA and 24MCA possess pharmaceutical properties including the anti-inflammatory and antioxidant activities (Abidi, 2001; Zhang et al., 2017). Their effect on *Fusarium* species is reported for the first time in this work where they had a moderate to weak antifungal activity (MIC between 250 and 1000 µg/ml). CA was reported with moderate to weak activity against clinical isolates of *Candida albicans*, *Trichophyton mentagrophytes* and *Aspergillus niger* (Ragasa et al., 2004), and inactive on the bacteria *Streptococcus aureus* and *Bacillus subtilis*, while 24MCA showed strong antiplasmodial activity on *Plasmodium falciparum* (Bickii et al., 2007).

The synergistic effect observed for CA and 24MCA might be used as a new strategy to reduce levels of PS needed for grain preservation. The best pairs of concentrations of CA+PS and 24MCA+PS offers the possibility to obtain a complete suppresion of fungal growth with PS concentrations of 188 and 375 µg/ml, respectively. Depending of the food matrix considered, PS can be incorporated to foods in a range of concentrations comprissed between 3000 and 300 µg/ml (EFSA, 2015). Hence, a significant reduction of PS can be achieved with its co-application together with the pentacyclic triterpenes. The reasons of the synergistic action were not elucidated in this work. The antifungal activity of PS lies in its incorporation into the fungal cells as sorbic acid which is intracellularly accumulated and finally produces a disruption in the pH homeostasis (Plumridge et al., 2004). Sorbic acid also is able to directly act on several cellular targets including enzymes of the carbohydrate metabolism, the antioxidant system, and the citrate cycle (Sofos and Busta, 1981). CA and 24MCA might primarily act on fungal membranes as other pentacyclic triterpenes and likely interact with membrane constituents leading to an increase in membrane permeability which favour the entrance of extracellular sustances and the leakage of cell solutes (Haraguchi et al., 1999). It can be hypothezised that the pentacyclic triterpenes increased sorbic acid uptake which readily enhanced its inhibitory effect on fungal physiology. This point requires further research. In the case of tebuconazole, its mixtures with CA and 24MCA showed an additive/indifferent interaction. Altogether, the joint action tests indicate that CA and 24MCA had a mode of action different from that of the commercial antifungals tested and might be used in mixtures with sorbic acid-based food preservatives.

Both CA and 24MCA blocked the biosynthesis of the mycotoxins produced by the *Fusarium* strains. The antimycotoxigenic effect of pentacyclic triterpenes against DON and fumonisins producing fungi has been scarcely explored. Only lupeol was reported and had a strong blocking effect on DON and fumonisins accumulation (Sakuda et al., 2016). CA and 24MCA might affect mycotoxin biosynthesis due to their high lipophilicities and antioxidant capacities. Lipophilicity aids the entrance of the secondary compound into the fungal cells while the antioxidant power is believed to relieve the oxidative stress needed for mycotoxins biosynthesis (Castro et al., 2020). However, accumulation of DON and fumonisins depends not only of the intrinsic molecular properties of a secondary metabolite but also of other factors provided for fungal growth such as nutrient availability, temperature and water activity (Belizan et al., 2019). For this reason, additional studies are needed for a better understanding of how CA and 24MCA impact DON and

fumonisins accumulation under several environmental conditions faced by the *Fusarium* species during storage of cereal grains.

This work reports for the first time the antifungal activity of CA and 24MCA. Although they showed a moderate to weak inhibitory effect on growth of *Fusarium* strains, they had a significant antimycotoxigenic effect. Their synergistic interactions with PS raise a promisory use of CA and 24MCA in food preservation against *F. verticillioides* and *F. graminearum* responsible of cereal ear rots.

AUTHOR'S CONTRIBUTION

DAS, CANC, RET designed the study. HLA performed and supervised the plant collections, and extracted the plant materials. CMJ, HLA, DAS performed antifungal and antimycotoxigenic assays. MSB, GRL, CANC, DAS, HLA did separation and identification of molecules. DAS, HLA and CMJ analysed the data. DAS drafted and reviewed the manuscript. All authors read and approved the final manuscript.

MATERIALS AND METHODS

Plant materials

Aerial parts (leaves and stems) of *Euphorbia serpens* (5 kg), *E. schickendantzii* (6 kg) and *E. collina* (6 kg) were collected during january and february 2014 in La Pampa province (Argentina). The plant species were sampled in Trenel (35°41′16′′ S, 64°07′58′′ W), Parque provincial Luro (36°90′03′′ S, 64°28′82′′ W) and Colonia Emilio Mitre (36° 20′27′′ S, 66° 27′ 89′′ W). The plant materials were identified by biologist Hebe Lina Alvarez and the voucher specimens SRFA158 (*E. serpens*), SRFA161 (*E. schickendantzii*) and SRFA164 (*E. collina*) were deposited at the Herbarium of the Faculty of Agronomy (National University of La Pampa, Argentina).

Microorganisms

Strains of *F. graminearum* (NRRL 28063 and LABI11) and *F. verticillioides* (NRRL 25457 and LABI7) were provided by the LABIFITO culture collection (National University of Tucumán, Argentina). The strains were maintained in SNA medium (Spezieller Nahrstoffarmer agar: 0.1% K_2HPO_4, 0.1% $NaNO_3$, 0.05% $MgSO_4$. $7H_2O$, 0.05% KCl, 0.02% glucose, 0.2% sucrose and 2% agar) at 4ºC. The stored strains were transferred and cultured at 30°C (*F. verticilliodes*) or 25°C (*F. graminearum*) on MPA medium (solid malt peptone agar medium: 1.5% malt extract, 0.5% peptone, 0.1% glucose and 1.8 % agar) for 7 days prior to use in bioassays.

Plant extracts

The aerial parts of the plants were dried at room temperature in the dark for a week. Then, they were ground to a coarse powder and stored in sealed flasks at -20°C until use. Each ground material (10 g) was sequentially extracted (2 x 100 ml) with hexane, ethyl acetate and metanol. The powdered plant material was exposed 48 h to each organic solvent, subsequently trapped in a Whatman 4 filter paper and dried at 40°C till complete dryness before immersion in the next organic solvent. Each filtered organic fraction was evaporated under reduced pressure at 40°C, the dry residues were weighed. The dry residues of the filtered organic fractions were stored at -15 °C in sealed flasks until use.

Broth microdilution tests

They were performed in 96-well, flat-bottom microplates following the M38-A document from the National Committee for Clinical Laboratory Standards with some modifications (NCCLS, 2002). Fungal colonies were grown in Petri dishes for 7 to 15 days in solid MPA medium in the darkness at 30°C (*F. verticillioides*) or 25°C (*F. graminearum*). Then, the fungal colonies were washed with 2 mL of sterile distilled water to obtain suspensions of microconidia (*F. verticillioides*) or macroconidia (*F. graminearum*). The asexual spores were counted in a Neubauer chamber, and the suspension was diluted in semiliquid YES medium (yeast-malt extract-sucrose: 2% yeast extract, 15% sucrose, 0.05 % magnesium sulphate, 0.125 % agar) to obtain a density of 1 x 10^4 spores mL^{-1}.The stock solutions of each organic extract were prepared in DMSO and diluted with culture medium to prepare two fold dilution series which were 2000, 1000, 500, 250, 125, 63 µg mL^{-1}, in semiliquid YES medium. The final volume in each well was 200 µL containing 2% DMSO. This volume corresponded to 100 µL of fungal spore suspension and 100 µL of a dilution of an organic plant extract. Growth controls were prepared by adding in each well 100 µL of YES medium plus 100 µL of spore suspension. Controls of sterility were 200 µL of YES medium per well. Each treatment (organic extracts or controls) included three wells per microplate. Each microplate was prepared twice. The microplates were incubated 72 h at 30°C (*F. verticillioides*) or 25°C (*F. graminearum*). Then, the minimum concentration of each organic fraction required to inhibit 100% of the microbial growth (MIC) was visually determined. The MIC values presented in tables are means of three replicates obtained from two experiments.

Isolation and identification of the antifungal constituents from the hexane extract of *Euphorbia collina*

The hexane extract (1.03 g) was suspended in 20 ml of hexane:ethyl acetate (90:10, v/v) and loaded on an hexane-stabilized column which was packed with silica gel 60 (75 g, 230-400 mesh, Merck, Darmstadt, Germany). The column was eluted with a gradient of hexane-ethyl acetate (840 mL, 88:12; 780 mL, 80:20; 760 mL, 75:25, v/v), with collection of 119 fractions (20 ml each). Fractions were grouped in 20 pools (G1-G20) according to the TLC patterns observed after development with hexane-ethyl acetate 88:12 (v/v) and 3:1 (v/v). Then, the antifungal activity of the pools was tested in microdilution tests at concentrations of 1500, 750, 375, 188, 94, 47 and 23 µg/ml. Pool G10 was evaporated under reduced pressure at 60°C, its dry residue was solubilized in dichloromethane and then injected in a gas chromatograph ThermoElectron Model trace GC ultra (Thermo Electron Corp, Madison, WI) equipped with a column DB-5 (phenyl methyl siloxane; 30 m x 0.25mm ID, 0.25 µm film thickness as stationary phase) and coupled to a mass spectrometry detector ThermoElectron Model Polaris Q (Thermo Electron Corp, Madison, WI). Helium was used as mobile phase at a flow of 1 ml/min; split ratio: 10:1; injection volume: 1 µl; temperature program: 100 °C (4 min), 100–280°C (ramp 10°C/min), 300°C (12 min); mass analyser: ion trap; ionization type: electron impact at 70 eV; method of acquisition: full scan: 50–500 a.m.u.; ionization time: 0.25 min. The constituents of pool G10 were identified by matching of their mass spectra fragmentation patterns with those stored in the Wiley/NIST database and data previously published (Zare et al., 2015). Retention index of each identified compound was calculated in relation to *n*-alkanes (Aristimuño Ficoseco et al., 2014).

The dry residue of G10 was dissolved in a small volume of 4% isopropanol in acetonitrile and filtered through a 0.22 µm PTFE membrane. The filtrate was used in multiple injections performed in a HPLC coupled to an ultraviolet detector set at 210 nm (Masohan and Bhatia, 1996) and equipped with an IB-SIL 5 C18 column (250 x 10 mm, 5 µm) from Phenomenex. Injections were performed through a Rheodyne injector fitted with a 500-µl loop. Flow was 2.8 mL/min. Fractions F1 and F2 corresponding to peaks were collected and dried under reduced pressure. A small aliquot of F1 and F2 was dissolved in dichloromethane and injected in GC-MS to check composition. F1, F2 and G10 were dissolved in DMSO and used for broth microdilution tests following the protocol previously described for the organic extracts.

Joint action of the hexane extract of *Euphorbia collina*, F1 and F2 with commercial antifungals

The hexane extract of *E. collina*, F1 and F2 were tested in combination with tebuconazole and potassium sorbate on strains of *F. verticillioides* and *F. graminearum* by the chessboard technique

and the inhibitory fractionated concentration (FICI) was calculated as: FICI = (Concentration of A in MIC_{A+B}/Concentration of A in MIC_A) + (Concentration of B in MIC_{A+B}/Concentration of B in MIC_B). Interpretation of FICI: ≤ 0.5, synergy; 0.5-4.0, no interaction; > 4.0, antagonism (Vitale et al., 2005).

Antimycotoxigenic effect of F1, F2 and their mixtures with potassium sorbate

The antifumonisin effect of F1, F2 and mixtures of these compounds with potassium sorbate on toxin production by *F. verticillioides* and *F. graminearum* was then tested. Strains were cultivated in a medium depicted by López-Errasquín et al. (2007) containing 0.05% malt extract, 0.1% yeast extract, 0.1% peptone, 0.1% KH_2PO_4, 0.03% $MgSO_4$ $7H_2O$, 0.03% KCl, 0.005% $ZnSO_4$ $7H_2O$, 0.001% $CuSO_4$ $5H_2O$ and 2% fructose. In the case of the *F. graminearum* strains, they were cultivated in 0.1% yeast extract, 0.1% peptone and 5% glucose (Ueno et al., 1975). F1, F2 and their mixtures with potassium sorbate were dissolved in 96% ethanol and added to 8 ml of each medium previously poured in 125 ml Erlenmmeyer flasks. Then, the media were inoculated with 1 ml of medium containing 10^4 conidia of *F. verticillioides* or *F. graminearum*. Concentrations of F1 and F2 were tested at 94, 188 and 375 µg/ml while the mixtures of F1+potassium sorbate and F2+potassium sorbate were assayed at 12+23, 23+47 and 47+94 µg/ml. The Erlenmeyers were shaken for a week at 150 rev min^{-1} under the absence of light at 25 °C. Then, their contents were centrifuged at 3000 g for 10 min. Mycotoxins were measured in the supernatants while the mycelial pellets were freeze dried during 48 h and weighed to establish the fungal biomass. Fumonisins and deoxynivalenol were measured with Elisa kits (Ridascreen Fast fumonisin and Fast DON, Biopharm, Germany) according to the manufacturer's instructions. They were expressed as µg mycotoxins/g of dry fungal biomass. The data recorded were subjected to ANOVA and differences among means were established by Dunnet T3 test. The statistical analyses were performed at $P = 0.05$.

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CONFLICT OF INTEREST DISCLOSURE

No conflict of interest declared

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Figure captions

Figure 1 Gas chromatography coupled to mass spectrometry of pool G10: (A) Chromatogram showing peaks at 14.708 and 15.339 min. Scanned mass spectra of these peaks are shown in (B) and (C), respectively.

Table 1. Values of inhibitory concentrations of 50% of fungal growth (IC_{50}) obtained for the ethyl acetate and hexane extracts of *Euphorbia collina* against strains of *Fusarium vertillioides* (NRRL 25457 and LABI7) and *F. graminearum* (NRRL 28063 and LABI11).

	IC_{50} (µg/ml)[1]			
	NRRL 25457	LABI7	NRRL 28063	LABI11
Ethyl acetate extract	1451 (1380-1520)	1325 (1270-1495)	1447 (1413-1495)	1467 (1407-1501)
Hexane extract	814 (781-925)	824 (791-915)	360 (295-394)	392 (315-454)
Tebuconazole	0.06 (0.04-0.07)	0.06 (0.04-0.07)	0.04 (0.02-0.05)	0.03 (0.01-0.04)
Potassium sorbate	1450 (1350-1513)	1490 (1310-1550)	1435 (1330-1490)	1446 (1390-1500)

[1]95% confidence intervals are indicated between parentheses.

Table 2. Values of minimum inhibitory concentration (MIC) obtained for pool G10 recovered from column chromatography of the hexanic extract of *Euphorbia collina* and fractions F1 and F2 separated from G10 after HPLC. The tests for antifungal activity were performed against strains of *Fusarium vertillioides* (NRRL 25457 and LABI7) and *F. graminearum* (NRRL 28063 and LABI11).

	MIC (µg/ml)[1]			
	NRRL 25457	LABI7	NRRL 28063	LABI11
G10	1500	1500	750	750
F1	1500	1500	750	750
F2	1500	1500	750	750
Potassium sorbate	3000	3000	3000	3000
Tebuconazole	2	2	2	2

[1]95% confidence intervals are indicated between parentheses.

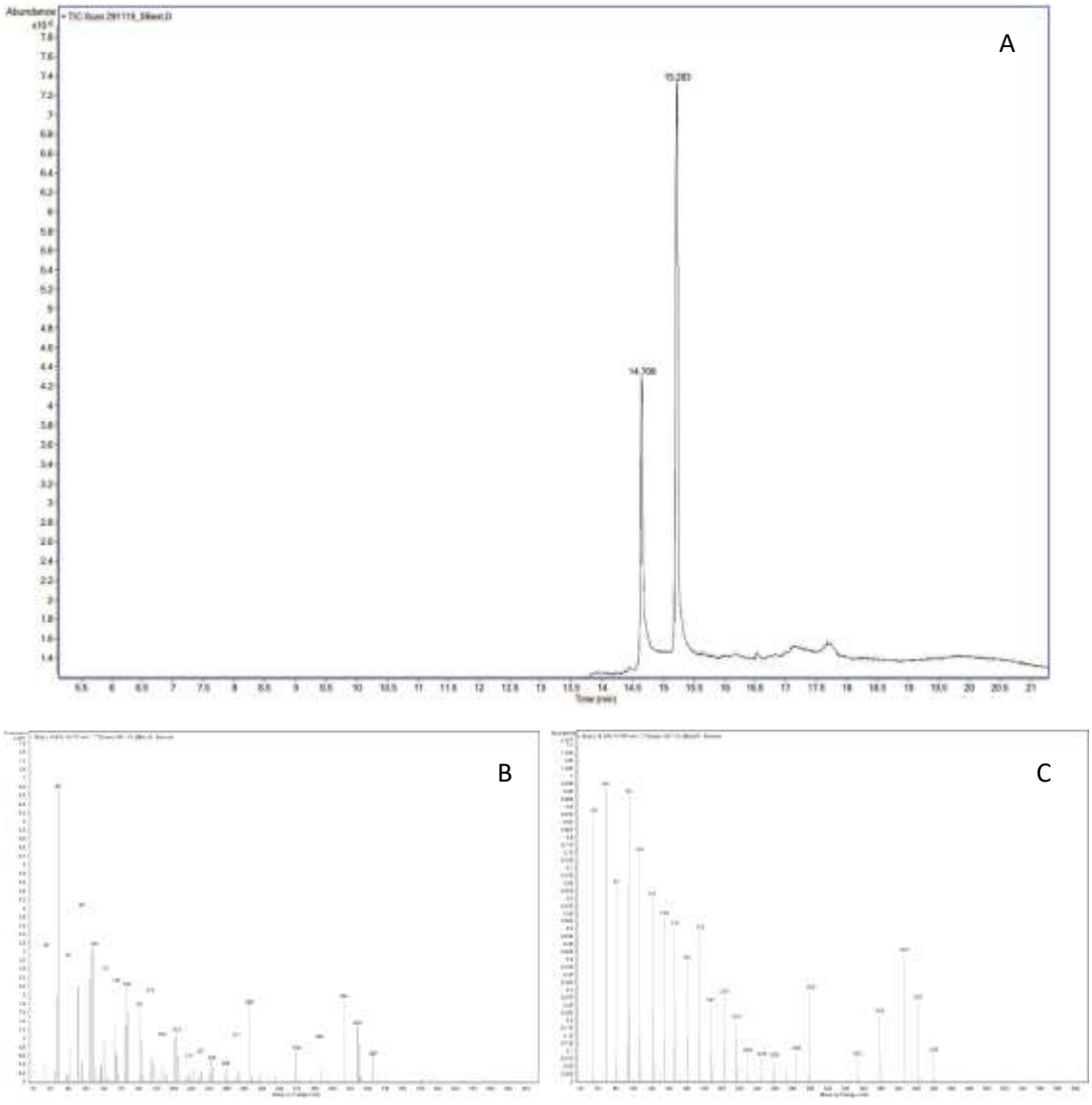

Figure 1

Table 3. GC-MS analysis of pool G10

Arithmetic Index	Purity (%)	$[M^+]$ m/z (%)	Major fragment ions, m/z (%)	Compound
2816	51	426 (11)	55 (46), 69 (100), 81 (42), 95 (60), 109 (46), 121 (38), 135 (35), 147 (32), 161 (25), 175 (30), 187 (15), 203 (18), 215 (9), 231 (10), 243 (9), 259 (8), 271 (15), 286 (18), 297 (5), 315 (5), 339 (29), 365 (15), 393 (29), 408 (20)	Cycloartenol
3108	49	440 (10)	55 (100), 69 (100), 81 (65), 95 (93), 107 (75), 121 (69), 135 (54), 147 (50), 161 (40), 175 (48), 187 (25), 203 (28), 216 (19), 229 (9), 245 (8), 259 (7), 285 (9), 300 (29), 353 (8), 379 (23), 407 (43), 422 (28)	24-methylene cycloartanol

Table 4. Joint action of commercial antifungals with F1 (98% cycloartenol) and F2 (97% 24-methylenecycloartenol) determined by the checkerboard technique against *Fusarium verticillioides* (NRRL 25457 and LABI7) and *F. graminearum* (NRRL 28063 and LABI11).

	FICI		Joint effect[1]	FICI		Joint effect[1]
	NRRL 25457	LABI7		NRRL 28063	LABI11	
Sorbate potassium						
+ F1	0.31	0.31	synergism	0.16	0.16	synergism
+ F2	0.13	0.13	synergism	0.13	0.13	synergism
Tebuconazole						
+ F1	1.00	1.00	additivism	1.00	1.00	additivism
+ F2	0.75	0.75	additivism	0.75	0.75	additivism

[1]Interpretation of FICI: ≤ 0.5, synergy; 0.5-4.0, no interaction; > 4.0, antagonism.

Table 5. Impact of sublethal concentrations of F1 (98% cycloartenol), F2 (97% 24-methylenecycloartenol) and their mixtures with potassium sorbate (PS) on production of deoxynivalenol (DON) and fumonisins (Fum) recorded for strains of *F. verticillioides* and *F. graminearum*. Potassium sorbate was included as control.

	Strains of *F. graminearum*				**Strains of *F. verticillioides***			
	NRRL 28063		**LABI11**		**NRRL 25457**		**LABI7**	
Concentration (µg/ml)	DON (µg/mg)[1]	Biomass (ref. control)	DON (µg/mg)[1]	Biomass (ref control)[2]	Fum (µg/mg)[1]	Biomass (ref. control)[2]	Fum (µg/mg)[1]	Biomass (ref. control)[2]
Control	0.79±0.01a	1.00±0.01	0.85±0.02a	1.00±0.01	3.91±0.01a	1.00±0.02	4.10±0.02a	1.00±0.01
F1								
94	0.15±0.01c	0.98±0.01	0.16±0.02b	0.95±0.01	4.10±0.02a	1.05±0.02	3.90±0.02a	1.00±0.01
188	ND	1.08±0.01	ND	0.50±0.01	2.58±0.02b	1.00±0.01	2.87±0.01b	1.00±0.01
375	ND	0.87±0.03	ND	0.89±0.03	ND	0.80±0.02	ND	0.90±0.03
F2								
94	0.20±0.01c	1.11±0.01	0.11±0.01b	0.98±0.01	3.89±0.04a	1.16±0.01	3.95±0.02a	1.15±0.02
188	ND	1.00±0.01	ND	1.00±0.01	2.89±0.04c	1.00±0.02	ND	1.00±0.03
375	ND	0.95±0.02	ND	0.95±0.02	ND	0.85±0.01	ND	0.80±0.01
F1+PS(2:1)								
47+23	0.45±0.01b	1.00±0.01	0.31±0.01c	1.00±0.01	2.50±0.02d	1.00±0.01	2.70±0.02b	1.00±0.01
94+47	0.41±0.02b	0.68±0.01	0.28±0.02c	0.75±0.01	1.50±0.02d	1.00±0.03	1.89±0.02c	1.00±0.01
188+94	-	NG	-	NG	0.76±0.02e	0.85±0.02	1.00±0.02f	0.79±0.03
F2+PS(1:2)								
12+23	0.32±0.02d	1.00±0.01	0.29±0.01c	1.05±0.01	2.70±0.01c	1.00±0.01	1.90±0.01c	1.08±0.01
23+47	0.21±0.02c	0.40±0.02	0.21±0.01b	0.45±0.02	1.45±0.01d	1.00±0.01	1.11±0.01d	1.00±0.01
47+94	0.16±0.01c	0.30±0.01	0.13±0.01b	0.28±0.01	0.56±0.01e	0.90±0.02	0.85±0.02e	0.95±0.02
PS (µg/ml)								
94	0.94±0.02a	1.10±0.01	0.86±0.01a	1.15±0.01	4.05±0.01a	1.20±0.01	4.21±0.01a	1.10±0.01
188	1.35±0.02c	1.00±0.02	0.99±0.01a	1.00±0.02	4.00±0.01a	1.08±0.02	4.10±0.01a	1.15±0.02
375	1.45±0.02c	1.08±0.01	1.25±0.01c	1.20±0.01	4.40±0.01a	1.10±0.01	5.00±0.01d	1.10±0.01

[1]µg of mycotoxin per mg of dry fungal biomass 60 mg of dry weight/Petri dish. ND: Not detected. NG: No growth
Means in the same column with the same letter are not significantly different (Dunnet T3 test, p = 0.05).
Data are reported as mean values ± standard deviation, based on two experiments where each treatment had three replications.