http://dx.doi.org/10.22161/ijeab/2.4.18 *ISSN: 2456-1878*

Insecticidal activities of diketopiperazines of *Nomuraea rileyi* entomopathogenic fungus

Karenina Marcinkevicius[3], Analía Salvatore[2], Alicia Bardón[1,3], Elena Cartagena,[1] Mario Arena,[1,4] Nancy Vera[1*]

[1]Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, 4000 Tucumán, Argentina.

[2] Facultad de Agronomia y Zootecnia. Florentino Ameghino s/n. Bº Mercantil. Campo Experimental (4105), El Manantial, Tucumán, Argentina

[3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Instituto de Química del Noroeste Argentino (INQUINOA). Ayacucho 471, 4000 Tucumán, Argentina.

[4]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Instituto de Biotecnología Farmacéutica y Alimentaria (INBIOFAL). Av. Gral Roca 1900, 4000 Tucumán, Argentina.

***Abstract*—** Entomopathogenic fungi are fungal organisms extensively used in various parts of the world as biopesticides against insect pests that cause important economic damage. Various secondary metabolites produced by these fungi have many potential biological activities. The present study was undertaken to evaluate the insecticidal activity of extracts and pure compounds from *Nomuraea rileyi* (Farlow) Samson entomopathogenic fungi against *Spodoptera frugiperda* Smith (Lepidoptera), *Ceratitis capitata* Wiedemann (Diptera) and *Tribolium castaneum* Herbst (Coleoptera), three insect pests that generate serious economic losses in the northwest of Argentina. Diketopiperazines were extracted from the culture free supernatant of the media with ethyl acetate. Antifeedant properties were detected in all extracts under dietary choice conditions (300 ug/ g of diet). The maximum antifeedant activity was noted in cycles (Pro-Val) (86.02) and cycle (Pro-Phe) (73.47), while the rest of the extracts and metabolites exhibited varying degrees of moderate or less toxic effects. The maximum oviposition deterrence against *C. capitata* (55.86%) was recorded with cycle (Pro-Phe) at a 50 µm/cm^2 dose. Culture medium extracts supplemented with insect remains and all pure compounds showed repellent action against *T. castaneum*. The main repellency was observed in phenylacetic acid and cycle (Pro-Val) with RI values of 42 and 41% respectively. The present study would suggest the possible utilization of entomopathogenic fungal metabolites as an effective agent for controlling insect pests that cause important economic losses.

Keywords*— *antifeedant, entomopathogenic fungi, insect pests, oviposition deterrence, repellency.

I. INTRODUCTION

In the last four decades, many research groups have concentrated their efforts in the search for bioactive products derived from natural sources that represent an alternative to conventional insecticides. The results of these investigations constitute new tools for pest control that differ fundamentally from conventional chemical control in that they are more ecosystem friendly.

Among the biological controllers are predatory insects and parasitoids, as well as insect pathogens, including viruses and different genera and species of protozoa, bacteria, fungi and nematodes (Asaff et al., 2002).

Entomopathogenic fungi (EF) are the main biological agents used in integrated pest management systems. To date, more than 750 species of entomopathogenic fungi belonging to almost 100 genera have been identified, most of which are classified among the Zigomicota (entomoptera), Deuteromicota (hyphomycetes) and Ascomicota (Roberts, 1989; Hegedus and Khachatourians, 1995; Khachatourians, 1996). However, only 10 of them have been or are currently being used in commercial or experimental insect control formulations (Lacey et al., 2001).

Although entomopathogenic fungi have many advantages they are highly sensitive a varying climatic conditions like extreme temperatures, drought and ultraviolet light. They need more demanding storage conditions than inorganic molecules to prevent pathogenicity loss. In general, biological insecticides do not kill instantly, but they achieve good levels of control between one and three weeks after application, depending on the pest and the environment. Hence, researchers have begun to look produced metabolites for fungus to control pests and avoid climatic dependance. A review of the literature in recent decades shows that a considerable number of low

molecular weight secondary metabolites isolated from insect pathogens have proved to have insecticidal activity (Gilliespie and Claydon, 1989). Many secondary metabolites produced by entomopathogenic fungi are common to several of them and have been detected in most cases by their in vitro production, which is significantly affected by the conditions and composition of the culture medium (Khachatourians, 1996).

Nomuraea rileyi (Farlow) Samson has been reported as a pathogen of more than 30 species of lepidopteran larvae, especially when they are in humid weather conditions (Devi et al., 2003). It has been mainly isolated from dead insects and cultivated soils, being very closely associated with important phytophagous species, such as *Spodoptera frugiperda* (J.E. Smith) in maize fields (Wyckhuys and O'Neil, 2006). Although the potentiality of the fungus as a biological control agent is recognized, it has not yet been widely used as a mycoinsecticide (Edelstein et al., 2004) because it is highly sensitive to nutritional and environmental conditions as compared to other entomopathogenic fungi and this characteristic limits its mass production. Previous studies have reported that the fungus *Nomuraea rileyi* produces active metabolites against insects (Ignoff et al., 1976; Wasti and Hartmann, 1978; Kucera and Sansinakova, 1968; Mohamed and Nelson, 1984; Ye et al., 1993), some metabolites showed toxic activity against larvae *of Heliohis zea*, *H. virescens* (Mohamed and Nelson, 1994) and Bombyxmori (Ye et al, 1993). Additionally, it is known that the addition of insect derived material in the broth culture could trigger the biosynthesis of bioactive compounds by EF (Lee et al., 2005; Kikuchi et al., 2004; Cartagena et al., 2014).

Spodoptera frugiperda (Lepidoptera), is a polyphagous species with a wide geographic distribution, from Argentina and Chile, to the south of the United States. Its preferential host is maize, causing important losses and putting at risk the productivity of the same. In Argentina it was declared a national pest in 1988 (Murua et al., 2003). Among crops attacked are sorghum, alfalfa, cotton, rice, soybean, peanut, tomato, pepper, poroto, onion, sunflower, cabbage, cauliflower, etc. It is the main pest of the northwest and northeast of Argentina (Willink et al., 1990).

The Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae) is a key pest that is distributed worldwide and attacks more than 250 species of fruits and vegetables (Morales et al., 2004), causing large economic losses.

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).

The objective of the present work was to evaluate the insecticidal activity of extracts and pure compounds isolated from cultivations of *Nomurae rileyi*, entomopathogenic fungus, with and without the addition of insect-derived material against *Spodoptera frugiperda* (Smith) (Lepidoptera), *Ceratitis capitata* (Wiedemann) (Diptera) and *Tribolium castañeum* (Herbst) (Coleoptera), insect plagues that generate serious economic losses in the Argentine northwest.

II. MATERIALS AND METHODS

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*

Nomuraea rileyi strain ARSEF 1972 were isolated from *Spodoptera frugiperda* [Lepidoptera: Noctuidae], in Bahia (Brazil) in 1985. The strains were assigned to Dr. Mario Arena, by Professor Richard A. Humber, ARSEF Director (ARS Collection of Entomopathogenic Fungal Cultures), New York (USA). *N. rileyi* was maintained to Sabouraud-maltose agar supplemented with 1% yeast extract (SMAY) 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 were determined using an improved Neubauer chamber and adjusted to 8.7 10^8 spores/Ml.

2.3 *Experimental culture media*

Three experimental culture media were developed:

Medium A: Sabouraud-maltose fortified with 1% yeast extract (SMY) + 3 % (v/v) of the suspension spores of *N. rileyi* ARSEF 1972.

Medium B: SMY + 1 % (w/v) of *S. frugiperda* cuticles (powder) + 3 % (v/v) of the suspension spores of *N. rileyi* ARSEF 1972.

Medium C: SMY + 1 % (w/v) of *S. frugiperda* cuticles (powder)

All culture media (A, B and C) were cultivated for 15 days at 25 °C at 180 rpm on a rotating shaker.

2.4 *Preparation of extracts from different culture media*

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 shows the experimental design of the extracts obtained.

2.5 *Isolation, purification and structural elucidation of fungal metabolites*

The ethyl acetate extract from the supernatant of Medium B (4,5 g) was subjected to silica gel CC (70–230 Mesh) with $CHCl_3$ and increasing amounts of EtOAc (0–100%) and finally MeOH, as eluents, to give 9 fractions of 10 mL each. The fractions III, IV, V and VI were selected for their activity to continue the isolation.

Fr. III (148.7 mg), which eluted with a mixture of $CHCl_3$–EtOAc (85:15), were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH–H_2O 65:35, 1.5 mL min^{-1}) to give compound **1** (58.1 mg, Rt 4 min).

Fr. IV (147.1 mg), which eluted with a mixture of $CHCl_3$–EtOAc (80:20), were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH–H_2O 2:1, 1.5 mL min^{-1}) to give compounds **2** (10.5 mg, Rt 3 min) and **4** (9.6 mg, Rt 11 min).

Fr. V (146.1 mg), which eluted with a mixture of $CHCl_3$–EtOAc (50:50), were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH–H_2O 45:55, 1.5 mL min^{-1}) to give compound **3** (18.2 mg, Rt 3 min).

Fr. VI (132.2 mg), which eluted with a mixture of $CHCl_3$–EtOAc (0:100), were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH–H_2O 50:50, 1.5 mL min^{-1}) to give compound **5** (5.6 mg, Rt 8 min).

The structures these compounds were completely elucidated by using extensive spectroscopic methods and by comparison with data previously reported in the literature (Adamczeski et al., 1995; Pedras et al., 2005; Huang et al., 2010; Yan et al,. 2004; Wang et al., 2010).

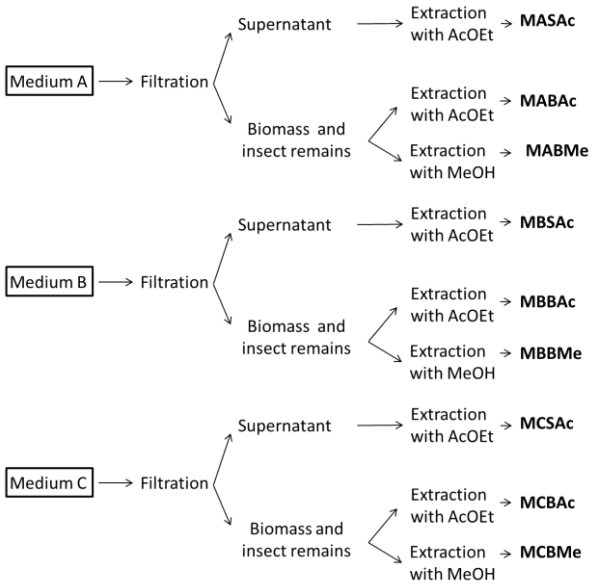

Fig.1: *Ethyl acetate extract from the medium supernatant without insect remains* ***(MASAc)****; Ethyl acetate extract from the medium biomass without insect remains* ***(MABAc)****; Methanolic extract from the medium biomass without insect remains* ***(MABMe)****; Ethyl acetate extract from the medium supernatant supplemented with insect remains* ***(MBSAc)****; Ethyl acetate extract from the medium biomass supplemented with insect remains* ***(MBBAc)****; Methanolic extract from the medium biomass supplemented with insect remains* ***(MBBMe)****, Ethyl acetate extract from the medium supernatant of insect remains* ***(MCSAc)****; Ethyl acetate extract from the medium biomass of insect remains* ***(MCBAc)****; Methanolic extract from the medium biomass of insect remains* ***(MCBMe)****.*

2.6 *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 ° C ± 2 ° C, 60 ± 10% relative humidity and a photoperiod of 12L: 2D.

T. castaneum was collected from flour contaminated by the insect. They were identified taxonomically, through entomological keys and raised in the laboratory under controlled conditions (25 ± 2) ° C and (65 ± 5) HR in brood chamber and fed a diet of self-rising flour.

2.7 *Antifeedant test against S. frugiperda (Choice test and No Choice test)*

http://dx.doi.org/10.22161/ijeab/2.4.18 *ISSN: 2456-1878*

The antifeedant activity was tested based on the methods described by Vera et al., (2008). A portion of artificial diet was mixed with acetone and, after solvent removal *in vacuo*, this portion was employed as control diet. Another portion was mixed with an acetone solution of each treatment, at 300 and 150 µg/ g of diet for extracts and pure respectively. In the no choice test, the same amount of control and treated diets were placed in a different glass tube with a larva inside. Larvae were allowed to eat and, when 50 % of control diet had been eaten, control and treated diets were removed from the tubes and weighted accurately. The experiment was carried out in 20 replicates. To evaluate the feeding behavior a "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.

2.8 *Insecticidal bioassay against S. frugiperda*

Insecticidal bioassay against *S. frugiperda* was investigated based on the methods described by Sosa et al., 2017. The duration (days) of the larval period, percentage the larval and pupal mortality and the number of malformed adults were registered at 300 µg/ g of diet for all the extracts.

2.9 *Effects of extracts on food consumption and utilization*

Ten days after the beginning of the experiment, the larval weight and diet eating were determined again, in order to record the relative consumption rate (RCR), relative growth rate (RGR), efficiency of conversion of ingested food (ECI), were calculated as follows (Farrar et al, 1989; Haouas et al., 2010).

2.10 *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/cm^2 and 25 µg pure compounds/cm^2 were deposited, respectivily. The inhibition of oviposition index (OI) was calculated: OI = [(1 − T/C) × 100]

2.12 *Bioassay food preference and repellency against T. castaneum*

This test was adopted according to the method previously described by Cartagena et al., 2014. The concentrations of extracts and pure compounds were 250 and 125 µg per g of diet, respectively. After 24 hours, count of the individuals present was performed on both diets (T and C) and food preference was assessed by calculating the food Preference Index (PI):

PI= (% ITD - % ICD) / (% ID + %ICD)

Where

% ITD = % insects in the treated diet

% ICD = % insects in the control diet

For PI value between - 1.00 and - 0.10 states that the substance is repellent; PI between - 0.10 and 0.10 + neutral substance is between 0.10 and + 1.00 + substance is attractant (Procopio et al., 2003; Stefanazzi et al., 2006).

The repellency is determined by the Repellency index (RI), where positive values indicate RI repellency and negative values, atractancia (Pascual Villalobos, 1998).

(C-T)/(C+T) x100

Where **C =** Insect in the control diet and **T =** Insect in the treated diet

2.13 *Statistical analysis*

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

III. RESULTS AND DISCUSSION

3.1 *Isolated metabolites*

The use of fungal biological control agents is a rapidly developing field and is increasingly adopted and accepted worldwide in the management of agricultural pests (Jaronski, 2010; Hajek and Delalibera, 2010). In most of the works carried out for the biological control of pests, concentrations of conidia of entomopathogenic fungi are used. There are few bibliographic records of fungal extracts used as insecticides.

N. rileyi, another potential entomopathogenic fungus. It has been shown that many insect species belonging to Lepidoptera, including *S. litura* and some belonging to Coleoptera, are susceptible to *N. rileyi* (Ignoffo, 1981). The host specificity of *N. rileyi* and its ecofriendly nature encourage its use in insect pest management. Wasti and Hartmann, 1978 and Mohamed and Nelson, 1984 have reported the toxic effects of mycelial extracts on *Lymantria dispar*, *Heliothis zea* and *H. virescens* larvae. Onofre in 2002 presented the insecticidal effect of *N. rileyi* on *A. gemmatalis* after ingestion. However, most studies to date have focused on the activity of *N. rileyi* as an insecticide and its use as a control agent, but very little is known about the metabolites responsible for this effect. Prompiboon in 2008 and Supakdamrongkul in 2010 were the first to isolate and identify *N. rileyi* metabolites that were active against *S. litura*.

http://dx.doi.org/10.22161/ijeab/2.4.18 *ISSN: 2456-1878*

In this work, after exhaustive chemical processing of the MBSAc extract (5.0387 g) with a yield of 414.71 mg/L, it was possible to isolate five pure compounds: phenylacetic Acid (**1**), 1-Phenylbuten-2,3-diol (**2**), cycle (Pro-Val) (**3**), cycle (Pro-Leu) (**4**) and cycle (Pro-Phe) (**5**). Compounds 3, 4 and 5 belong to the diketopiperazine family (DKPs) and were reported for the first time in *N. rileyi* (Fig. 2).

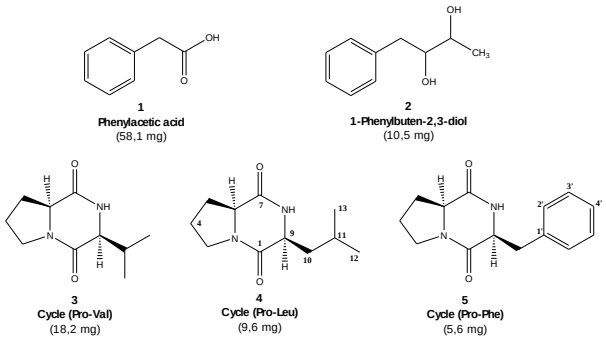

Fig.2: *Compounds isolated from ethyl acetate extract from the medium supernatant supplemented with insect remains* ***(MBSAc)***

Diketopiperazines are naturally occurring cyclic dipeptides, many of which show a wide range of antimicrobial, antiviral, antitumor and immunosuppressive activities (Prasad, 1995). They have been isolated from yeasts, lichens, fungi, bacteria and marine sponges (Mitova et al., 2005) and their skeleton is generated by the cyclization of two L-α-amino acids, so that most naturally occurring DKPs have a cis configuration (Bull et al., 1998).

Santamarina, 2002 and Gimenez, 2000 carried out tests against *Oncopeltus fasciatus* with extracts obtained from different strains, such as *Aspegillus*, *Penicillum*, *Fusarium*, *Alternaria* and *Trichoderma harzianum*, obtaining positive results. *Beauveria bassiana*, is one of the most studied entomopathogenic fungi. It produces metabolites such as bassianin, beauvericin, bassionolide, beauveroiolide, bassacridin, oosporein, and tenellin (Jeffs and Khachatourians, 1997; Quesada-Moraga and Vey, 2004). This would indicate that the production of active metabolites is involved in the antagonistic effect of these agents.

3.2 *Antifeedant activity*

In the Choice Test all the extracts tested (300 µg/g of diet) presented an anti-alimentary effect with FEI values above 42%. When the insects were fed one diet, only extracts supplemented with insect showed intake inhibition but it was significantly lower. However, compounds **2**-**5** isolated from the MBSAc extract were active in both tests (150 µg/g of diet). Diketopiperazines **3** and **5** were the most active in both tests (FEI_{CH} = 86 and FEI_{NCH} 81%) and (FEI_{CH} = 73 and FEI_{NCH} 75%), respectively. Compound **1** was the only one that stimulated ingestion in both tests (Table1).

A higher antifeedant index normally indicates a decreased rate of feeding. An antifeedant is a chemical that inhibits feeding without killing the insect directly, but causing it to die through starvation when it remains near the treated foliage (Yasui et al., 1998; Pavunraj et al., 2012). Antifeedants offer first line of crop protection against notorious insects. Any substance that reduces food consumption by an insect can be considered an antifeedant or feeding deterrent (Isman, 2002). In general, antifeedants have profound adverse effects on insect feeding behavior (Hummelbrunner and Isman, 2001). Antifeedants can be described as allomone substances which inhibit feeding and do not kill the insect pests directly, but rather limit their developmental potential considerably and act as a phagodeterrent or phagorepellent over test as well as permanent insect pests feeding on the plant (Lakshmanan et al., 2012).

3.3 *Insecticidal bioassay against S. frugiperda*

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 lethal and sublethal effects produced by the substances until the insects reached their last stage Table 2. The larval period was not altered by the extracts tested. The AcOEt extracts from the biomass and the supernatants of both culture conditions showed a larval mortality between 10 and 20%. Malformation of adult insects was observed. The MABMe extract showed the moderate percentage of malformation (40%), which would result in a reduction of the viable and fertile population of the insect.

3.4 *Oviposition Deterrent test*

The extracts from the fungus-insect medium were the most active, showing moderate to strong action with deterrence values higher than 50% at 50 µg / cm^2. The highest percentage belonged to MBBAc (OII 79%). Other authors have reported a reduction in the oviposition rate of *C. capitata* in fruits treated with commercial formulations containing conidia of *Beauveria bassiana* (Falchi et al., 2015). They also evaluated the efficacy of the commercial bioinsecticide "Naturalis" based on the *B. bassiana* ATCC 74040 strain against *C. capitata* (Wiedemann) fruit oviposition. In laboratory conditions, females of the Mediterranean fly preferred to place 5 to 3 times more eggs in untreated fruits than in fruits treated with co-formulants of the bioinsecticide (Ortu et al., 2009). Isolates of *Metarhizium anisopliae* and *B. bassiana* were active against adults and pupae of *C.*

http://dx.doi.org/10.22161/ijeab/2.4.18 *ISSN: 2456-1878*

capitata (Lacey et al., 2001; Ortu et al., 2009; Ekesi et al., 2002; 2005; Dimbi et al., 2003; Konstantopoulou and Mazomenos, 2005) while *Paecilomyces fumosoroseus* (Wize) has been shown to reduce fertility and fecundity of the Mediterranean fruit fly (Castillo et al., 2000). However, since there are no reports of insecticidal activity of *N. rileyi* on dipterans, our results would be the first to report on the activity of *N. rileyi* extracts in the inhibition of *C. capitata* oviposition. All compounds tested showed a greater or lesser effect on the inhibition of oviposition of *C. capitata* at the concentration of 25 µg / cm^2 with percentages ranging from 16 to 70%, the most active being 1-phenylbuten-2,3-diol compound (2), followed by 5 and 1. This activity could be attributed to the presence of an aromatic ring, a structural similarity that these compounds possess.

3.5 *Bioassay food preference and repellency against T. castaneum*

When analyzing the results obtained in *T. castaneum*, the medium-insect extracts of *Nomuraea* were found to be repellent. The methanolic extract of the MBBMe biomass proved to be the most active, while the extracts from the control-fungus medium proved to be attractive (Table 4). The activity of *Paecilomyces fumosoroseus* on adults and larvae of *T. confusum* and *B. bassiana* against *Capnodis tenebrionis* (Coleopterus) are reported in previous works (Michalaki et al., 2007; Marannino et al., 2006).

Most accounts of insecticidal activity of *N. rileyi* are mainly on Lepidoptera. However, Ignoffo, 1981 reported its activity on two *Hypera punctata* and *Leptinotarsa decemlineata* beetles. Cartagena, 2014 shows that the addition of *T. castaneum* components (2% w / v) in a culture of the fungus *Aspergillus parasiticus* MOR3 induces the production of insect repellent substances mentioned above. This would suggest the specific biosynthesis of fungal metabolites in order to control the insect. In this work, all compounds tested showed repellent action, the most active being compounds 1 and 3 with RI values of 42 and 41% and PI values of -0.43 and -0.42 respectively, with no mortality at 125 µg / g diet in any of the compounds tested (Table 4).

There is little information on the insecticidal activity of DKPs. Only Cycloechinulin, was reported to be effective in the control of coleoptera and lepidoptera such as *H. zea* and *Carpophilus hemipterus* (de Guzman et al., 1993). Three DKPs isolated from *Eurotium cristatum* with the isopentyl group substituted by indole residues, showed cytotoxic activity against *Artemia salina* (Chinese Patent No: CN102675293 2012; Chinese Pat-ent No: CN102669110, 2012). Recent studies revealed that Cycle (Trp-Phe) exhibited dose-dependent anti-alimentary, larvicidal and pupicidal activity against *H. armigera*. In addition, the purified compound prolonged the larval and pupal period when compared to the untreated control (Sathya et al., 2016).

IV. CONCLUSIONS

Our results are the first account of insecticidal activity of *N rileyi* extracts on three species of insect pests. In addition, isolated dikepiperazines are reported for the first time in this fungus. Although there are papers on the activity of some DPKs on lepidopterans and beetles, no publications were found on the insecticidal activities of the three DPKs studied in this work. Hence, this would be the first study to publish the insecticidal activity of these compounds, besides being the first report on the insecticidal activity of DPKs in Diptera, specifically *Ceratitis capitata*.

These results would be promising for the development of control agents for *S. frugiperda*, *C. capitata* and *T. castañeum*, pests that generate serious economic losses in the northwest of Argentina. In turn, these results show the potential of diketopiperazines as leading structures for the synthesis of new, more potent and environmentally friendly insecticides.

Conflict of interest

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

ACKNOWLEDGMENTS

Work in Argentina was suportet by Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET).

.

REFERENCES

[1] Adamczeski, M., Reed, A.R., Crews, P., 1995. New and known diketopiperazines from the Caribbean sponge, *Calyx cf. podatypa*. J. Nat. Prod. 58, 201– 208.

[2] Asaff, A., Reyes-Vidal, Y., López, E., López, M., 2002. Guerra entre Insectos y microorganismos: una estrategia natural para el control de plagas. Avance y Perspectiva 21, 291−295.

[3] Bull, S.D., Davies, S.G., Parkin, R.M., Sanchez-Sancho, F.F., 1998. The biosynthetic origin of diketopiperazines derived from D-proline. J. Chem. Soc. 1, 2313-2320.

[4] Cartagena, E., Marcinkevicius, K., Luciardi, C., Rodríguez, G., Bardón, A., Arena, M.E., 2014. Activity of a novel compound produced by *Aspergillus* parasiticus in the presence of red flour beetle *Tribolium castaneum*

against *Pseudomonas aeruginosa* and coleopteran insects. J Pest Sci 87, 521-530.

[5] Castillo, M.A., Moya, P., Hernandez, E., Primo-Yufera, E., 2000. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopatogenic fungi and their extracts. Biological Control 19: 274-282.

[6] CN102669110(2012) Institute of Oceanology Chinese Academy of Sciences. Chinese Patent N| CN102669110. The application of an indoly diketopiperazine compound. China Patent and Trademark Office, Shangai, China.

[7] CN102675293(2012) Institute of Oceanology Chinese Academy of Sciences. Chinese Patent N° CN102675293. An indolyl diketopiperazine like derivative, its preparation method and application. China Patent and Trademark Office, Shangai, China.

[8] de Guzman, F.S., Dowd, P.F., Gloer, J.B., Wicklow, D.T., 1993. Cycloechinulin antiinsectan metabolite US5196420 A

[9] Devi, U., Mohan, C., Padmavath, J., Ramesh, K., 2003. Susceptibility to Fungi of Cotton Bollworms before and after a natural epizootic of the Entomopathogenic Fungus *Nomuraea rileyi* (Hyphomycetes). Biocontrol Sci Technol 13, 367-371.

[10] Dimbi, S., Maniana, N.K., Lux, S.A., Ekesi, S., Mueke, J.K., 2003. Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, to three adult fruit fly species: *Ceratitis capitata* (Wiedemann), *C. rosa* var. fasciventris Karsch and *C. cosyra* (Walker) (Diptera: Tephritidae). Mycopathologia 156, 375-382.

[11] Edelstein, J., Lecuona, R., Trumper, E., 2004. Selection of culture media and in vitro assessment of temperature dependent development of *Nomuraea* rileyi. Neotropical Entomology 33, 737-742.

[12] Ekesi, S., Maniania, N.K., Lux, S.A. 2002. Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Science and Technology 12, 7-17.

[13] Ekesi, S., Maniania, N.K., Mohamed, S.A., Lux, S.A., 2005. Effect of soil application of different formulations of *Metarhizium anisopliae* on African tephritid fruit flies and their associated endoparasitoids. Biological Control 35, 83-91.

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

[15] Farrar RR, Barbour JD, Kenedy GG (1989) Quantifying food consumption and growth in insects. Annal. Entomol. Soc. Am. 82: 593-598.

[16] Gilliespie, A.T., Claydon, N., 1989. The use of entomogenous fungi for pest control and the role of toxins in pathogenesis. Pesticida Science 27, 203-215.

[17] Gimenez, A.B., Garcia Breijo, J., Rosello, J., Santamarina, M.P., 2000. Investigacion y detección de fúngicos con actividad bactericida, fungicida e insecticida. Departamento de Biologia vegetal: Escuela Universitaria de ingeniería Tecnica Agricola de la Universidad politécnica de Valencia.

[18] Hajek, A.E., Delalibera, I., 2010. Fungal pathogens as classical biological control agents against arthropods. BioControl 55, 147–158.

[19] Haouas D, Flamini G, Ben Halima-Kamel M, Ben Hamouda MH (2010) Feeding perturbation and toxic activity of five Chrysanthemum species crude extracts against *Spodoptera littoralis* (Boisduval) (Lepidoptera; Noctuidae). Crop Protection 29: 992–997.

[20] Hegedus, D., Khachatourians, G., 1995. Biotechnology Advances 13, 455−490.

[21] Huang, R., Ma, W., Dong, J., Zhou, X., Xu, T., Lee, K.J., Yang, X., Xu, S., Liu, Y., 2010. A New 1,4-Diazepine from South China Sea Marine Sponge Callyspongia Species. Molecules 15, 871-877.

[22] Hummelbrunner, L.A., Isman, M.B., 2001. Acute, sublethal, antifeedant and synergestic effects of monoterpenoid essential oil compounds on the tobacco cutworm *Spodoptera litura* (Lepidoptera: Noctudiae). Journal of Agricultural and Food Chemistry 49, 715-720.

[23] Ignoffo, C.M., 1981. *Nomuraea rileyi* as microbial insecticide. In: Burges HD (ed) Microbial control of pests and plant diseases 1970–1980. Academic Press Inc, London, pp 513–538.

[24] Isman, M.B., 2002. Insect antifeedants. Pesticide Outlook 13, 152-157.

[25] Jaronski, S.T., 2010. Ecological factors in the inundative use of fungal entomopathogens. Biocontrol 55, 159–185.

Jeffs, L.B., Khachatourians, G.G. 1997. Toxic properties of Beauveria pigments on erythrocyte membranes. Toxicon 35: 1351–1356.

[26] Khachatourians, G.G., 1996. Biochemistry and molecular biology of entomopathogenic fungi. In The Mycota VI. Human and Animal Relationships, Howard/Miller eds., Springer-Verlag, Berlin pp 331−364.

[27] Kikuchi, H., Miyagawa, Y., Nakamura, K., Sahashi, Y., Inatomi, S., Oshima, Y., 2004. A novel carbon skeletal trichothecane, tenuipesine A, isolated from an entomopathogenic fungus, Paecilomyces tenuipes. Org Lett 6, 4531–4533.

[28] Konstantopoulou, M.A., Mazomenos, B.E., 2005. Evaluation of *Beauveria bassiana* and *B. brongniartii* strains and four wild type fungal species against adults of

Bactrocera oleae and *Ceratitis capitata*. Biocontrol 50: 293-305.

[29] Kucera, M. and Samsinakova, A., 1968. Toxins of the entomophagous fungus *Beauveria bassiana.* J. Inver. Pathol., 12, 316-320.

[30] Kumar, R., Kumar, A., Shekhar Prasa, C., Kishore Dubey, N., Samant, R. 2008. Insecticidal activity *Aegle marmelos* (L.) Correa essential oil against four stored grain insect pests. Int J Food Saf 10, 39–49

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

[32] Lakshmanan, S., Krishnappa, K., Elumalai, K., 2012. Certain plant essential oils against antifeedant activity of *Spodoptera litura* (Fab.), *Helicoverpa armigera* (Hub.) and *Achaea janata* (Linn.) (Lepidoptera: Noctuidae). International Journal of Current Life Sciences 2, 5-11.

[33] Lee, S., Nakajima, I., Ihara, F., Kinoshita, H., Nihira, T., 2005. Cultivation of entomopathogenic fungi for the search of antibacterial compounds. Mycopathologia 160, 321–325

[34] Marannino, P., Santiago-Álvarez, C., de Lillo, E., Quesada-Moraga, E. 2006. A new bioassay method reveals pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* against early stages of *Capnodis tenebrionis* (Coleoptera; Buprestidae). Journal of Invertebrate Pathology 93, 210–213.

[35] Michalaki, M.P., Athanassiou, C.G., Steenberg, T., Buchelos, C.T. 2007. Effect of *Paecilomyces fumosoroseus* (Wise) Brown and Smith (Ascomycota: Hypocreales) alone or in combination with diatomaceous earth against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Biological Control 40: 280–286.

[36] Mitova, M., Tutito, M.L., Infusini, G., Marino, G., De Rosa, S., 2005. Exocellular peptides from antarctic psychrophile *Pseudoalteromonas haloplanktis*. Marine Biotechnology 7: 523-531.

[37] Mohamed, A.K.A., Nelson, F.R.S., 1984. Toxic effect of *Nomuraea rileyi* extract on Heliothis spp. J Agric Entomol 1, 349–353.

[38] Morales, P., Cemeli, M., Godoy, F., and Salas, B., 2004. A list of Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), host plants based on the records of INIACENIAP Museum of Insects of Agricultural Interest. Entomotropica 19: 51-54.

[39] Murúa, G., 2014. Principal plaga de maíz en el NOA: Spodoptera frugiperda. Resumen preparado para el 2do Taller de Insectos en Maíz, 14 pp.

[40] Onofre, S.B., Gonzalez, R.R., Messias, C.L., Azevedo, J.L., de Barros, N.M., 2002. LC_{50} of the peptide produced by the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson active against third instar larvae of *Anticarsia gemmatalis* (Lep.: Noctuidae). Braz Arch Biol Technol 45, 269–275.

[41] Ortu, S., Cocco, A., Dau, R., 2009. Evaluation of the entomopathogenic fungus *Beauveria bassiana* strain ATCC 74040 for the management of *Ceratitis capitata*. Bulletin of Insectology 62, 245-252.

[42] Padín, S.B., Fusé, C., Urrutia, M.I., Dal Bello, G.M., 2013. Toxicity and repellency of nine m medicinal plants against *Tribolium castaneum* in stored wheat. Bull Insectol 66, 45–49.

Prasad, C., 1995. Bioactive Cyclic Dipeptides. Peptides 16, 151-164.

[43] Pascual Villalobos, M., 1998. Repelencia, inhibición del crecimiento y toxicidad de extractos vegetales en larvas de *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae). Boletín de Sanidad Vegetal Plagas 24, 143-154.

[44] Pavunraj, M., Baskar, K., Ignacimuthu, S., 2012. Efficacy of *Melochia corchorifolia* L. (Sterculiaceae) on feeding behavior of four Lepidoptera pests. International Journal of Agricultural Research 7, 58-68.

[45] Pedras, M.S.C., Yu, Y., Liu, J., Tandron Moya, Y.A., 2005. Metabolites Produced by the Phytopathogenic Fungus *Rhizoctonia solani*: Isolation, Chemical Structure Determination, Syntheses and Bioactivity. Z. Naturforsch. 60c, 717-722.

[46] Procópio, S., Vendramin, J., Ribeiro, J., Santos, J., 2003. Bioactibidade de diversos pós de origen vegetal em relacáo o *Sitophilus seamaiz* Mots (Coleoptera: Curculionidae). Ciencia Agrotécnica 27, 1231-1236.

[47] Prompiboon, P., Bhumiratana, A., Ruchirawat, S., Boucias, D.G., Wiwat, C., 2008. Isolation of ergosterol peroxide from *Nomuraea rileyi* infected larvae of *tobacco cutworm*. World Journal of Microbiology and Biotechnology 24, 2909-2917.

[48] Quesada-Moraga, E., Vey, A., 2004. Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana*. Mycol Res 108, 441–452.

[49] Roberts, D., 1989. World picture of biological control of insects by fungi. Memorias do Instuto Oswaldo Cruz 84, 89−100.

[50] 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. Revista Iberoamericana de Micología 19, 99-103.

[51] Sathya, A., Vijayabharathi, R., Kumari, B.R., Srinivas, V., Sharma, H.C., Palanisamy Sathyadevi, P., Gopalakrishnan, S., 2016. Assessment of a diketopiperazine, cyclo(Trp-Phe) from treptomyces

griseoplanus SAI-25 againt cotton bollworm, Helicoverpa armigera Lepidoptera: Noctuidae). Appl Entomol Zool. 51, 11.

[52] 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*. Journal of chemical ecology 34, 539-548.

[53] Sosa, A., Costa, M., Salvatore, A., Bardón, A., Borkosky, S., Vera, N., 2017. Insecticidal effects of eudesmanes from *Pluchea sagittalis* (Asteraceae) on *Spodoptera frugiperda* and *Ceratitis capitata*. International Journal of Environment, Agriculture and Biotechnology 2: 361-369.

[54] Stefanazzi, N., Stadler, T., Ferrero, A., 2011. Repellent and feeding deterrent activity of essential oils against the stored–grain pests *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). Pest Manag Sci 67, 639–646.

[55] Stefanazzi, N., Gutierrez, M., Stadler, T., Bonini, A., Ferrero, A., 2006. Actividad biológica del aceite esencial de *Tagetes terniflora* Kunth (Ateraceae) en *Tribolium castaneum* Herbst (Insecto, Coleoptera, Tenebrionidae). Boletín de Sanidad Vegetal Plagas 32: 439-447.

[56] Supakdamrongkul, P., Bhumiratana, A., Wiwat, C. 2010. Characterization of an extracellular lipase from the biocontrol fungus, *Nomuraea rileyi* MJ, and its toxicity toward *Spodoptera litura*. J Invertebr Pathol., 105: 228-35.

[57] Vera N, Misico R, Gonzalez M, Asakawa Y, Bardon A (2008) Eudesmanes from *Pluchea sagittalis*. Their antifeedant activity on *Spodoptera frugiperda*. Phytochemistry 69: 1689–1694.

[58] Wang, G., Dai, S., Chen, M., Wu, H., Xie, L., Luo, X., Li, X., 2010. Two diketopiperazine cyclo(pro-phe) isomers from marine bacteria *Bacillus subtilis* sp. 13-2. Chem Nat Compd 46, 583.

[59] Wasti, S.S., Hartmann, G.C., 1978. Host-parasite interactions between larvae of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantridae) and the entomogenous fungus, *Nomuraea rileyi* (Farlow) Samson (Monilials: Moniliaceae). Appl Entomol Zool (Jpn) 1323–28.

[60] Willink, E.; Costilla, M.; Osores, V., 1990. Principales plagas del maíz: Daños, pérdidas y recomendaciones para la siembra. Avance Agroindustrial 42, 17-19.

[61] Wyckhuys, K.A., O'Neil, R.J., 2006. Population dynamics of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) and associated arthropod natural enemies in Honduran subsistence maize. Crop Protection 25, 1180-1190.

[62] Yan, P., Song, Y., Sakuno, E., Nakajima, H., Nakagawa, H., Yabe, K., 2004. Cyclo (L -Leucyl- L -Prolyl) Produced by *Achromobacter xylosoxidans* Inhibits Aflatoxin Production by *Aspergillus parasiticus*. Applied and Environmental Microbiology 70, 7466–7473.

[63] Yasui, H., Kato, A., Yazawa, M., 1998. Antifeedants to armyworm, *Spodoptera litura* and *Pseudaletia separata*, from bitter gourd leaves, *Momordica charantia*. Journal of Chemical Ecology 24, 803-813.

[64] Ye, M.Z., Han, G.Y., Fu, C.L., Bao, J.R., 1993. Insecticidal toxin produced by the entomogenous fungus *Nomuraea rileyi*. Acta Agric. Univ. Zheijian., 19, 76-79.

http://dx.doi.org/10.22161/ijeab/2.4.18 *ISSN: 2456-1878*

Table.1: Antifeedant activity of extracts and pure compounds of culture from N. rileyi ARSEF 1972 on S. frugiperda

		FEEDING DETERRENCE INDEX	
		FEI_{CH} Choice test (%)	FEI_{NCH} No choice test (%)
Extracts (300 µg/g)	MASAc	73.92 ± 7.91a	-7.41 ± 0.71a
	MABAc	69.92 ± 11.73a	-21.73 ± 2.15b
	MABMe	63.51 ± 12.60a	-34,32 ± 8,63c
	MBSAc	61.70 ± 17.11a	21.41 ± 4.22d
	MBBAc	75.28 ± 13.56a	22.30 ± 4.93d
	MBBMe	42.43 ± 17.56b	10.13 ± 3.81e
Compounds (150 µg/g)	1	-24,39 ± 4,04a	-20,04 ± 5,24a
	2	58,91 ± 8,58b	65,64 ± 8,02b
	3	86,02 ± 7,92c	81,66 ± 6,61b
	4	49,85±5,79b	42,74±6,78c
	5	73,47±7,57c	75,21±6,91b

Feeding election index ± SEM (n = 20). * The values in a column with the same letter do not present significant differences (P> 0.05, Tukey Multiple Range Test).* FEI (%) = Feeding election index = [(1 − T/C) × 100], where C and T represent the amount of control and treated diets, respectively, consumed during the test.

Table.2: Effect of N. rileyi ARSEF 1972 culture extracts, incorporated in an artificial diet, on larval development of S. frugiperda larvae, pupae and adult insects.

	PHYSIOLOGICAL INDEX			LETHAL AND SUB-LETHAL EFFECTS				
Extracts (300 µg/g)	RCR (mg/mg/days)*	RGR (mg/mg/days*	ECI (%)*	Larvae period (days)*	Pupae period (days)*	Larvae Mortality (%)	Pupae Mortality (%)	Malformation Adults (%)
Control	**0.51±0.06a**	**0.25±0.03a**	**21.11±2.91a**	**15.41±1.41a**	**11.15±1.21a**	**5**	**-**	**5**
MASAc	0.59±0.05b	0.18±0.01b	31.63±3.72b	15.13±2.61a	10.31±1.32a	11	-	23
MABAc	0.57±0.06b	0.18±0.01b	32.23±3.51b	14.11±1.31a	10.62±1.61a	20	-	14
MABMe	0.52±0.08a	0.19±0.01b	31.14±4.40b	15.92±1.13a	10.42±1.44a	-	17	40
MBSAc	0.59±0.04b	0.23±0.02c	37.40±4.51b	13.32±1.02a	11.73±1.24a	20	5	20
MBBAc	0.49±0.04a	0.22±0.01c	32.21±6.43b	14.51±1.33a	11.61±1.24a	10	25	20
MBBMe	0.58±0.04b	0.23±0.01c	37.11±3.15b	13.72±1.22a	11.62±1.23a	5	-	35

ECI: Efficiency of conversion of ingested food; **RGR**: Relative growth rate. **RCR**: Relative consumption rate.
*Values in a column with the same letter do not show significant differences (P> 0.05, according to Tukey's Multiple Range Test). The values in the columns represent the mean ± SEM (n = 20).

http://dx.doi.org/10.22161/ijeab/2.4.18 *ISSN: 2456-1878*

Table.3: *Effect of culture extracts and compounds from N. rileyi ARSEF 1972 on the oviposition behavior of C. capitata*

		OVIPOSITION INHIBITION		
		Number of Eggs Laid on the Treated Fruit*	Number of Eggs Laid on the Control Fruit*	OII (%)*
Extracts (50 µg/cm^2)	MASAc	207.33 ± 12.06c	501.33 ± 20.13c	58.63 ± 2.17a
	MABAc	252.00 ± 28.00d	437.33 ± 22.03 d	42.26 ± 7.47c
	MABMe	148.00 ± 7.21e	404.00 ± 14.42d	63.30 ± 2.92a
	MBSAc	98.00 ± 9.17a	269.33 ± 34.02a	63.14 ± 6.29a
	MBBAc	45.00 ± 4.24b	216.00 ± 0.00b	79.17 ± 1.96b
	MBBMe	113.33 ± 6.11a	320.00 ± 53.81a	64.11 ± 4.51a
Compounds (25 µg/cm^2)	1	117.33± 8.33a	208.00± 22.27a	43.43± 2.79a
	2	61.00± 1.41b	248.00± 5.66b	75.39± 1.13b
	3	180.00± 8.00c	214.67± 8.33a	16.13± 2.70d
	4	174.67±6.11c	248.00± 6.93b	29.57± 1.64c
	5	56.67± 3.06b	189.33± 8.33a	70.05± 1.60e

The values in the columns represent the mean ± SEM (n=3).*Values in a column with the same letter do not show significant differences (P> 0.05, according to Tukey's Multiple Range Test). OI (%) = Inhibition of oviposition index = [(1 − T/C) × 100], where C and T represent the amount of eggs in the treated and control oviposition substrate respectively.

Table.4: *Effect of the extracts and compounds of N. rileyi ARSEF 1972 on the behavior of T. castaneum*

		FOOD PREFERENCE AND REPELLENCY INDICES		
		RI (%)*[a]	PI (%)*[b]	
Extracts (250 µg/g)	MASAc	-27,56 ±2.61d	0.28±0.02d	attractive
	MABAc	-26.39 ±1.96d	0.26±0.03d	attractive
	MABMe	-27.16 ±4.01d	0.27±0.04d	attractive
	MBSAc	30.00 ±7.07a	-0.30±0.07a	repellency
	MBBAc	17.75 ±4.90b	-0.18±0.05a	repellency
	MBBMc	50.35 ±5.93c	-0.50±0.06c	repellency
Compounds (125 µg/g)	1	42.50±3.49a	-0.43±0.04a	repellency
	2	32.50±3.50b	-0.33±0.04b	repellency
	3	41.79±2.54a	-0.42±0.02a	repellency
	4	27.50±3.54b	-0.28±0.04b	repellency
	5	17.50±3.54c	-0.18±0.04c	repellency

The values in the columns represent the mean ± SEM (n=3).*Values in a column with the same letter do not show significant differences (P> 0.05, according to Tukey's Multiple Range Test). [a]Positive values indicate repellency. [b]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.