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ORIGINAL PAPER

Activity of a novel compound produced by *Aspergillus parasiticus* in the presence of red flour beetle *Tribolium castaneum* against *Pseudomonas aeruginosa* and coleopteran insects

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Abstract *Aspergillus* is a promising source of bioactive secondary metabolites. The addition of insect-derived material in the broth culture could trigger the biosynthesis of insecticidal and antimicrobial compounds by entomopathogenic fungus (EF). Insects inhabit diverse niches and interact with various bacteria, for this reason, when a fungus kills an insect should inhibit the insect's gut bacteria to prevent the insect consumption by bacteria. According with this hypothesis, the EF is able to produce substances that inhibit bacteria growth or bacteria virulence strategies. In the present, investigation is demonstrated that the addition of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) components (2 % w/v) in a culture of saprophytic fungus *Aspergillus parasiticus* MOR 3 induces the production of a novel compound present inside mycelium 2-(4-bromophenyl)-2-oxoethyl benzoate, that is reported here for the first time as a natural product of *A. parasiticus*. In addition, increased significantly the fungal extracellular production of undecyl 4-fluorobenzoate. The mycelium extract (ME) at 250 μg per g of diet on adult population of *T. castaneum* produced an alteration of the feeding behavior of coleopteran insects (Repellency index = +30). The fraction derived from ME (Fr2) that contained the organobromine compound, 2-(4-bromophenyl)-2-oxoethyl benzoate had the highest inhibition of the *Pseudomonas aeruginosa* virulence factors like elastase enzyme (49 %) at 5 μg/ml and biofilm formation (43 %) at 100 μg/ml. The results suggest that the extract from mycelium of a non-aflatoxigenic *A. parasiticus* MOR 3 strain is a potential candidate as food coleopteran repellent as well as an anti-virulence strategy of *P. aeruginosa*.

Keywords *Aspergillus parasiticus* · Fungal induction · Repellent substances · *Tribolium castaneum* Herbst · *Pseudomonas aeruginosa* · Biofilm · Elastase activity

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Introduction

Entomopathogenic fungi (EF) have been used and developed worldwide as biocontrol agents for invertebrate pest control. The use of EF is among the most promising alternatives, since they combine low mammalian toxicity, high effectiveness and natural origin (Moore et al. 2000). EF show potential like control agents for insects. However, their efficiency could be affected by many environmental factors, such as solar radiation, temperature, and humidity (Inglis et al. 2001). Many fungi are known to produce bioactive secondary metabolites; these metabolites serve different functions, depending on the ecological niche of the fungus. Fungi and fungal derived products can be highly toxic to insects. The biological control of these compounds can thus be an effective and ecological friendly approach, which can be used as an option to minimize the insect population (Soni and Prakash 2011).

Aspergillus parasiticus is one of the least studied among EF. However, it was reported that *A. parasiticus* reduced the amount of plant tissue consumed by *Chortoicetes*

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terminifera indicating the possibility of deterrence of feeding (Guruligappa et al. 2010). Various species of *Aspergillus* produce metabolites that are toxic to insects (Tanada and Kaya 1993), and other fungi are known to produce metabolites that may deter insect feeding (Kaur et al. 2013). However, considering that several strains of *Aspergillus flavus* and some strains of *A. parasiticus* produce aflatoxin, only non-toxigenic strains should be used (Degola et al. 2009).

Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) is one of the most widespread and destructive pests of stored products, feeding on different stored grain, and grain (Padín et al. 2013). In Argentine, it is one key pest of stored grain in the ports (Stefanazzi et al. 2011). *T. castaneum* known as "the red flour beetles" attack stored-grain products causing deterioration, especially loss of quality and weight during storage. In addition, they may cause an allergic response (Kumar et al. 2008). Bulk stored grain and processed food commodities can be negatively affected by stored-product insects during storage and processing (Hagstrum and Flinn 1995). Chemical pesticide application (as pyrrole chlorfenapyr) is the most common method to pest control. Recent studies demonstrated that the mortality generally increased with starvation time, the presence of a food source led to decreased mortality, and *Tribolium confusum* was more susceptible than *T. castaneum* (Frank 2013).The overuse of pesticides has resulted in environmental toxic waste, insecticide resistance as well as undesirable effects on human health (Wijayaratne et al. 2012). Moreover, various types of storage interfere with the insecticide efficacy (Jankov et al. 2013).

It is well known that the addition of insect-derived material in the broth culture could trigger the biosynthesis of antimicrobial compounds by EF (Lee et al. 2005; Kikuchi et al. 2004). Insects inhabit diverse niches and interact with various bacteria, and when a fungus kills an insect its gut bacteria could consume it before the fungus. Inside the insect gut 18 bacterial species from genera *Pseudomonas*, *Burkholderia,* and *Serratia* were found (Pereira de Oliveira et al. 2001). To prevent insect consumption by bacteria, the EF need to produce substances to inhibit bacterial growth or pathogenicity strategies.

Antipathogenic compounds do not kill bacteria or stop their growth. They rather control bacterial virulence factors like biofilm, elastase activity, and prevent the development of resistant strains (Otto 2004). Biofilms allow microorganisms to trap nutrients and withstand hostile environmental conditions, a key feature for their survival. Another virulence factor is *Pseudomonas* elastase, also known as pseudolysin or LasB, is a metalloprotease, which has long been recognized as a key virulence factor produced by *P. aeruginosa* (Stewart and Costerton 2001). This secreted protease degrades a broad range of host tissue proteins and key biomolecules involved in innate immunity such as immunoglobulins, complement factors, and cytokines (Cathcart et al. 2009).

This study was based on the assumption that insect–derived material would facilitate the onset of bioactive metabolite production in EF. The aim was to induce the production of repellent and antipathogenic substances through the inclusion of coleopteran insect-derived materials in the culture medium of a non-aflatoxigenic *A. parasiticus* MOR 3 strain.

Methods and materials

Fungal strain

Aspergillus parasiticus MOR 3 was isolated from exoskeleton of a decomposing *Spodoptera frugiperda* (L.) insect. The strain was classified by morphological criteria and a microscope method. An HPLC method (Braga et al. 2005) was used to determine the inability to produce aflatoxins for this strain, in the Mycology Laboratory (Cátedra de Micología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina).

Insects

The stored-grain pest *T. castaneum* (Herbst) was collected from samples of self rising flour, and reared under controlled conditions of (25 ± 2) °C and (65 ± 5) relative humid (RH) with alternating light and dark periods of 12 h, in the Laboratory of Entomology (Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina).

EF growth conditions

Aspergillus parasiticus MOR 3 was maintained on potato dextrose agar (PDA) slants. The spores produced were placed in an aqueous solution of 0.05 % Tween 80. After homogenising, the suspension was counted using a Neubauer chamber and adjusted to 8.7 × 107 spores/ml.

Three experimental culture media were developed. Medium A (control):potato dextrose. Medium B:potato dextrose plus 2 % (w/v) of *T. castaneum* cuticles (powder). Medium B (control of powdered):potato dextrose plus 2 % (w/v) of powdered *T. castaneum* cuticles. Medium A and B were inoculated with 2 % (v/v) of the resuspended spores of *A. parasiticus* MOR 3. Medium C (without spores):potato dextrose plus 2 % (w/v) of *T. castaneum* cuticles (powder), was used as control. Erlenmeyer flasks of 500 ml were cultivated for 15 days at 25 °C at 180 rpm on a rotating shaker.

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Extraction of fungal metabolites from culture broth of *A. parasiticus* MOR 3

After the incubation period as described above, the mycelium and insoluble materials were separated by filtration. The filtrate media were extracted with $CHCl_3$ twice. The $CHCl_3$ extracts dried over anhydrous Na_2SO_4, and were evaporated under reduced pressure at room temperature in order to obtain the culture broth chloroform extract (BE). According to the culture medium used to obtain the extract, the samples were denominated as BEA, BEB, and BEC, respectively.

On the other hand, the mycelium and insoluble materials were extracted with $CHCl_3$ twice, in order to obtain the culture mycelium extract (ME). According to the culture medium used to obtain the ME and/or insoluble materials, the samples were denominated as MEA, MEB, and MEC, respectively.

Identification of volatile metabolites from extracts of *A. parasiticus* MOR 3

The extracts obtained were analyzed by gas chromatography techniques. GC and GC–MS (EI) analysis were carried out using a Thermo electron Trace™ 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 60 °C during 1 min. A temperature programing was applied from 60 to 300 °C at a rate flow of 10 °C/min, and finally 300 °C for 5 min. Carrier gas was helium (flow 1 ml/min). Injection mode split-less with surge (30 s, surge pressure 100 kPa). The main volatile constituents were determined by comparison of their mass spectra with standard data of NIST GC/MS library.

Purification of metabolites from fungal induced extract

The extract MEB was purified by preparative thin-layer chromatography (PTLC) using precoated plates Merck silica gel 60 F254 (20 × 20 cm), and mixture of $CHCl_3$ + AcOEt (50 + 2.5 ml), as mobile phase.

The plate was analyzed under UV light at 254 nm. The fractions Fr1 (R_f 0.33), Fr2 (R_f 0.45), and Fr3 (R_f 0.68) were obtained by desorption with $CHCl_3$ (thrice) and dried in order to identify the main constituents by GC–MS technique.

Food preference and repellency bioassay

The extracts MEB, MEA, BEB, and BEA were subjected to insect bioassay. For the bioassay, we used a glass apparatus with a center cubicle connected symmetrically to another four cubicles. 2 g of flour treated with 1 ml of a chloroform solution of extract was placed in two of them to obtain a concentration of 250 µg per g of diet (Treatment). 2 g of flour impregnated with 1 ml of chloroform was placed in the two remaining cubicles (Control). Previously, both diets were left at room temperature, for 24 h to eliminate the chloroform. In the central division, 40 adult insects of *T. castaneum* were placed. After 24 h, food preference was assessed through the calculation of preference index (PI) with the following formula:

$$PI = (\%ITD - \%ICD)/(\%ITD + \%ICD)$$

where %ITD = % insect in treated diet; %ICD = % insects in the control diet. PI values between −1.00 and −0.10 indicate that the extract is repellent; between −0.10 and +0.10 the extract is neutral. And, if the PI is between +0.10 and +1.00 the extract is attractive (Procopio et al. 2003).

The repellency was also established through the calculation of repellency index (RI), according to the following formula:

$$RI = (C - T)/(C + T) \times 100$$

where C = Insect in the control diet, T = Insects in the treated diet. Positive values indicate repellency (Pascual-Villalobos 1998; Stefanazzi et al. 2011).

Bacterial growth

Overnight cultures of *P. aeruginosa* ATCC 27853 were diluted to reach 2.5 × 10^6 CFU/ml in Luria–Bertani (LB) medium. The diluted culture (190 µl) was placed in each of the 96 wells of a microtitre polystyrene plate. Solutions containing 1 and 0.1 mg/ml of extracts and the three fractions (Fr1-3 from MEB) in DMSO–distilled water (1:1) were prepared separately and 10 µl of each was pipetted to the plastic microtitre plate wells individually (eight replicates). 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. Bacteria grew in LB medium at 37 °C and growth was detected as turbidity (560 nm) using a microtitre plate reader (Power Wave XS2, Biotek, VT, USA) and by direct counting of CFU/ml determined by plating 0.1 ml of the inoculation onto LB agar (pH 6.0). The maximum level of DMSO to which the cells were exposed was 2.5 %.

Biofilm formation assay

For biofilm quantification, a micro method based on a protocol previously reported was employed (O'Toole and

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Kolter 1998). Biofilms formed after 24 h incubation of bacterial cultures prepared as described 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 microtitre plate reader (Power Wave XS2, Biotek, VT, USA). Azithromycin a known quorum-sensing inhibitor, was incorporated in the bioassay at 5 µg/ml, as a control in the same experimental conditions employed to evaluate the compounds (Tateda et al. 2001).

Elastase B activity

Elastolytic activity was determined using a modification of the method previously described (Caballero et al. 2001). One hundred microliter of the substrate, elastin Congo red (Sigma) dissolved in Tris–HCl (pH 8.0) at a concentration of 5 mg/ml was mixed with 100 µl of cell-free supernatant obtained from *P. aeruginosa* ATCC 27853 culture grown during 24 h, in LB media containing 50 or 5 µg/ml of extracts or fractions, respectively. The reaction mixture (200 µl) was incubated at 37 °C for 24 h and centrifuged at 13,000 rpm for 10 min. The absorbance (495 nm) of the supernatant is a measure of the enzyme activity.

Statistical analysis

Differences between means were evaluated by analysis of variance (ANOVA). For group comparison tests were used Tukey and subtle differences were used. In all statistical analysis, P values >0.05 were not considered significant. The Analytical Software Statistix 7.1, 2002 for Windows was used. All the data are normal values. The Analytical Software Statistix 7.1, 2002 for Windows was used.

Results

GC–MS analysis of the fungal extracts

The yield of extract from mycelium of *A. parasiticus* grown with insect cuticles (MEB) was of 0.07 g/l and higher than that of MEA (0.04 g/l). The extract MEB and the derived Fr2 had a sweet fragrance with flower notes and was analyzed by GC–MS (EI). The total ion current (TIC) showed a main peak (32.4 %) with the pattern of substitution with bromine, that is correlated with the MS spectrum of structure **1** (Fig. 1), named 2-(4-bromophenyl)-2-oxoethyl benzoate ($C_{15}H_{11}BrO_3$). This compound has never been reported as a natural product. However, the phenyl ethyl benzoate nucleus is a known natural insecticide from the Zingiberaceae plants *Kaempferia rotunda* L. and *K. angustifolia* Roscoe (Nugroho et al. 1996; Woerdenbag et al. 2004), and constituent of many essential oils. Today, it is used in appropriate doses, as cosmetic, and as flavor and fragrance agents due to its pleasant aroma of roses and honey. On the other hand, it is important to note that the organobromine chemicals are produced naturally by an array of biological and other chemical processes in our environment. Some of these compounds are identical to man-made organobromine compounds, such as bromophenols, but many others are entirely new molecular entities, often possessing biological properties. These compounds are produced naturally by marine creatures and seaweed, plants, fungi, lichen, algae, bacteria, microbes, and some mammals. Many of these organobromine compounds are used in chemical defense, to facilitate food gathering, or as hormones (Gribble 2000).

In contrast, compound **1** was not detected by GC–MS analysis of MEA and MEC.

The compound **1** from Fr2 (retention time: 20.85 min, 33.7 %) was isolated by PTLC of MEB and was active under UV light (254 nm). The MS spectrum exhibited the main peaks at *m/z* 185 ($C_7H_4^{81}BrO+$), 183 ($C_7H_4^{79}BrO+$, 100 %), 157 ($C_6H_4^{81}Br+$), 155 ($C_6H_4^{79}Br+$), 105 (C_7H_50+), 77 (C_6H_5+), and 51 (C_4H_3+). These mass fragments justified the structure **1** (Fig. 1).

The broth culture extract from induced medium BEB was obtained with a yield of 0.03 g/l; while BEA had a lower yield (0.006 g/l). Undecyl 4-fluorobenzoate (**2**) was the main compound of BEB (retention time: 13.42 min, 29 %). This compound was also detected in BEA (Fig. 2), but in lower concentration (20 %). Probably, the compound **2** is a fungal catabolic product of environmental contaminant 4-fluortoluene used in pesticides and pharmaceutical but also in electronic industry (Prenafeta-Boldú et al. 2001).

Food preference and repellency bioassay

The ME MEB had repellent effect at 250 µg per g of diet on adult population of *T. castaneum.* This extract produced an alteration of the feeding behavior of coleopteran insects, which was determined by a preference index of −0.3 (Table 1). The RI was also measured, and indicated that this extract was repellent, as shown in Table 1 (RI = +30). These results are in agreement with previous literature that informed that structural related compounds, as benzyl benzoate is a good repellent against *T. castaneum* (Caballero-Gallardo et al. 2011). In addition, bromine compounds

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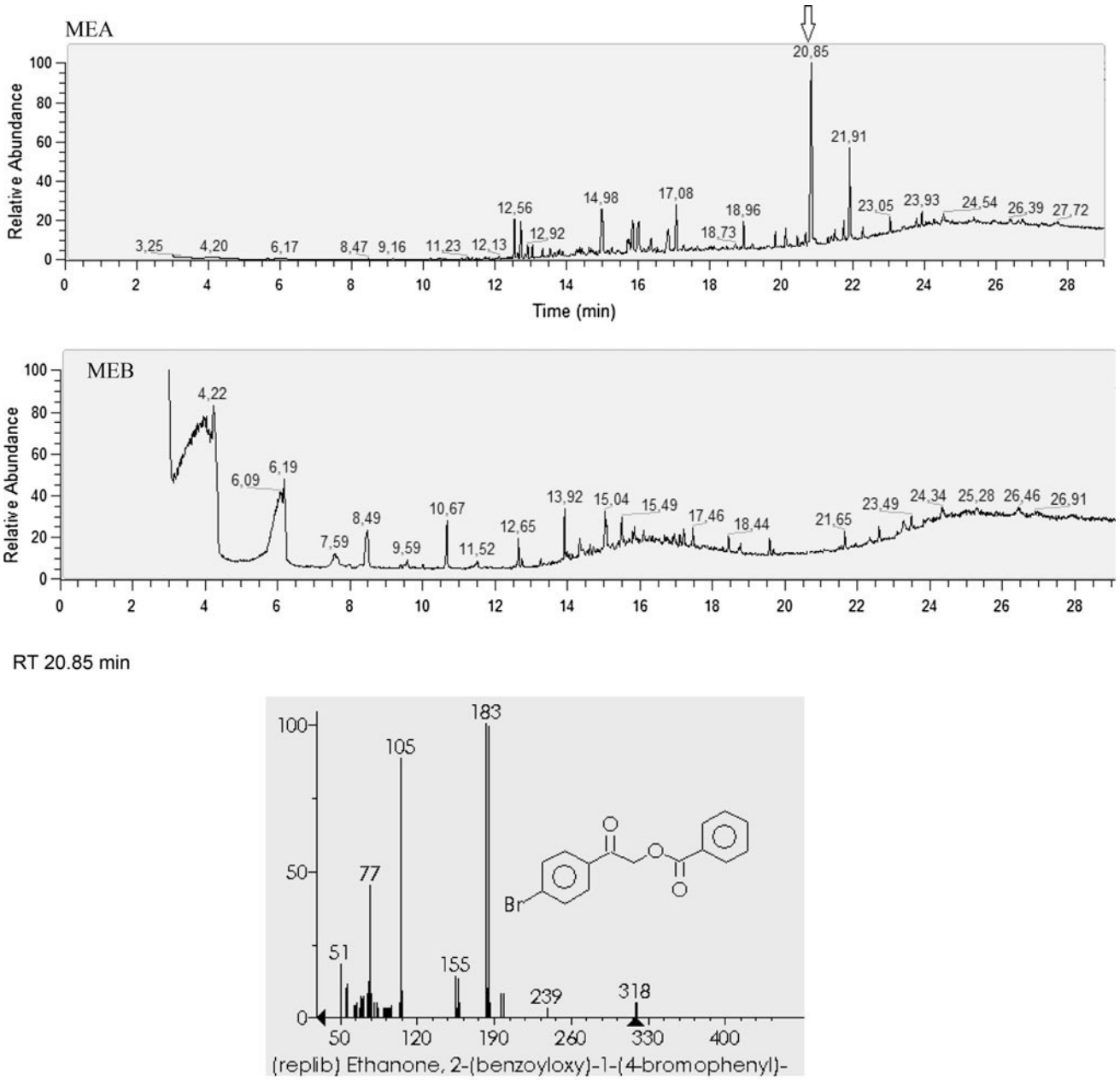

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Fig. 1 Total ion currents of MEB and MEA from *A. parasiticus* MOR 3, respectively, and mass spectrum of main compound **1** (20.85 min)

as methyl bromine known as gaseous fumigant was extensively used as tool for the control post-harvest of stored-grain pest (MBTOC 2010), but because of its contribution to stratospheric ozone depletion, it was phased out in developed countries by 2005, and its phase out in developing countries is scheduled by 2015 (Navarro 2012). The extract MEA did not exhibit repellent effect against adults of *T. castaneum* as we expected (PI = +0.1; RI = −14). Coherently, the extract BEA was neutral (PI = +0.1; RI = −12), and BEB had a repellent effect (PI = −0.4; RI = +35). It is important to note that BEB showed an increase of undecyl 4-fluorobenzoate concentration (Fig. 2).

Bacterial growth

The effects of extracts (MEB, MEA, BEB, and BEA) and fractions Fr1-3 on the bacterial growth of *P. aeruginosa* ATCC 27853, in comparison with the control experiments, are shown in Fig. 3. Neither the broth culture or mycelial

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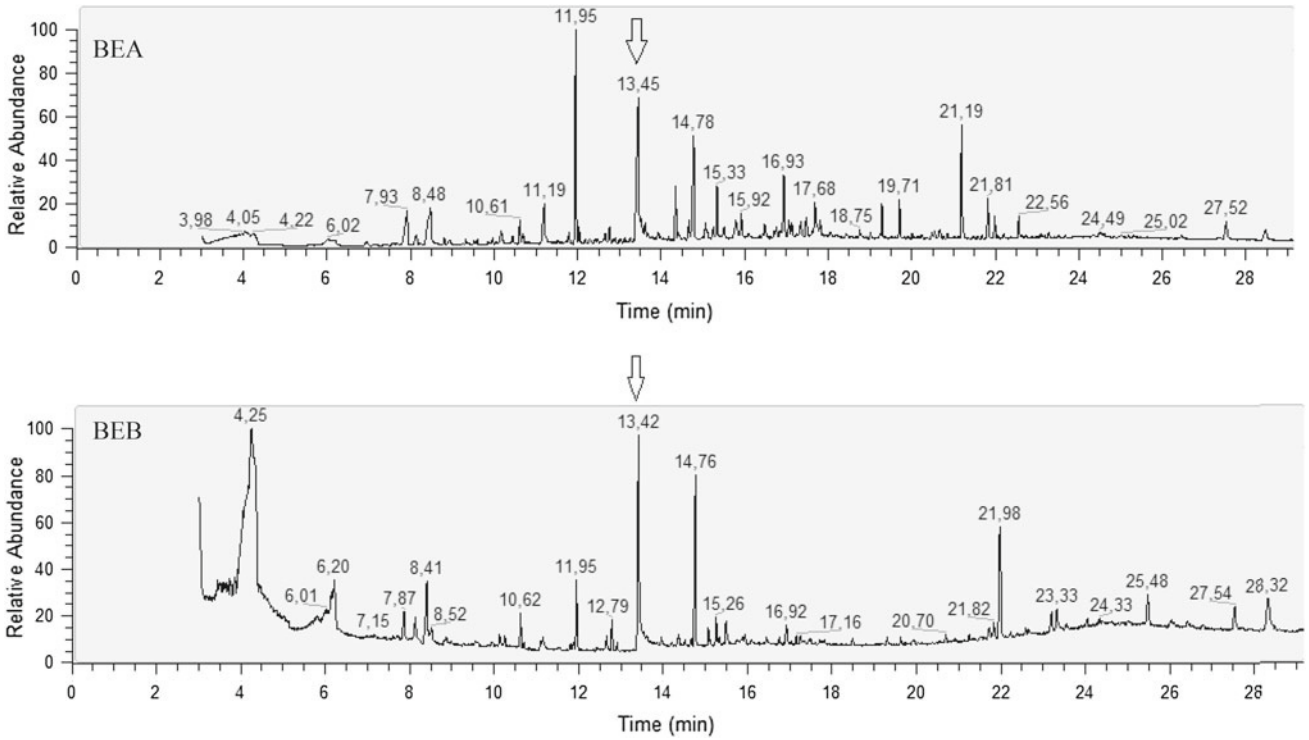

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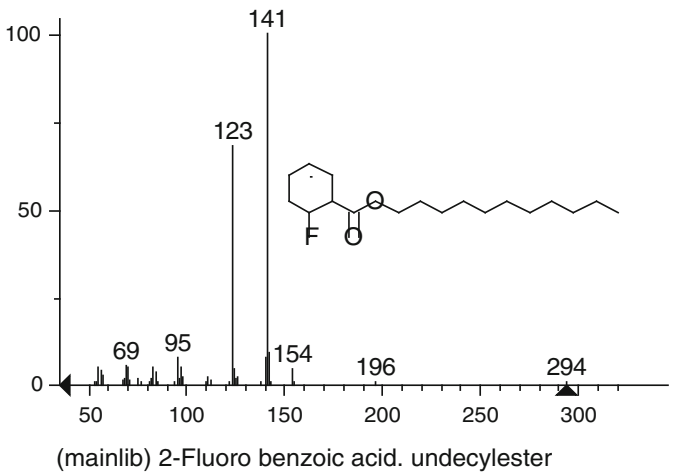

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Fig. 2 Total ion currents of BEA and BEB from *A. parasiticus* MOR 3, respectively, and mass spectrum of main compound **2** (13.4 min)

extract or fractions were able to inhibit significantly the bacterial growth.

Biofilm formation

Absorbance measurements of biofilm formed after 24 h incubation are shown in Fig. 4. Between the extracts from broth culture, only induced media (BEB) produced a decrease of the amount of biofilm (25 %). With respect to the ME, MEA, and MEB inhibited 33 and 37 % of the biofilm formation, respectively. The fractions obtained of mycelium from induced media (Fr1, Fr2, and Fr3) showed significant biofilm formation diminution (30, 43, and 9 %, respectively).

Elastase activity in culture supernatants

All the samples studied inhibit partially the elastase activity (Fig. 5). The broth culture extract BEB had two times higher inhibition with respect to BEA. However, not

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Table 1 Food preference and repellency indices

Mean number of insects			Statistic *P* values	PI[a]	RI[b]	
Control diet	Treated diet					
17.7 ± 1.5	BEA	22.3 ± 1.5	0.0201	+0.1	−12	Neutral
27.0 ± 1.0	BEB	13.0 ± 1.0	0.0001	–0.4	35	Repellent
17.3 ± 0.6	MEA	22.7 ± 0.6	0.0003	+0.1	−14	Neutral
25.3 ± 0.6	MEB	13.7 ± 0.6	0.0000	−0.3	30	Repellent

BEA extract from broth culture, *BEB* extract from broth culture obtained by fungal induction, *MEA* extract from fungal mycelium, *MEB* extract from fungal mycelium obtained by induction

Significant differences ($P < 0.05$) in the mean number of adults of *T. castaneum* in the treated diet compared with the control diet were measurements in all cases

[a] PI values between −1.00 and −0.10 indicate that the extract is repellent; between −0.10 and +0.10 the extract is neutral. And, if the PI is between +0.10 and +1.00 the extract is attractive

[b] Positive values indicate repellency

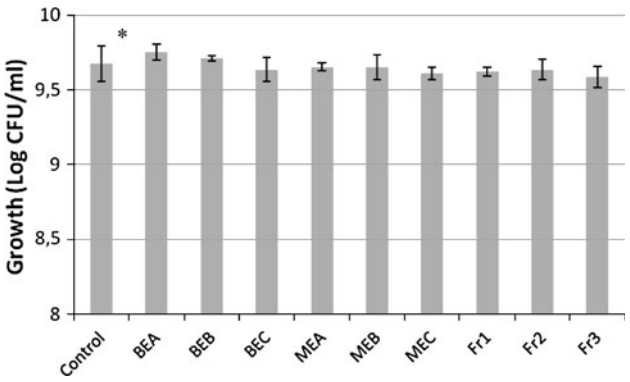

Fig. 3 Effect of the $CHCl_3$ extracts from *A. parasiticus* MOR 3 and fractions (Fr1-3) on *P. aeruginosa* growth. *BEA* extract from broth culture, *BEB* extract from broth culture obtained by fungal induction, *BEC* extract from media plus insect without inoculation, *MEA* extract from fungal mycelium, *MEB* extract from fungal mycelium obtained by induction, *MEC* extract from media plus insect without inoculation. *Fr1-3* fractions1-3 from MEB. The *error bars* indicate standard deviation (SD). *No significant differences were observed between the different cultures conditions

significant difference was observed due to the insect inductive effect in the ME. The fraction that contained the organobromine compound **1** (Fr2) had the highest inhibition of the enzyme (49 %), as well as the best biofilm formation inhibition.

Discussion

The development of EF is adversely affected by many environmental factors (Michalaki et al. 2007; Moore et al. 2000). Therefore, currently the uses of specific fungal metabolites or extracts instead of EF are investigated. In presence of insect constituents, the fungi may increase active responses that promote the production of constitutively expressed metabolites and/or stimulate the de novo synthesis of additional compounds. It is presumed that specific insect–derived compounds are recognized by the host fungus and thereupon activate a defensive response. The most studied EF, *Beauveria bassiana,* produces many insecticidal metabolites such as, bassianin, beauvericin, bassionolide, beauveroiolide, bassacridin, oosporein, and tenellin (Jeffs and Khachatourians 1997; Quesada-Moraga and Vey 2004; Guruligappa et al. 2010).

Fungal metabolites of *B. bassiana* diminish the presence of different larvae insects in opium poppy, in banana, in tunneling, and in corn (Quesada-Moraga et al. 2009; Akello et al. 2008; Cherry et al. 2004). On the other hand, strains of *Purpureocillium lilacinum* may be considered good candidates for biologic control in the ecosystem of stored maize (Barra et al. 2013).

Aspergillus parasiticus can colonize plants from soil-borne inoculums (Guruligappa et al. 2010). Based on these findings, it is supposed that the plant inoculation with a non-aflatoxigenic strain of *A. parasiticus* could be used as biological control. Moreover, *A. parasiticus* could be isolated from inoculated leaves of cotton, bean, corn, tomato, and pumpkin. The toxicity of some *A. parasiticus* toxins precludes the widespread use of this fungus and only should be used non-aflatoxigenic strains (Pitt and Hocking 2009).

Various species of *Aspergillus* produce metabolites that are toxic to insects (Tanada and Kaya 1993). Some fungal metabolites may deter insect feeding (Daisy et al. 2002).The presence of *A. parasiticus* reduced the amount of plant tissue consumed by *C. terminifera* indicating the possibility of deterrence of feeding (Guruligappa et al. 2010). In agreement, with the previous results we observed an antifeedant effect of extracts from cultured mycelium and broth with insect-derived material, suggesting a

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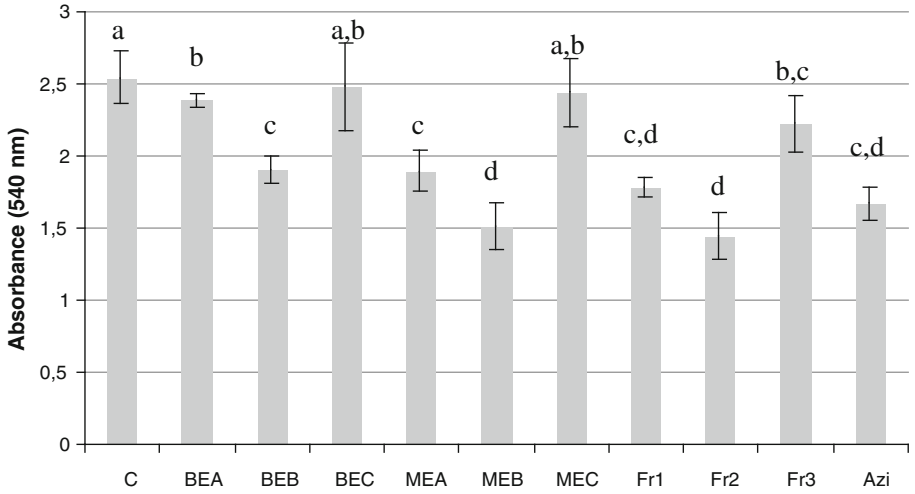

Fig. 4 Effect of the $CHCl_3$ extracts from *A. parasiticus* MOR 3 and fractions (Fr1-3) on *P. aeruginosa* biofilm production. *BEA* extract from broth culture, *BEB* extract from broth culture obtained by fungal induction, *BEC* extract from media plus insect without inoculation, *MEA* extract from fungal mycelium, *MEB* extract from fungal mycelium obtained by induction, *MEC* extract from media plus insect without inoculation. *Fr1-3* fractions1-3 from MEB. *Azi* azithromycin (5 µg/ml). The *error bars* indicate standard deviation (SD). Mean ± SD for the biofilm formation with no common letters (*a–d*) differ significantly ($P < 0.05$)

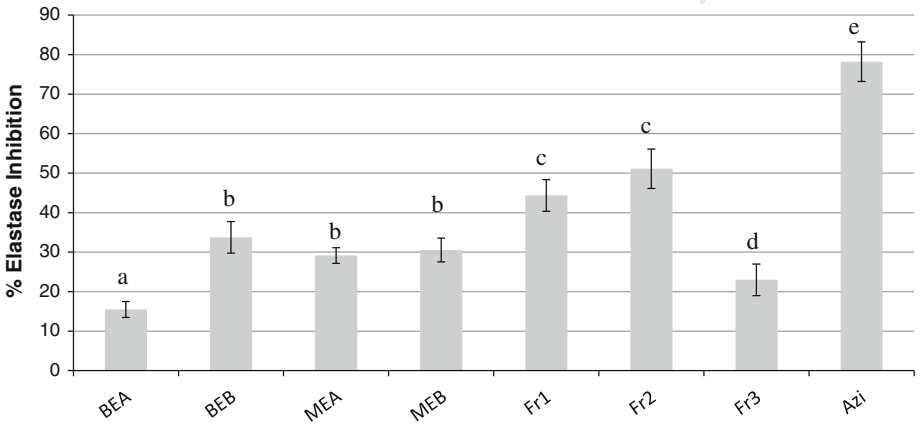

Fig. 5 Elastase activity percentage of supernatant of *P. aeruginosa* incubated 24 h in the presence of extracts of *A. parasiticus* MOR 3 and fractions (Fr1-3). *BEA* extract from broth culture, *BEB* extract from broth culture obtained by fungal induction, *BEC* extract from media plus insect without inoculation, *MEA* extract from fungal mycelium, *MEB* extract from fungal mycelium obtained by induction, *MEC* extract from media plus insect without inoculation. *Fr1-3* Fractions 1-3 from MEB. *Azi* azithromycin (5 µg/ml). The results are the mean ($n = 4$) (standard deviation). Mean ± SD for the elastase activity with no common letters (*a–e*) differs significantly ($P < 0.05$)

specific induction of the fungal metabolites biosynthesis in order to control the insect.

On the other hand, the results obtained in this investigation were consistent with studies of Lee et al. (2008) who suggested that the insect–derived material was a good medium component for culturing the EF *Metarhizium anisopliae* and raised the possibility that different types of insect–derived materials may lead to the discovery of a more diverse array of bioactive metabolites. Compounds expressed in response to antagonistic insects have been demonstrated for plant–herbivore interactions (Howe and Jander 2008). In the present study, it was demonstrated that the addition of *T. castaneum* Herbst components (2 % w/v) in a culture of saprophytic fungus *A. parasiticus* induces the production of repellent substances against the aforementioned insect, as 2-(4-bromophenyl)-2-oxoethyl benzoate which is reported here for the first time as natural product form *A. parasiticus*. This result is important to take into account because red flour beetle is a cosmopolitan pest found in grain products in storage, processing and retail facilities.

With respect to the antibacterial activity of extracts and fractions of *A. parasiticus*, the biofilm inhibition was not correlated with the bacterial growth. These results suggest

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an effect on the mechanism involved in biofilm formation, Quorum sensing, more than an antibiotic property. The biofilm formation is the main strategy for bacterial infection. According to these results, the fungal extracts and fractions could be considered as antipathogenic substances, and are in agreement with previous reports that indicate the influence of natural compounds (isolated from plants) which inhibit the biofilm formation without altering the bacterial growth (Gilabert et al. 2011; Amaya et al. 2012).

In concordance with our hypothesis, Lee et al. (2005) demonstrated that some EF, including *M. anisopliae* HF293, began to produce or produced more antibacterial compounds when they were cultivated in media containing insect–derived material. The results provide the evidence of some link between the modification of extracts due to the presence of part of the insect and the fungal protection against the bacteria present in the insect and their virulence strategies (biofilm and elastase).

No relationship seems to exist between the food repellency and anti–pathogenesis shown by *A. parasiticus* against *T. castaneum* and *P. aeruginosa*, respectively.

In summary, the obtained results support the hypothesis that treating fungi with insect–derived material as elicitors may enable us to identify novel compounds with biological activity and that the extract from mycelium of non-aflatoxigenic *A. parasiticus* MOR 3 is a potential candidate as coleopteran insect repellent, and it could control opportunist bacterial pathogenesis (biofilm and elastase inhibition).

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