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*, *Euphorbia schickendantzii* and *Euphorbia collina* were sequentially extracted with hexane, ethyl acetate and methanol. The extracts were tested against strains of *Fusarium verticillioides* and *Fusarium 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 mycotoxigenic risk for control of the *Fusarium* species.

**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.

**INTRODUCTION**

*Fusarium verticillioides* and *Fusarium 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. *Fusarium 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 leukaencephalomalacia 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
Plant antifungals against moulds

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 LAB111) and F. verticillioides (NRRL 25457 and LAB17) were provided by the LABIFITO culture collection (National University of Tucumán, Argentina). The strains were maintained in SNA medium (Spezieller Nahrstoffarmer agar: 0.1% K2HPO4, 0.1% NaNO3, 0.05% MgSO4, 7H2O, 0.05% KCl, 0.02% glucose, 0.2% sucrose and 2% agar) at 20°C. The stored strains were transferred and cultured at 30°C (F. verticillioides) 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 × 100 ml) with hexane, ethyl acetate and methanol. 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 7–15 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 semisolid YES medium (yeast-malt extract-sucrose: 2% yeast extract, 15% sucrose, 0.05% magnesium sulphate, 0.125% agar) to obtain a density of 1 × 10⁴ spores per ml. 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 semisolid 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.

as potassium sorbate during grain storage. Nevertheless, 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. schickendantzii and E. collina against Fusarium strains and identified the bioactive compounds involved.
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 many). The column was eluted with a gradient of hexane: ethyl acetate (90 : 10, v/v) and loaded on an gel 60 (75 g, 230–400 mesh, Merck, Darmstadt, Germany) according to the manufacturer’s instructions and equipped with an IB-SIL 5 C18 (Phenomenex). 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 a diode array detector (model 1200, Agilent) 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⁻¹. 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 checkerboard technique and the inhibitory fractionated concentration (FICI) was calculated as: FICI = (Concentration of A in MICₐ/B/Concentration of A in MICₐ)+/(Concentration of B in MICₐ/B/Concentration of B in MICₐ). 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-Errasquin et al. (2007) containing 0.05% malt extract, 0.1% yeast extract, 0.1% peptone, 0.1% KH₂PO₄, 0.03% MgSO₄·7H₂O, 0.03% KCl, 0.005% ZnSO₄·7H₂O, 0.001% CuSO₄·5H₂O 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 Erlenmeyer flasks. Then, the media were inoculated with 1 ml of medium containing 10⁷ conidia of *F. verticillioides* or *F. graminearum*. Concentrations of F1 and F2 were tested at 94, 188 and 375 µg ml⁻¹ while the mixtures of F1 + potassium sorbate and F2 + potassium sorbate were assayed at 12 + 23, 23 + 47 and 47 + 94 µg ml⁻¹. 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.

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 IC50s 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 IC50s that were similar to the values recorded for potassium sorbate and two to four fold higher than the IC50s 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 *Fusarium verticillioides* were similarly inhibited by pool G10, F1 and F2. The same was observed for strains of *F. graminearum*. GC-MS analysis displayed in Fig. 1 and Table 3 indicated that G10 was a mixture of 51% cycloartenol (CA) and 49% 24-methylenecycloartenol (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⁻¹) and F2 + PS (94 + 188 µg ml⁻¹) on *F. graminearum*, and F1 + PS (188 + 94 µg ml⁻¹) and F2 + PS (94 + 188 µg ml⁻¹) on *F. verticillioides*. The partial concentrations at which PS participates in these blends mean a lowering of 1/8 × MIC to 1/16 × 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⁻¹ and at 188 µg ml⁻¹ the biosynthesis of fumonisins. The mixtures strongly reduced the accumulation of mycotoxins although complete suppression 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⁻¹ and 94 + 47 µg ml⁻¹, 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⁻¹, while the lowest levels of fumonisins were achieved at 47 + 94 µg ml⁻¹.

### Table 1

|                      | NNRRL 25457 | LABI7 | NNRRL 28063 | LABI11 |
|----------------------|-------------|-------|-------------|--------|
| Ethyl acetate extract| 1451 (1380–1520) | 1325 (1270–1495) | 1447 (1413–1495) | 11467 (1407–1501) |
| Hexane extract       | 814 (781–925) | 824 (791–915) | 360 (295–394) | 392 (315–454) |
| Tebuconazole         | 0.06 (0.06) | 0.06 (0.06) | 0.04 (0.04–0.05) | 0.03 (0.03) |
| Potassium sorbate    | 1450 (1350–1513) | 1490 (1310–1550) | 1435 (1330–1450) | 1466 (1390–1500) |

95% confidence intervals are indicated between parentheses.

### Table 2

|                      | NNRRL 25457 | LABI7 | NNRRL 28063 | LABI11 |
|----------------------|-------------|-------|-------------|--------|
| G10                  | 1500 (1325–1675) | 1500 (1425–1575) | 750 (725–775) | 750 (725–775) |
| F1                   | 1500 (1325–1675) | 1500 (1425–1575) | 750 (725–775) | 750 (725–775) |
| F2                   | 1500 (1325–1675) | 1500 (1425–1575) | 750 (725–775) | 750 (725–775) |
| Potassium sorbate    | 3000 (2800–3200) | 3000 (2800–3200) | 3000 (2800–3200) | 3000 (2800–3200) |
| Tebuconazole         | 2 (2) | 2 (2) | 2 (2) | 2 (2) |
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. verticilloides* 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 comprised between 3.9 and

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**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.
12.3 mg ml<sup>−1</sup> in macrodilution assays (Bakhuni et al. 1974; Payne et al. 2015). Methanolic extracts of <i>E. schickendantzii</i> and <i>E. collina</i> were previously tested for antihelmintic activity which was moderate on <i>Ancylostoma caninum</i> at concentrations equal and higher than 7.5 mg ml<sup>−1</sup> (Alvarez et al. 2018). The methanolic extract of <i>E. schickendantzii</i> also moderately inhibit the motility of <i>Haemonchus</i> spp at concentrations of 1–4 mg ml<sup>−1</sup> (Lamberti et al. 2009). As far as we know, the current work reports for the first time antifungal activity associated to <i>E. schickendantzii</i> and <i>E. collina</i>. The search for the antifungal agents responsible of the antifungal activity of the hexane extract of <i>E. collina</i> 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 <i>Euphorbia</i> 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 <i>Fusarium</i> 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<sup>−1</sup>). CA was reported with moderate to weak activity against clinical isolates of <i>Candida albicans</i>, <i>Trichophyton mentagrophytes</i> and <i>Aspergillus niger</i> (Ragasa et al. 2004), and inactive on the bacteria <i>Streptococcus aureus</i> and <i>Bacillus subtilis</i>, while 24MCA showed strong antiplasmodial activity on <i>Plasmodium falciparum</i> (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 suppression of fungal growth with PS concentrations of 188 and 375 µg ml<sup>−1</sup>, respectively. Depending of the food matrix considered, PS can be incorporated to foods in a range of concentrations comprised between 3000 and 300 µg ml<sup>−1</sup> (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 hypothesized that the pentacyclic triterpenes increased sorbic acid uptake which readily enhanced its inhibitory effect on fungal physiology. This point requires further research.
Table 5  Impact of sublethal concentrations of F1 (98% cycloartenol), F2 (97% 24–methylene cycloartenol) and their mixtures with potassium sorbate (PS) on production of deoxynivalenol (DON) and fumonisins (Fum) recorded for strains of Fusarium verticillioides and Fusarium graminearum. Potassium sorbate was included as control.

| Concentration (µg mL⁻¹) | Strains of Fusarium graminearum | Strains of Fusarium verticillioides |
|------------------------|--------------------------------|-----------------------------------|
|                        | NRRL 28063 | LABI11 | NRRL 25457 | LABI7 |
| Control                |           |        |            |       |
| F1                     |           |        |            |       |
| 94                     | 0.79 ± 0.01a | 1.00 ± 0.01 | 0.85 ± 0.02a | 1.00 ± 0.01 |
| 188                    | ND        | 1.08 ± 0.01 | ND        | 0.50 ± 0.01 |
| 375                    | ND        | 0.87 ± 0.03 | ND        | 0.89 ± 0.03 |
| F2                     |           |        |            |       |
| 94                     | 0.20 ± 0.01c | 1.11 ± 0.01 | 0.11 ± 0.01b | 0.98 ± 0.01 |
| 188                    | ND        | 1.00 ± 0.01 | ND        | 1.00 ± 0.01 |
| 375                    | ND        | 0.95 ± 0.02 | ND        | 0.95 ± 0.02 |
| F1 + PS (2:1)          |           |        |            |       |
| 47 + 23                | 0.45 ± 0.01b | 1.00 ± 0.01 | 0.31 ± 0.01c | 1.00 ± 0.01 |
| 94 + 47                | 0.41 ± 0.02b | 0.68 ± 0.01 | 0.28 ± 0.02c | 0.75 ± 0.01 |
| 188 + 94               | ND        | NG      | ND        | NG      |
| PS (µg mL⁻¹)           |           |        |            |       |
| 94                     | 0.94 ± 0.02a | 1.10 ± 0.01 | 0.86 ± 0.01a | 1.15 ± 0.01 |
| 188                    | 1.35 ± 0.02c | 1.00 ± 0.02 | 0.99 ± 0.01a | 1.00 ± 0.02 |
| 375                    | 1.45 ± 0.02c | 1.08 ± 0.01 | 1.28 ± 0.01c | 1.20 ± 0.01 |

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.

ND, not detected; ng, no growth.

* µg of mycotoxin per mg of dry fungal biomass 60 mg of dry weight/Petri dish.

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 antimitoxogenic 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 (Belizán 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 antimitoxogenic effect. Their synergistic interactions with PS raise a promising use of CA and 24MCA in food preservation against F. verticillioides and F. graminearum responsible of cereal ear rots.

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Author contributions

DAS, CANC, RET designed the study. HLA performed and supervised the plant collections, and extracted the plant materials. CMJ, HLA, DAS performed antifungal and antimiycotoxigenic 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.

Conflict of Interest

No conflict of interest declared.

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