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Phytochemical investigation and biological activities of *Fusarium SP.* An entomogenous fungus

Karenina Marcinkevicius[a], Analia Salvatore[b], Gabriela Murua[c], Mario Arena[d,e], Nancy Vera[d,*]

[a] Instituto de Química del Noroeste Argentino (INQUINOA)-(CONICET), Ayacucho 471, 4000, Tucumán, Argentina
[b] Facultad de Agronomía y Zootecnia, Florentino Ameghino s/n. Bº Mercantil, Campo Experimental, 4105, El Manantial, Tucumán, Argentina
[c] Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Instituto de Tecnologia Agroindustrial del Noroeste Argentino (ITA-NOA) – (CONICET), Av. William Cross 3150, T4101XAC, Las Talitas, Tucumán, Argentina
[d] Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, 4000, Tucumán, Argentina
[e] Instituto de Biotecnología Farmaceutica y alimentaria (INBIOFAL-CONICET), Av. Kirchner 1900 (4000). Tucumán, Argentina

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ABSTRACT

Entomopathogenic fungi have an incredible capacity to produce biologically active metabolites. The present study was undertaken to evaluate the insecticidal activity of extracts, fractions and pure compounds from *Fusarium sp*. ARSEF 3300 entomopathogenic fungi against *Spodoptera frugiperda* Smith (Lepidoptera) and *Ceratitis capitata* Wiedemann (Diptera). Additionally, antimicrobial activity was determined against *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 27853. The culture media developed in the absence and presence of remains of the *S. frugiperda* insect were called fungus (H) and fungus-insect (HI), respectively. Volatile compounds in the extracts obtained with ethyl acetate were identified by GC-MS. Seven compounds of known chemical structure were isolated: cholesterol (1), campesterol (2), palmitic acid (3), cis-Oleic acid (4), stearic acid (5), ester propyl myristate (6) and cis-9, cis-12 Linoleic acid (7). The ethyl acetate extract of the HI supernatant of *Fusarium sp*., showed the highest ingestion dissuading activity in *S. frugiperda* (83% at 300 µg/g of diet) and the highest oviposition deterrence in *C. capitata* (50% at 50 µg/cm^2). Extracts of H and HI supernatants from *Fusarium sp*. inhibited the growth of the *P. aeruginosa* (53.64% and 45.39%) and *S. aureus* (76.08% and 79.61%) at 400 µg/mL. Palmitic acid (46.23% and 38.59%), cis-Oleic acid (49.95% and 42.33%) and stearic acid (50.44% and 39.72%) showed the highest inhibition of growth and biofilm production in *S. aureus* at 100 µg/mL. Our results suggest the possible utilization of entomopathogenic fungal metabolites in the control of insect pests and human health.

1. Introduction

Within the practices of modern agriculture, pesticides play an important role in the stabilization and increase of agricultural yield. However, many of these compounds are highly detrimental to human health and ecosystems. Also, the emergence of resistant insects has led to the use of increasing doses or more toxic products. Currently there is a growing trend towards an integrated pest management. Microbial products are now being applied in every sphere of pesticide use. Recently, a large number of insect pathogenic microorganisms referred to as entomopathogens have been identified as possible biological control agents for insects (Bidochka and Khatchatourians, 1991). Among these agents, fungal entomopathogens had been reported to have great potentials for insect's control (Prior and Greathead, 1989; Lacey et al., 2001).

The genus of Fusarium is commonly known as a group of phytopathogenic fungi. However, it is now known that a large number of them act as entomopathogens, covering a wide range of hosts including Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera and Lepidoptera (Majumbar et al., 2008). In the literature there are reports of the action of different strains of Fusarium on lepidoptera, such as *F. chlamydosporum, F. equiseti, F. graminearum, F. moniliforme, F. oxysporum, F. poae, F. semitectum, F. sacchari,* y *F. solani*. Currently, several authors (Vega et al., 2008; Cherry et al., 2004) suggest that the effect of entomopathogenic fungi on insects is due to the presence of fungal metabolites that provoke feeding deterrence or antibiosis. The factors responsible for mycosis in insects are complex and involve the production of volatile and non-volatile metabolites (Crespo et al., 2008). Fusarium includes various species/strains that are able to produce potent secondary metabolites, such as trichothecenes (Desjardins et al.,

* Corresponding author. Tel: +54 381- 424-8169; fax: +54 381- 424-8169.
E-mail addresses: nrvera@fbqf.unt.edu.ar, veranr@gmail.com (N. Vera).

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2003; Foroud and Eudes, 2009; Desjardins and Proctor, 2001), fumonisins (Proctor et al., 2003; Desjardins and Proctor, 2001); Fusaproliferina (Pascale et al., 2002); Fusarocromanona (Bryden et al., 2001) and enniatins (Bottalico and Perrone, 2002; Logrieco et al., 2002; Nicholson et al., 2004). Insects are often used as reliable organisms for screening the potential pathogenicity and toxicity of fungi (Anand et al., 2009). The application of these metabolites in human medicine and as controllers of insect vectors and their bioactivity represents a wide field for scientific research (Vey et al., 2002; Lee et al., 2008).

In this context, the objective of our study was to investigate the antimicrobial and insecticide activities of the extracts of *Fusarium sp*. to access for the potential production of bioactive secondary metabolites that may serve as leads for novel drug discovery and bioinsecticides.

The current article reports the chemical characterization by GC MS of the organic extracts obtained from *Fusarium sp.*, its insecticidal properties against *S. frugiperda* (Lepidoptera) and *C. capitata* Wiedemann) (Diptera Tephritidae), key pests that cause great economic losses in the northwest northeast of Argentina and antipathogenic activity observed against human pathogenic microorganisms (*P. aeruginosa* and *S. aureus*). We also carried out the bioassay-guided isolation and elucidation of the structures of the major secondary metabolites.

2. Experimental section

2.1. General

NMR spectra were recorded on a Bruker AC spectrometer operating at 300 MHz for 1H and 125 MHz for 13 C with TMS as internal standard in $CDCl_3$. The mass spectra were recorded on a THERMO POLARIS Q (EIMS). For HPLC separation of mixtures, Waters equipment was used. Detection was accomplished by the use of refractive index detector. Column Phenomenex Luna C8 (5 µm, 10 mm i.d. x 250 mm), was used. Retention time was measured from the solvent peak.

2.2. Culture of entomopathogenic fungi

Fusarium sp. strain ARSEF 3300 [NRRL 25102] were isolated from *S. frugiperda* [Lepidoptera: Noctuidae], in Colima (Mexico) in 1988. The strains were assigned to Dr. Mario Arena, by Professor Richard A. Humber, ARSEF Director (ARS Collection of Entomopathogenic Fungal Cultures), New York (USA). *Fusarium sp.* was maintained to Potato-dextrose agar 1% (PDA) and was incubated in an oven at 25 ± 2 °C for 14–15 days until they developed dense sporulation. The spores were resuspended with sterile water containing 0.05% Tween 80. The desired spore concentration was determined using an improved Neubauer chamber and adjusted to 8.5×10^8 spores/mL.

2.3. Preparation of extracts from different culture media

Three experimental culture media were developed, Medium A (Potato dextrose broth medium 1% + 3% [v/v] of the suspension spores of *Fusarium sp*. ARSEF 3300), Medium B (medium A + 1% [w/v] of *S. frugiperda* cuticles) and Medium C (Potato dextrose broth medium 1% + 1% [w/v] of *S. frugiperda* cuticles) was used as a control medium. All culture media were cultivated for 15 days at 25 °C at 180 rpm on a rotating shaker. After the incubation period, the biomass and insoluble residues were separated to the supernatants by filtration. The filtrate media obtained from the different culture media was carried out three liquid-liquid extractions with ethyl acetate (AcOEt) in equal parts. To the biomass and insoluble residues were made solid-liquid extractions with AcOEt and then with methanol (MeOH). The organic phases obtained were then evaporated on a rotary evaporator under reduced pressure. The weight and yield of the dry extracts were determined for each case (Fig. 1).

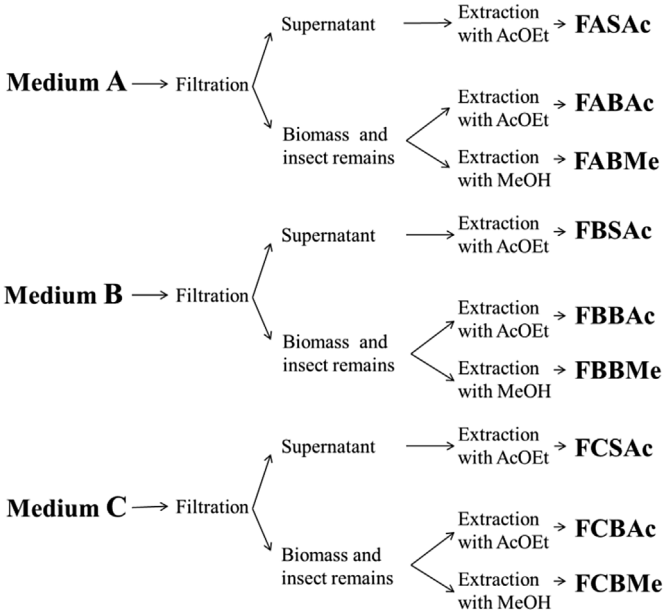

Fig. 1. Ethyl acetate extract from the medium supernatant without insect remains **(FASAc)**; Ethyl acetate extract from the medium biomass without insect remains **(FABAc)**; Methanolic extract from the medium biomass without insect remains **(FABMe)**; Ethyl acetate extract from the medium supernatant supplemented with insect remains **(FBSAc)**; Ethyl acetate extract from the medium biomass supplemented with insect remains **(FBBAc)**; Methanolic extract from the medium biomass supplemented with insect remains **(FBBMe)**. Ethyl acetate extract from the medium supernatant of insect remains **(FCSAc)**; Ethyl acetate extract from the medium biomass of insect remains **(FCBAc)**; Methanolic extract from the medium biomass of insect remains **(FCBMe)**.

2.4. Gas chromatography-mass spectrometry analysis of extracts

The extracts obtained were analyzed by gas chromatography techniques. GC and GC/MS (EI) analysis were carried out using a Thermo electron Trace^{TM}Ultra couple with split–split-less injector and Polaris Q ion trap mass spectrometer equipped with a DB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 µm). The initial temperature of the column was 40 °C during 4 min. A temperature programing was applied from 40 to 280 °C at a rate flow of 10 °C/min, and finally 300 °C for 5 min. Carrier gas was helium (flow 1 mL/min). The injector was heated to 280 °C and was on split mode with a split ratio of 1:10, and the injection volume was 1 µL. The interface temperature should be 300 °C. Mass spectra were taken over the m/z 50–500 range with an ionizing voltage of 70 eV. Kovat's retention index was calculated using chromatographed standard hydrocarbons. The individual compounds were identified by MS and their identity was confirmed by comparison of their RIs, relative to C_9-C_{30} n-alkanes, and mass spectra with authentic samples or with data already available in the NIST 2014 Mass Spectral Library and in the literature (Adams, 2007).

2.5. Isolation, purification and structural elucidation of fungal metabolites

The ethyl acetate extract from the supernatant of the medium (FBSAc) (650 mg) was subjected to silica gel CC (70–230 Mesh) with CH_2Cl_2 and increasing amounts of EtOAc (0–100%) and finally MeOH, as eluents, to give XVI fractions of 10 mL each. The fractions FVI, FVII, FXI and FXII were selected for their antimicrobial activity to continue the isolation (Fig. 2). The fractions FIV, FV and FVIII were also active in bacteria but were not selected to continue their chemical study due to their low weight.

Fr. VI (42.3 mg), which eluted with a mixture of CH_2Cl_2-AcOEt (95:5), were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH 100%, 1.5 mL min^{-1}) to give compound **1** (5.6 mg,

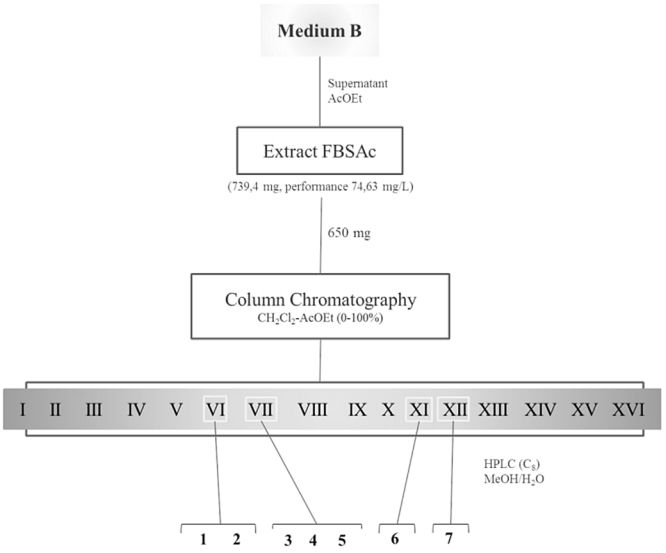

Fig. 2. Processing scheme of the medium supernatant supplemented with insect remains (**FBSAc**).

Rt 12.88 min) and compound **2** (1.0 mg, Rt 15.34 min).

Fr. VII (52.4 mg), which eluted with a mixture of CH_2Cl_2-AcOEt (95:5), were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH-H_2O 92,5:7,5, 1.5 mL.min^{-1}) to give compounds **3** (5.7 mg, Rt 7.54 min) compounds **4** (6.5 mg, Rt 9,20 min) and **5** (2.0 mg, Rt 5.64 min).

Fr. XI (13.3 mg), which eluted with a mixture of CH_2Cl_2-AcOEt 70:30, were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH-H_2O 80:20, 1.5 mL. min^{-1}) to give compound **6** (0.5 mg, Rt 10.67 min).

Fr. XII (17.2 mg), which eluted with a mixture of CH2Cl2-AcOEt 50:50, were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH-H_2O 80:20, 1.5 mL.min^{-1}) to give compound **7** (1.0 mg, Rt 12.45 min).

The structures these compounds were completely elucidated by using extensive spectroscopic methods and by comparison with data previously reported in the literature (Wilson et al., 1996; Gangwal et al., 2010; Cole and Schweikert, 2003; Suttiarporn et al., 2015).

2.6. Bioassays

2.6.1. Insect rearing

S. frugiperda larvae were obtained from our laboratory population. The larval diet consisted of a mixture of yeast (3 g), bean boiled and milled (250 g), wheat germ (12.5 g), agar agar (12.5 g), ascorbic acid (1.5 g), methyl p-hydroxybenzoate (1.5 g), formaldehyde (4 mL of a 38% water solution), and water (500 mL).

The colony of *C. capitata* used in the bioassays derived from the laboratory of the Experimental Agroindustrial "Obispo Colombres" station. It was initiated with pupae of oranges infested obtained in northwestern Argentina. Adults were fed with a solution prepared with water and a mixture of sugar and hydrolyzed protein ratio (3: 1) diet.

The brood chamber is maintained at 24 ± 2 °C, 60 ± 10% relative humidity and a photoperiod of 12 L: 2D.

2.6.2. Insecticidal bioassay against S. frugiperda

Third instars larvae of homogeneous size were weighed and individually placed in glass tubes. Treated and control diets (prepared as described for choice conditions) were also weighted and offered to larvae in each tube (20 replicates for control and 20 for each treatment). Tubes were kept at 27 °C ± 1 °C in a chamber (70–75% relative humidity, with a photoperiod of 16/8 h light-dark cycle). Every two days faecal matter was eliminated and every addition of diet with the corresponding weight was recorded. At the end of experiment (ten days period), larvae were weighted and food consumption was determined. Nutritional indices, namely relative consumption rate (RCR), relative growth rate (RGR) and efficiency of conversion of ingested food index (ECI) were calculated according to Nathan and Sehoon (2006), as follows:

[RGR = (A − B)/t], which gives the average of larval weight increment per hours [A = final larval weight, B = initial larval weight and t = experimental period in hours].
[RCR = D/t], is the average of the larval diet consumed per day where D is the total weight of food consumed during the experiment and t = experimental period in hours.
[ECI = (ΔB/D) * 100], where ΔB change in larval weight (mg) and D is the total weight of food consumed during the experiment. Additional observations were recorded on sublethal effects as larval, pupal and adult deformities. Lifecycle measurements, such as time to larval duration, time to pupal duration and adult emergence were measure. Finally, larval and pupal mortality were also recorded (Marcinkevicius et al., 2017).

2.6.3. Antifeedant test against S. frugiperda (choice test and No choice test)

The antifeedant activity was tested based on the methods described by Marcinkevicius et al. (2017). Different concentrations of extract (300 μg/g of diet) and pure compounds (100 μg/g of diet) were prepared by dissolving in acetone and tested against third instars larvae of *S. frugiperda*. In the no choice test, the same amount of control and treated diets were placed in a different glass tube with a larva inside. The experiment was carried out in 20 replicates. Feeding election index was calculated as FEI = (1 – T/C) 100, where C and T represent the amounts eaten of control and treated diets, respectively.

2.6.4. Oviposition-deterrent activity against C. capitata

Oviposition deterrent activity against *C. capitata* was investigated based on the method described by Socolsky et al. (2008). Artificial fruits (oviposition substrates) were prepared. The surface of the wrapped cylinder was pricked with a needle and treated with an acetone or methanol solution of the sample to be tested. An amount of 50 μg of extracts and 25 μg of pure compounds/cm^2 were deposited. The index of oviposition inhibition (OI) was calculated: OI = [(1 – T/C) × 100].

2.7. Antimicrobial activity

2.7.1. Bacterial growth

Overnight cultures of *P. aeruginosa* ATCC 27853 (Gram negative bacilli) and *S. aureus* ATTC 6538P (Gram positive cocci) were diluted to reach 2.5 × 10^6 CFU/mL in Luria–Bertani (LB) and Mueller-Hinton (MH) media, respectively. The diluted culture (190 μL) was placed in each of the 96 wells of a micro titer polystyrene plate. Solutions of extracts in DMSO–distilled water (1:1) were prepared separately and 10 μL of each was pipetted to the plastic micro titer plate wells individually (eight replicates) in order to reach final concentrations of 400, 200, 100, and 50 μg/mL. Control wells (eight replicates) contained the diluted culture (190 μL) and 10 μL of a solution of DMSO–water (1:1) in which the final concentration of DMSO is 2.5%. Medium control was prepared using sterile LB and MH for to each microorganisms. After 24 h incubation at 37 °C, bacterial growth was detected as turbidity (600 nm) using a micro titer plate reader (Power Wave XS2, Biotek, VT, USA).

2.7.2. Biofilm formation assay

For biofilm quantification, a micro method based on a protocol previously reported was employed (O'Toole and Kolter, 1998). Biofilms formed after 24 h incubation of bacterial cultures prepared as described

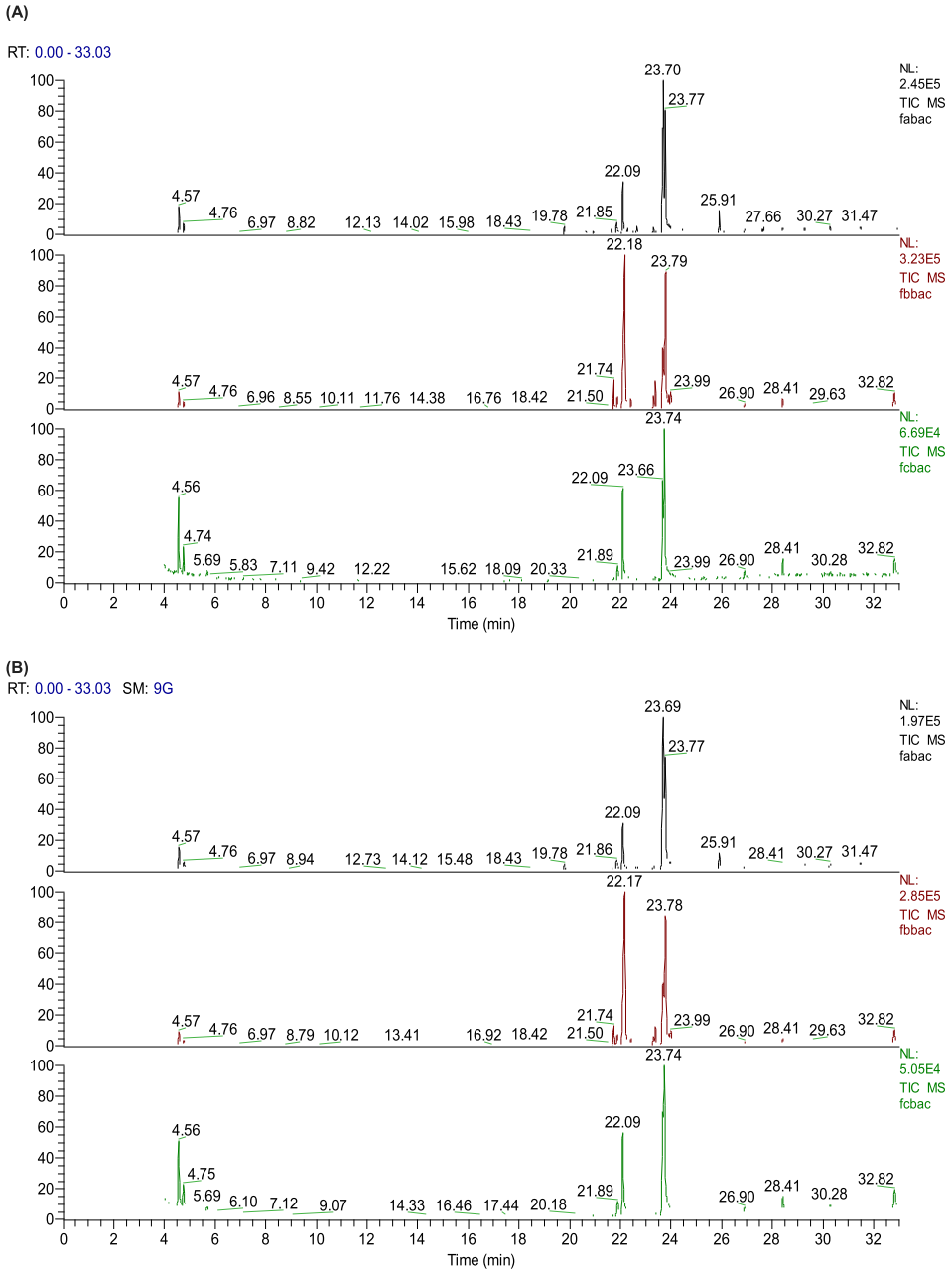

Fig. 3. (A) Volatile components profile of extracts of AcOEt supernatants per GC/MS; **(B)** Volatile components profile of extracts of AcOEt biomasses per GC/MS.

in the previous paragraph, were stained with 20 µL of an aqueous solution of crystal violet, (0.1% w/v) for 20 min. After washing with water, the liquid was discarded from the wells and the material that remained fixed to the polystyrene (containing biofilm) was washed with PBS (thrice). Crystal violet bound to biofilm was removed from each well employing 200 µL absolute ethanol during 30 min at 37 °C with shaking. Absorbance (540 nm) of ethanol solutions of crystal violet was determined using a microtiter plate reader (Power Wave XS2. Biotek, Vermont, USA). Ciprofloxacin, a known biofilm inhibitor, was incorporated in the same bioassay as a positive control at 5 µg/mL. At this concentration, ciprofloxacin inhibited the biofilm formation but did not significantly modify the bacterial growth (Sandasi et al., 2011).

2.8. Statistical analysis

Results are reported as Mean ± SEM. The differences in the mean values were evaluated by analysis of variance (ANOVA). Tukey test was used for all pair wise multiple comparisons of groups. In all statistical analysis P values > 0.05 were considered not significant.

3. Results and discussion

3.1. Chemical studies

The Fig. 1 shows the experimental design and yields of the extracts obtained. A total of 9 extracts were obtained, 3 for each culture medium. The chemical composition of ethyl acetate extracts was determined by GC/MS analysis. Supernatant extracts were richer in volatile components than biomass extracts with similarities in their chemical constituents (Fig. 3A and B). From mass spectral analysis thirty compounds were identified, mainly straight chain hydrocarbons and some fatty acids and their esters such as linoleic, oleic and eicosanoic acids (Table 1). These results are consistent with those obtained by Tayung et al. (2011), for *F. solani*. After the bio directed chemical

Table 1
List of compounds identified from the ethyl acetate extracts using GC-MS analysis.

RI	Compound	Peak area (%) Extracts					
		FASAc	FBSAc	FCSAc	FABAc	FBBAc	FCBAc
763	Toluene	7.31	3.15	4.91	4.19	1.85	10.73
771	Isobutyl acetate	1.75	-	1.68	1.39	-	3.59
1481	8.8-Dimethyl-9-methylene-1.5-cycloundecadiene	-	-	7.31	-	-	-
1496	n-pentadecane	-	1.10	1.47	-	-	-
1719	1.5.5.8-Tetramethyl-3.7-cycloundecadien-1-ol	54.06	24.68	-	1.18	-	-
1796	2.6-Dimethylheptadecane	1.88	1.58	1.00	-	-	-
1806	Propyl myristate	1.26	-	12.18	-	-	-
1917	Palmitic acid. methyl ester	-	-	-	-	2.38	-
1959	Palmitic acid	4.83	12.64	22.43	10.44	-	17.91
1968	Methyl isoheptadecanoate	-	-	-	-	37.58	-
2095	Linoleic acid. methyl ester	-	-	-	-	1.07	-
2106	Methyl cis-6-octadecenoate	-	-	-	-	2.37	-
2149	Linoleic acid	5.15	9.68	9.43	37.73	11.67	14.67
2160	Oleic Acid	7.04	11.49	23.76	23.78	25.39	35.3
2198	Stearic acid	2.83	3.85	2.58	1.95	3.63	3.63
2201	Arachidonic acid methyl ester	-	-	-	3.41	-	-
2392	2-hydroxy-3-tert-butyl-5-methylphenyl	-	-	-	2.45	-	-
2594	7-n-Hexyleicosane	-	8.48	-	2.20	-	-
3089	Cholesterol	-	4.81	1.31	-	3.19	5.29
Total		**86.11**	**81.46**	**88.06**	**88.72**	**89.13**	**91.12**

RI. retention index relative to C 9 - C 30n-alkane on DB-5 column.

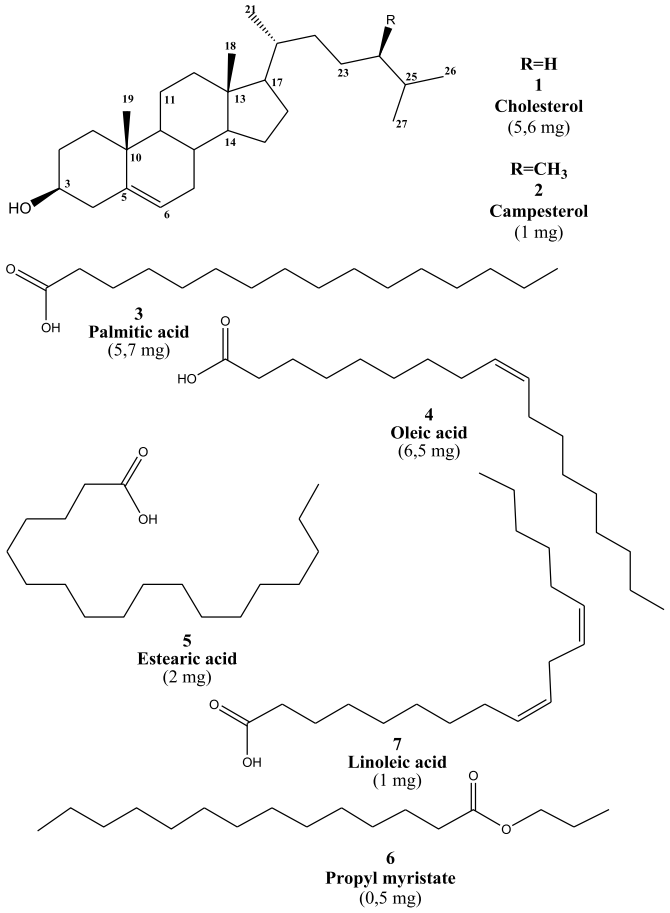

Fig. 4. Compounds isolated from ethyl acetate extract from the medium supernatant supplemented with insect remains (FBSAc).

processing Fig. 2, seven major compounds of the most active fractions were isolated: two sterols, cholesterol (**1**) and campesterol (**2**), four fatty acids, palmitic acid (**3**), cis-Oleic acid (**4**), stearic acid (**5**) and cis-9, cis-12 Linoleic acid (**7**), and a propyl myristate ester (**6**). NMR spectroscopic data and the rotational power of these compounds coincide with data previously described in the literature (Fig. 4). Although none of the compounds are new, this would be the first report of a chemical analysis for *Fusarium sp*. ARSEF 3300, isolated entomopathogenic strain of *S. frugiperda*.

Numerous species of the genus of Fusarium produce a wide chemical range of phytotoxic compounds, such as fusaric acid, fumonisins, beauvericin, eniantines and trichothecenes. They are mainly described in phytopathogenic strains most of the isolated mycotoxins were reported from species that colonize stored grains (Neuhold et al., 1997; Desjardins and Hohn, 1997). Other authors also reported the isolation of cholesterol and campesterol in other fungi (Nes and Parish, 1989; Cole and Schweikert, 2003).

3.2. Insecticidal bioassay against S. frugiperda

In the literature there are reports of the action of different strains of Fusarium on lepidoptera. Ameen (2012), tested isolates of nine strains of Fusarium, reporting pathogenicity of all isolates against larvae *Galleria mellonella*. Rajesh and Bhupendra (2009) published 100% mortality in eggs of *Spodoptera litura* by *F. lateritium*, *M. anisopliae*, and *C. cardinalis* using a conidia suspension of 106 conidia/mL. However, in most of the works carried out for the biological control of pests, concentrations of conidia of the entomopathogenic fungus are used.

As shown in Table 2, the extracts tested at 300 µg/g showed no significant differences in the physiological indices (RCR, RGR, ECI) with respect to the control. The test was continued by analyzing the lethal and sublethal effects produced by the substances until the insects reached their last stage. The larval period was not altered by the extracts tested at tested doses. Larval mortality was low, but high mortality rates, between 35 and 50%, were observed in the pupal state. There was adult insect malformation and the FBSAc extract showed the highest percentage at 25% which would result in a reduction of the viable and fertile population of the insect. Other authors also conducted tests with extracts from different strains of *Aspegillus, Penicillum, Fusarium, Alternaria* and *Trichoderma harzianum*, against *Oncopeltus fasciatus* with positive results (Santamarina et al., 2002; Gimenez et al., 2000).

3.3. Antifeedant test against S. frugiperda (Choice test and No choice test)

The effect of the extracts and pure compounds obtained from

Table 2
Effect of *Fusarium sp.* ARSEF 3300 extracts on the development of *S. frugiperda*.

Extracts (300 µg/g)	Physiological index			Lethal and sub-lethal effects				
	RCR (mg/mg/days)*	RGR (mg/mg/days)*	ECI (%)*	Larvae period (days)*	Pupae period (days)*	Larvae Mortality(%)	Pupae Mortality(%)	Malformation Adults (%)
Control	0.67 ± 0.08a	0.15 ± 0.02a	24.01 ± 2.50a	18.40 ± 1.21a	15.80 ± 2.20a	5	-	-
FASAc	0.63 ± 0.05a	0.15 ± 0.02a	24.02 ± 3.18a	16.66 ± 0.94a	15.41 ± 1.32a	10	50	5
FABAc	0.65 ± 0.07a	0.15 ± 0.01a	21.57 ± 4.26a	18.06 ± 0.96a	16.30 ± 1.05a	5	35	5
FABMe	0.62 ± 0.06a	0.15 ± 0.01a	25.40 ± 3.42a	17.16 ± 1.06a	16.12 ± 1.04a	5	45	10
FBSAc	0.71 ± 0.05a	0.15 ± 0.02a	37.40 ± 4.51b	17.05 ± 1.00a	15.33 ± 1.03a	10	50	25
FBBAc	0.68 ± 0.05a	0.15 ± 0.01a	32.21 ± 6.43a	19.42 ± 1.29a	15.82 ± 1.19a	5	35	10
FBBMe	0.68 ± 0.08a	0.15 ± 0.01a	37.11 ± 3.15b	17.77 ± 1.31a	16.01 ± 1.90a	5	50	5

The values in the columns represent the mean ± SEM (n = 20). *Values in a column with the same letter do not show a significant difference (P > 0.05. according to Tukey's multiple range test).

Table 3
Antifeedant activity of extracts of culture from *Fusarium sp.* ARSEF 3300 on *S. frugiperda*.

Feeding deterrence index		
Extracts (300 µg/g)	FEI_{CH} Choice test(%)	FEI_{NCH} No choice test(%)
FASAc	33.50 ± 7.13a	53.10 ± 7.23a
FABAc	81.60 ± 11.17b	49.60 ± 4.96a
FABMe	44.30 ± 7.17a	52.03 ± 2.71a
FBSAc	83.13 ± 9.19b	53.53 ± 5.19a
FBBAc	71.56 ± 12.36b	52.55 ± 7.18a
FBBMe	53.35 ± 8.31c	47.65 ± 6.75a

Feeding election index ± SEM (n = 20). The values in a column with the same letter do not present a significant difference (P > 0.05. Tukey's multiple range test).

Table 4
Effect of culture extracts from *Fusarium sp.* ARSEF 3300 on the oviposition behavior of *C. capitata*.

	Oviposition inhibition		
Extracts (50 µg/cm^2)	N° of eggs laid on the treated fruit*	N° of eggs laid on the control fruit*	OI (%)*
FASAc	134.67 ± 12.06a	341.33 ± 12.20a	58.61 ± 5.88a
FABAc	152.67 ± 3.06b	246.67 ± 18.90b	37.92 ± 3.67b
FABMe	122.67 ± 8.33a	242.67 ± 12.22b	49.46 ± 3.87a
FBSAc	110.67 ± 8.33c	282.00 ± 19.08b	60.76 ± 0.91a
FBBAc	122.00 ± 2.83a	268.00 ± 1.00b	54.48 ± 1.06a
FBBMe	122.67 ± 8.33a	260.33 ± 14.41b	54.71 ± 6.18a

The values in the columns represent the mean ± SEM (n = 3).*Values in a column with the same letter do not show a significant difference (P > 0.05. according to Tukey's multiple range test).

Fusarium sp. on the dietary behavior of the insect were evaluated under the Choice and No Choice Test of the diet at a concentration of 300 µg extract/g of diet. In the Choice Test all the extracts tested presented an anti-alimentary effect with FEI values greater than 33%. The inhibition percentage of the highest intake was produced by the FBSAc extract with an FEI of 83%. In the non-choice condition, all the extracts tested showed moderate anti-food activity without significant differences between them with FEI values around 52% (Table 3). The ecological effect of natural products on the quantification of antifeedants is of enormous importance in the field of insect pest management (IPM programme because they never kill the target insects pests directly, but allow them to be available to their natural enemies (predator and parasites) and thus help in the safeguarding of natural balance. Furthermore, monophagous, oligophagous and polyphagous insects die of starvation due to the application of antifeedants on their food plants.

3.4. Oviposition-deterrent activity against C. capitata

Table 4 shows the results of the deterrent activity of *C. capitata* oviposition. They are described using the index of the oviposition inhibition (OI) calculated as the ratio between the number of eggs on the treated substrate and the number of eggs on the control substrate and expressed as a percentage. This index takes positive values for oviposition dissuasive substances and negative ones for attractants.

All the extracts tested were moderate to strong oviposition inhibitors of *C. capitata* at a 50 µg/cm^2 concentration with percentages between 37 and 60%. The extracts of insect supplemented media were active, but FBSAc showed the highest oviposition inhibition at 60.76%.

There are reports of *F. solani* strain action on *Teanops myopaeformis* (Diptera), but no works specifically on *C. capitata* (Majumdar et al., 2008). Other authors reported a reduction in the oviposition rate of *C. capitata* Wiedemann in fruits treated with commercial formulations

Table 5
Effect of extracts from *Fusarium sp.* ARSEF 3300 on bacterial growth.

Extracts	*Pseudomonas aeruginosa*				*Staphylococcus aureus*			
	400 µg/mL	200 µg/mL	100 µg/mL	50 µg/mL	400 µg/mL	200 µg/mL	100 µg/mL	50 µg/mL
FASAc	0.478 ± 0.045	0.620 ± 0.045	0.743 ± 0.046	0.850 ± 0.050	0.244 ± 0.015	0.615 ± 0.036	0.819 ± 0.049	0.845 ± 0.074
FABAc	1.002 ± 0.074a	0.998 ± 0.002a	0.994 ± 0.054a	0.977 ± 0.050a	0.309 ± 0.024	0.533 ± 0.000	0.770 ± 0.021	0.868 ± 0.001
FABMe	0.834 ± 0.038	0.931 ± 0.016a	0.976 ± 0.035a	0.986 ± 0.052a	0.912 ± 0.033	0.963 ± 0.060b	1.014 ± 0.036b	1.017 ± 0.036b
FBSAc	0.563 ± 0.018	0.597 ± 0.041	0.744 ± 0.063	0.854 ± 0.021	0.208 ± 0.030	0.283 ± 0.083	0.508 ± 0.051	0.533 ± 0.028
FBBAc	0.890 ± 0.061a	0.911 ± 0.095a	0.956 ± 0.042a	0.962 ± 0.050a	0.243 ± 0.031	0.607 ± 0.045	0.765 ± 0.052	0.817 ± 0.046
FBBMe	0.860 ± 0.033	0.877 ± 0.005	0.933 ± 0.083a	0.962 ± 0.018a	0.510 ± 0.044	0.846 ± 0.008	0.935 ± 0.069b	0.958 ± 0.051b
FCSAc	0.810 ± 0.061	0.862 ± 0.030	0.864 ± 0.059	0.868 ± 0.073	0.842 ± 0.016	0.850 ± 0.037	0.858 ± 0.027	0.889 ± 0.020
FCBAc	0.955 ± 0.026a	0.955 ± 0.062a	0.955 ± 0.085a	0.958 ± 0.046a	0.842 ± 0.042	0.848 ± 0.056	0.848 ± 0.009	0.869 ± 0.069
FCBMe	1.058 ± 0.010a	1.072 ± 0.034a	1.074 ± 0.028a	1.081 ± 0.043a	1.227 ± 0.040	1.227 ± 0.040	1.227 ± 0.040	1.221 ± 0.042
Control	1.031 ± 0.074a				1.020 ± 0.050b			
Ciprofloxacin	0.062 ± 0.014				0.093 ± 0.009			

Effects of extracts (after 24 h incubation) on *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 6538P (Absorbance at 600 nm) were determined. Ciprofloxacin concentration: 5 mg/ml. Data are expressed as means ± standard deviation (n = 8). Values with the same letter do not show a significant difference compared to each control (P > 0.05. Tukey's multiple range test).

Table 6
Effect of extracts from *Fusarium sp.* ARSEF 3300 on biofilm formation.

Extracts	*Pseudomonas aeruginosa*				*Staphylococcus aureus*			
	400 µg/mL	200 µg/mL	100 µg/mL	50 µg/mL	400 µg/mL	200 µg/mL	100 µg/mL	50 µg/mL
FASAc	0.959 ± 0.049	1.130 ± 0.049	1.370 ± 0.050	1.374 ± 0.055	0.567 ± 0.023	0.626 ± 0.022	0.755 ± 0.028	0.765 ± 0.008
FABAc	1.976 ± 0.080	1.829 ± 0.002	1.372 ± 0.059*	1.217 ± 0.054	0.426 ± 0.032	0.484 ± 0.052	0.501 ± 0.037	0.540 ± 0.058
FABMe	1.860 ± 0.060	1.767 ± 0.025	1.757 ± 0.055	1.644 ± 0.082	0.501 ± 0.015	0.527 ± 0.051	0.547 ± 0.031	0.757 ± 0.028
FBSAc	1.001 ± 0.019	1.078 ± 0.062	1.355 ± 0.069*	1.373 ± 0.022a	0.385 ± 0.059	0.384 ± 0.060	0.391 ± 0.079	0.386 ± 0.081
FBBAc	1.589 ± 0.094	1.765 ± 0.148	1.898 ± 0.066	1.892 ± 0.079	0.405 ± 0.059	0.528 ± 0.055	0.551 ± 0.001	0.572 ± 0.034
FBBMe	1.612 ± 0.052	1.927 ± 0.008	2.144 ± 0.130	2.201 ± 0.028	0.484 ± 0.053	0.502 ± 0.059	0.502 ± 0.059	0.502 ± 0.051
FCSAc	1.368 ± 0.066a	1.378 ± 0.033a	1.430 ± 0.064a	1.575 ± 0.079	0.567 ± 0.042	0.572 ± 0.066	0.587 ± 0.028	0.604 ± 0.042
FCBAc	1.567 ± 0.028	1.684 ± 0.067	1.779 ± 0.093	1.851 ± 0.050	0.574 ± 0.079	0.576 ± 0.065	0.581 ± 0.040	0.587 ± 0.062
FCBMe	2.309 ± 0.016	2.280 ± 0.053	1.934 ± 0.044	1.623 ± 0.067	0.538 ± 0.079	0.551 ± 0.002	0.579 ± 0.060	0.694 ± 0.078
Control	1.375 ± 0.083a				1.235 ± 0.043			
Ciprofloxacin	0.073 ± 0.014				0.083 ± 0.016			

Effects of extracts (after 24 h incubation) on *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 6538P biofilm (determined with crystal violet at 0.1%) were determined. Ciprofloxacin concentration: 5 mg/mL. Data are expressed as means ± standard deviation (n = 8). Values with the same letter do not show a significant difference compared to each control (P > 0.05. Tukey's multiple range test).

Table 7
Effect of pure compounds on bacterial growth.

Compounds	*Pseudomonas aeruginosa*				*Staphylococcus aureus*			
	100 µg/mL	50 µg/mL	10 µg/mL	5 µg/mL	100 µg/mL	50 µg/mL	10 µg/mL	5 µg/mL
1	0.868 ± 0.019	0.884 ± 0.025	0.920 ± 0.012	0.938 ± 0.049	0.706 ± 0.034	0.746 ± 0.032	0.943 ± 0.024b	0.965 ± 0.036b
2	0.868 ± 0.027	0.895 ± 0.006	0.963 ± 0.003	0.992 ± 0.009a	0.714 ± 0.034	0.812 ± 0.026	0.891 ± 0.052	0.976 ± 0.060b
3	0.876 ± 0.006	0.927 ± 0.013	0.983 ± 0.006	1.000 ± 0.015a	0.548 ± 0.029	0.577 ± 0.021	0.916 ± 0.008	0.940 ± 0.030b
4	0.918 ± 0.021	0.984 ± 0.004	0.999 ± 0.008a	1.013 ± 0.008a	0.510 ± 0.027b	0.585 ± 0.013b	0.799 ± 0.033	0.976 ± 0.060b
5	0.835 ± 0.009	0.886 ± 0.008	1.002 ± 0.009a	1.013 ± 0.013a	0.505 ± 0.031	0.612 ± 0.041	0.720 ± 0.030	0.951 ± 0.060b
7	1.114 ± 0.013	1.079 ± 0.003a	1.057 ± 0.016a	1.060 ± 0.017a	0.788 ± 0.028	0.888 ± 0.035	1.011 ± 0.074b	1.016 ± 0.041b
Control	1.036 ± 0.083a				1.019 ± 0.012b			
Ciprofloxacin	0.078 ± 0.003				0.090 ± 0.005			

Effects of pure compounds (after 24 h incubation) on *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 6538P (Absorbance at 600 nm) were determined. Ciprofloxacin concentration: 5 mg/mL. Data are expressed as means ± standard deviation (n = 8). Values with the same letter do not show a significant difference compared to each control (P > 0.05. Tukey's multiple range test).

Table 8
Effect of pure compounds on biofilm formation.

Compounds	*Pseudomonas aeruginosa*				*Staphylococcus aureus*			
	100 µg/mL	50 µg/mL	10 µg/mL	5 µg/mL	100 µg/mL	50 µg/mL	10 µg/mL	5 µg/mL
1	0.950 ± 0.044	1.043 ± 0.026	1.100 ± 0.044	1.239 ± 0.038	0.911 ± 0.024	1.054 ± 0.041	1.064 ± 0.005	1.074 ± 0.027
2	1.529 ± 0.020	1.426 ± 0.050a	1.351 ± 0.049a	1.258 ± 0.034	1.225 ± 0.005b	1.199 ± 0.036b	1.220 ± 0.028b	1.204 ± 0.034b
3	0.935 ± 0.035	1.130 ± 0.023	1.227 ± 0.044	1.409 ± 0.006a	0.753 ± 0.015	0.762 ± 0.033	0.795 ± 0.012	0.938 ± 0.017
4	0.814 ± 0.039	0.930 ± 0.023	1.064 ± 0.025	1.098 ± 0.057	0.707 ± 0.009	0.793 ± 0.010	0.896 ± 0.030	1.039 ± 0.045
5	1.530 ± 0.030	1.439 ± 0.040a	1.424 ± 0.044a	1.394 ± 0.010*	0.739 ± 0.019	0.822 ± 0.016	0.841 ± 0.022	1.127 ± 0.034
7	0.826 ± 0.059	0.915 ± 0.014	1.021 ± 0.051	1.085 ± 0.043	0.903 ± 0.020	0.952 ± 0.022	1.020 ± 0.022	0.090 ± 0.017
Control	1.375 ± 0.023a				1.226 ± 0.009b			
Ciprofloxacin	0.093 ± 0.003				0.090 ± 0.009			

Effects of pure compounds (after 24 h incubation) on *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 6538P biofilm (determined with crystal violet at 0.1%) were determined. Ciprofloxacin concentration: 5 mg/mL. Data are expressed as means ± standard deviation (n = 8). Values with the same letter do not show a significant difference compared to each control (P > 0.05. Tukey's multiple range test).

containing conidia of *Beauveria bassiana* (Falchi et al., 2015). Isolates from *Metarhizium anisopliae* (Metschnikoff) Sorokin and *B. bassiana* (Balsamo) Vuillemin were active against adults and pupae of *C. capitata* (Almeida et al., 2007).

The isolated pure compounds showed no strong insecticidal activity in any of the experimental models of insects used in this work (data not shown)

3.5. Antimicrobial activity

When evaluating extract activity against *S. aureus* and *P. aeruginosa*, that of the supernatants was the most active. The greatest growth inhibition was observed in *S. aureus* where FASAc, FABAc, FBSAc, and FBBAc extracts were the most active with inhibitions higher than 70%. Additionally, the FBSAc extract was active in the 4 concentrations tested with inhibitions between 80 and 48% at 400 µg/mL (Table 5).

Most of the extracts stimulated *P. aeruginosa* biofilm production, with only MASAc and MBSAc being active at 400 and 200 µg/mL and inhibiting its formation. In the case of *S. aureus* all extracts inhibited the formation of biofilm up to about 40%. FBSAc was the most active with an inhibition of up to 70% at the four concentrations tested (Table 6).

Merlin et al. (2013) reported that dichloromethane extracts of *F. solani* were active against *Enterococcus fecalis*. Ratnaweera et al. (2015) reported growth inhibition of three Gram-positive bacteria: *Bacillus subtilis* (UBC 344), *S. aureus* (ATCC 43300) and *S. aureus* MRSA (ATCC 33591) by the ethyl acetate extract of *Fusarium sp*. although it was inactive against Gram-negative bacteria *E. coli* (UBC 8161) and *P. aeruginosa* (ATCC 27853). Other authors like Hateet et al., 2014 reported activity of the ethyl acetate extract of *F. solani* isolated from tomato flats, against *E. coli* (ATCC 25922) and *S. aureus* (NCTC 6571), while Musavi and Balakrishnan (2014) observed antimicrobial activity of an extract of *F. oxysporum* NFX06 against *E. coli* (ATCC 25922), *S. aureus*

(ATCC 25923) and *P. aeruginosa* (ATCC 27853).

The pure compounds isolated from Fusarium (1–5), inhibited the growth of *P. aeruginosa* at 100, 50 and 10 µg/mL, while biofilm production was inhibited by compounds 1, 3, 4, 7 at the four concentrations tested, the most active being compound 5 (stearic acid) in growth and compounds 4 (cis-oleic acid) and 7 (cis-9, cis-12 linoleic) in biofilm. All compounds, except number 2 in biofilm, inhibited *S. aureus* growth and biofilm formation at 100 and 50 µg/mL. Compounds 3, 4 and 5 were the most active at 100 µg/mL in both tests (Tables 7 and 8).

There are studies that report the antimicrobial activity of some isolated metabolites of different genera of *Fusarium*, such as esquesetin, a metabolite derived from tetramic acid, isolated from *Fusarium sp*. with antimicrobial activity against *Bacillus subtilis*, *S. aureus* and *S. aureus* (MRSA) (Ratnaweera et al., 2015). Tayung et al. (2011) studied the metabolites produced by *F. solani*, which were identified as volatile hydrocarbons. These volatile hydrocarbons have also been reported previously in some endophytic fungi with antimicrobial activity against the pathogenic bacteria of plants and humans (Strobel et al., 2001; Stinson et al., 2003).

The mode of action of insect pathogenic fungi varies and kills the insect by different ways such as causing starvation to toxin production. These insect pathogenic fungi produce many toxins and extracellular enzymes such as proteases and chitinases. Some structures and general processes are involved in the penetration of host cuticle and the mechanisms of each fungus may also differ. After the penetration of germ tube through the cuticle and insect epidermis, the fungus multiplies into the body cavity of insect. Toxins are produced by some insect pathogenic fungi and more of them aid to increase pathogenesis and play an insecticidal role. While some other such fungi produce antimicrobial metabolites.

Our results lead us to believe that the isolated main compounds would not be responsible for the insecticidal activity observed in the extracts. However, they could facilitate the action of other active compounds present in them. Hence, the production of active metabolites would be involved in the antagonistic effect of these agents.

4. Conclusions

In general, fungi have the ability to produce a wide variety of molecules in both natural and synthetic media. This work would be the first report of a chemical analysis for *Fusarium sp*. ARSEF 3300, isolated entomopathogenic strain of *S. frugiperda*. It should also be remarked that deformed adults affect insect reproduction resulting a population control. In regard to *C. capitata* bioassay, a moderate to high oviposition deterrent activity was observed. However, more studies are necessary to find the most effective concentration to improve this activity. The sites and mode of action of these compounds are being investigated and probably correspond to a combination of antifeedant or deterrent action, as well as insecticidal and antimicrobial activities. While some natural insecticides are found on the market, the search for new compounds with activity against a variety of insects is always necessary to prevent the emergence of resistance in insects or to ensure the immediate availability of natural insecticides through more widely available sources. On the other hand, the information on the chemical-structural characteristics and the mechanisms of action of the metabolites can also be used as a source of information for the molecular design of novel drugs or synthetic insecticides, so that they have a greater selectivity for specific pests to promote phytosanization, safety and food safety that consumers demand.

Conflict of interest

The authors declare that there are no conflicts of interest and they have no actual or potential competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101084.

References

Adams, R., 2007. Identification of Essential Oil Components by Gas Chromatography/ Quadrupole Mass Spectrometry, fourth ed. Allured Publishing Corporation, Carol Stream, IL.

Almeida, J.E.M., Filho, A.B., Oliveira, F.C., Raga, A., 2007. Pathogenicity of the entomopathogenic fungi and nematode on medfly Ceratitis capitata (Wied.) (Diptera: Tephritidae). BioAssay [online]. http://www.bioassay.org.br/articles/2.7.

Ameen, M.K.M., 2012. Screening of Fusarium isolates pathogenicity in vitro by using the larvae of *Galleria Mellonella* L. J. Basrah Res. (Sciences) 38, 19–28.

Anand, R., Prasad, B., Tiwary, B.N., 2009. Relative susceptibility of *Spodoptera litura* pupae to selected entomopathogenic fungi. BioControl 54, 85–92.

Bidochka, M.J., Khatchatourians, G.G., 1991. Microbial and Protozoan pathogens of grasshoppers and locusts as potential biocontrol agents. Biocontrol Sci. Technol. 1, 243–249.

Bottalico, A., Perrone, G., 2002. Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur. J. Plant Pathol. 108, 611–624.

Bryden, W.L., Logrieco, A., Abbas, H.K., Porter, J.K., Vesonder, R.F., Richard, J.L., Cole, R.J., 2001. Other significant Fusarium mycotoxins. In: Summerell, B.A. (Ed.), Fusarium. APS Press, St. Paul, Minnesota, pp. 360–392.

Cherry, A.J., Banito, A., Djegui, D., Lomer, C., 2004. Suppression of the stem-borer *Sesamia calamistis* (Lepidoptera; Noctuidae) in maize following seed dressing, topical application and stem injection with African isolates of *Beauveria bassiana*. Int. J. Pest Manag. 50, 67–73.

Cole, R.J., Schweikert, M.A., 2003. Handbook of Secondary Fungal Metabolites, vol. 2. Academic Press, USA-California, pp. 1–91.

Crespo, R., Pedrinia, N., Juarez, M.P., Dal Bello, G.M., 2008. Volatile organic compounds released by the entomopathogenic fungus *Beauveria bassiana*. Microbiol. Res. 163, 148–151.

Desjardins, A.E., Hohn, T.M., 1997. Mycotoxins in plant pathogenesis. Mol. Plant Microbe Interact. 10, 147–152.

Desjardins, A.E., Proctor, R.H., 2001. Biochemistry andgenetcs of Fusarium toxins. In: Summerell, B.A. (Ed.), Fusarium. APS Press, St. Paul, Minnesota, pp. 122–137.

Desjardins, A.E., 2003. Gibberella from A (venaceae) to Z (eae). Annu. Rev. Phytopathol. 41, 177–198.

Falchi, G., Marche, M.G., Mura, M.E., Ruiu, L., 2015. Hydrophobins from aerial conidia of *Beauveria bassiana* interfere with *Ceratitis capitata* oviposition behavior. Biol. Control 81, 37–43.

Foroud, N.A., Eudes, F., 2009. Trichothecenes in cereals grains. Int. J. Mol. Sci. 10, 147–173.

Gangwal, A., Parmar, S.K., Sheth, N.R., 2010. Scholars research library. der Pharmacia letter 2 (1), 307–317.

Giménez, A.B., Garcia Breijo, J., Rosello, J., Santamarina, M.P., 2000. Investigación y detección de fúngicos con actividad bactericida, fungicida e insecticida. Departamento de Biología vegetal: Escuela Universitaria de ingeniería Técnica Agrícola de la Universidad politécnica de Valencia.

Lacey, L.A., Frutos, R., Kaya, H.K., Vail, P., 2001. Insect pathogens as biological control agents: do they have a future? Biol. Control 21, 230–248.

Lee, I.H., Hung, Y.H., Chou, C.C., 2008. Solid-state fermentation with fungi to enhance the antioxidative activity, total phenolic and anthocyanin contents of black bean. Int. J. Food Microbiol. 121, 150–156.

Logrieco, A., Mulè, G., Moretti, A., Bottalico, A., 2002. Toxigenic Fusarium species and mycotoxins associated with maize ear rot in Europe. Eur. J. Plant Pathol. 108, 597–609.

Majumdar, A., Boetel, M.A., Jaronski, S.T., 2008. Discovery of *Fusarium solani* as a naturally occurring pathogen of sugar beet root maggot (Diptera: ulidiidae) pupae: prevalence and baseline susceptibility. J. Invertebr. Pathol. 97, 1–8.

Marcinkevicius, K., Salvatore, A., Bardon, A., Cartagena, E., Arena, M., Vera, N., 2017. Insecticidal activities of diketopiperazines of *Nomuraea rileyi* entomopathogenic fungus. Int. J. Environ. Agric. Biothecnol. 2 (4), 1586–1596.

Merlin, J.N., Christhudas, I.V.S.N., Kumar, P.P., Agastian, P., 2013. Optimization of growth and bioactive metabolite production: *Fusarium solani*. Asian J. Pharmaceut. Clin. Res. 6 (3), 98–103.

Musavi, S.F., Balakrishnan, R.M., 2014. A study on the antimicrobial potentials of an endophytic fungus *Fusarium oxysporum* NFX 06. J. Med. Bioeng. 3 (3), 162–166.

Nathan and Sehoon (2006).

Neuhold, G., Fidesser, M., Krska, R., 1997. Head blight (Fusarium spp.) on wheat: investigation on the relationship between disease symptoms and mycotoxin content.

Cereal Res. Commun. 25, 459–465.
Nes, W.D., Parish, E.J., 1989. Analysis of Sterols and Other Biologically Significant Steroids. Academic Press Inc., New York, NY, pp. 341.
Nicholson, P., Simpson, D.R., Wilson, A.H., Chandler, E., Thomsett, M., 2004. Detection and differentiation of trichothecene and enniantin-producing Fusarium species on small-grain cereals. Eur. J. Plant Pathol. 110, 503–514.
O'Toole, G.A., Kolter, R., 1998. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. 30, 295–304.
Pascale, M., Visconti, A., Pronczuk, M., Wisniewska, H., Chelkowski, J., 2002. Accumulation offumonisins, beauvericin and fusaproliferin in maize hybrids inoculated under field conditions with *Fusarium proliferatum*. Mycol. Res. 106, 1026–1030.
Proctor, R.H., Brown, D.W., Plattner, R.D., Desjardins, A.E., 2003. Coexpression of 15 contiguous genes delineates a fumonisin biosynthetic gene cluster in *Gibberella moniliformis*. Fungal Genet. Biol. 38, 237–249.
Prior, C., Greathead, D.J., 1989. Biological control of locusts: the potential for the exploitation of pathogen. FAO Plant Prot. Bull. 37, 37–48.
Rajesh, A., Bhupendra, N.T., 2009. Pathogenicity of entomopathogenic fungi to eggs and larvae of *Spodoptera litura*, the common cutworm. Biocontrol Sci. Technol. 19, 919–929.
Ratnaweera, P.B., Dilip de Silva, E., Williams, D.E., Andersen, R.J., 2015. Antimicrobial activities of endophytic fungi obtained from the arid zone invasive plant *Opuntia dillenii* and the isolation of equisetin, from endophytic *Fusarium sp*. BMC Complement Altern. Med. 15, 220.
Sandasi, M., Leonard, C.M., Viljoen, A.M., 2008. The effect of five common essential oil components on *Listeria monocytogenes* biofilms. Food Control 19, 1070–1075.
Santamarina, M.P., Rosello, J., Lacer, R., Sanchis, V., 2004. Actividad antagonista de *Penicillium oxalicum* Corrie and Thom, *Penicillium decumbens* Thom y *Trichoderma harzianum* Rifai frente a hongos, bacterias e insectos *in vitro*. Rev. Iberoam. De. Micol. 19, 99–103.
Socolsky, C., Fascio, M.L., D'Accorso, N.B., Salvatore, A., Willink, E., Asakawa, Y., Bardón, A., 2008. Effects of p-vinylphenyl glycosides and other related compounds on the oviposition behavior of *Ceratitis capitata*. J. Chem. Ecol. 34, 539–548.
Stinson, M., Ezra, D., Hess, W.M., Sears, J., Strobel, G., 2003. An endophytic *Gliocladium sp*. of *Eucryphia cordifolia* producing selective volatile antimicrobial compounds. Plant Sci. 165, 913–933.
Strobel, G.A., Dirksie, E., Sears, J., Markworth, C., 2001. Volatile antimicrobials from *Muscodor albus*, a novel endophytic fungus. Microbiology 147, 2943–2950.
Suttiarporn, P., Chumpolsri, W., Mahatheeranont, S., Luangkamin, S., Teepsawang, S., Lardkamolkarn, V., 2015. Structures of phytosterols and triterpenoids with potential anti-cancer activity in bran of black non-glutinous rice. Nutrients 7, 1672–1687.
Tayung, K., Barik, B.P., Jha, D.K., Deka, D.C., 2011. Identification and characterization of antimicrobial metabolite from an endophytic fungus, *Fusarium solani* isolated from bark of *Himalayan yew*. Mycosphere 2 (3), 203–213.
Vega, F.E., Posada, F., Aime, M.C., Pava-Ripolli, M., Infante, F., Rehner, Stephen A., 2008. Entomopathogenic fungal endophytes. Biol. Control 46, 72–82.
Vey, A., Matha, V., Dumas, C., 2002. Effects of the peptide mycotixin destruxin E on insect haemocytes and on dynamics and efficiency of the multicellular immune reaction. J. Invertebr. Pathol. 80, 177–187.
Wilson, W.K., Sumpter, R.M., Warren, J.J., Rogers, P.S., Ruan, B., Schroepfer, G.J., 1996. Analysis of unsaturated C27 sterols by nuclear magnetic resonance spectroscopy. J. Lipid Res. 37, 1529–1555.