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Short Communication

Potential insecticidal toxins extracted from some isolates of *Akanthomyces*

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Abstract

Entomopathogenic fungi (EPF) are key elements for pest management in sustainable agriculture. Many EPFs can produce secondary metabolites with toxic effects, playing an essential role in their pathogenicity. In this study, the metabolites of some EPFs, including three isolates of *Akanthomyces lecanii* species (PAL6, PAL7, and PAL8) and one isolate of *A. muscarius* species (AGM5), were biochemically analyzed. Extracellular metabolites were isolated from the fungal cells of the two-week-old culture of each isolate, and the chemical constituents were identified by high-performance liquid chromatography (HPLC). During the retention interval of 10-13 minutes, four different peaks were observed within metabolites of the isolates PAL6, PAL7, and PAL8. In the case of the AGM5 isolate; however, one peak was observed at 11.53 retention time. Comparative analysis showed the presence of insecticidal toxic cyclic peptides such as bassianolide within the fungal metabolites. Moreover, one peak with a new identity was detected within the metabolites from the fungal isolates of PAL6, PAL7, and PAL8 in the retention interval of 5 minutes from the isolate AGM5 at 11.53 retention times. The results demonstrated that producing toxic compounds such as bassianolide by the EPF probably contributed to their insecticidal effects.

Keywords: Mycotoxins; cyclic peptides; bioinsecticide; entomopathogenic fungi

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Introduction

Many entomopathogenic fungi (EPFs), as the most important microbial control agents of insect pests, systematically belong to the phylum Ascomycota, order Hypocreales, and family Cordycipitaceae (Litwin et al., 2020). The genus *Akanthomyces* is an important EPF from the family Cordycipitaceae (Ascomycota, Hypocreales), infecting spiders and insects in various ecosystems (Aini et al., 2020). The genus that was first established by Lebert (1858) can parasitize many insect orders, including Hemiptera, Coleoptera, Lepidoptera and Orthoptera (Mongkolsamrit et al., 2018). Among the genera, *A. lecanii* Zimmerman and *A. muscarius* Petch are two important and widespread EPFs of hemipteran pests (Broumandnia et al., 2021). Three Iranian-originated isolates of *A. lecanii* (PAL6, PAL7, and PAL8) and one isolate of *A. muscarius* (AGM5) were previously isolated from hemipteran citrus pests, *Pulvinaria aurantii* Cock. (Hemiptera: Coccidae) and *Aphis gossypii* Glover (Hemiptera: Aphididae). The appropriate pathogenicity of the isolates against *Bemisia tabaci* Gen. (Hem., Aleyrodidae) (Broumandnia et al., 2021), *Tribolium castaneum* Herbst (Col., Tenebrionidae) (Broumandnia & Rajabpour, 2020) and *Aphis fabae* Scopoli (Hemiptera: Aphididae) (Soltani et al., 2022) have been previously reported.

Similar to other EPFs, *Akanthomyces* fungi can produce some secondary metabolites that are considered mycotoxins. Some metabolites have considerable insecticidal effects and lead to higher and faster insect host mortality in comparison with nontoxigenic fungi (Wang et al., 2018). Furthermore, the antifeedant properties of some active metabolites of EPF have been documented (Berestetskiy & Hu, 2021). In some cases, metabolites induce host plant systematic resistance to sap-feeding plants, e.g., whiteflies, by triggering related genes (Abdulle et al., 2021). In total, secondary metabolites play important roles in host plant resistance against insect pests. Some bioinsecticides have been developed based on mycotoxin constituents derived from EPFs, e.g., beauvericin from *Beauveria bassiana* Balsamo and cytochalasin C from *Metarhizium anisopliae* Metschn. and *Tolypocladium inflatum* Gams (Boguś et al., 2021). Due to many problems and limitations associated with chemical insecticides, bioinsecticides can play an important role in the eco-friendly control of insect pests for developing integrated pest management (IPM) programs. Determinations of mycotoxin constituents are the basic information before bioinsecticide production. The chemical compounds of some mycotoxins derived from *Acanthomyces* fungi were previously studied. For instance, constituents of bioactive metabolites extracted from mycelia of *A. evansii* comprised phenolic compounds (terphenylin, deoxy terphenylin, terprenin 2, terprenin epoxide), bipeptide (cyclo-tyrosylprolyl), and simple aromatic compounds (acetyl hydroxybenzamide, 4-hydroxybenzaldehyde) (Effendi, 2012). It has been reported that many of the bioactive metabolites derived from *Akanthomyces* act as cuticle-degrading proteases to facilitate penetration of EPF mycelia (Keppanan et al., 2019). This study aimed to detect the insecticidal bioactive compounds derived from pathogenic isolates of *Akanthomyces*.

Materials and methods

Fungal culture and maintenance

Different isolates of pathogenic fungi from *P. aurantii* and *A. gossypii* were isolated in citrus orchards of northern Iran, 36°54'24.2"N 50°39'26.7"E, and their pathogenicity was confirmed against *T. castaneum* (Broumandnia and Rajabpour, 2020), *B. tabaci* (Broumandnia et al., 2021) and *A. fabae* (Soltani et al., 2022). Morphological and molecular investigations have indicated that EPFs belong to three fungal isolates of *A. lecanii* species (PAL6, PAL7 and PAL8) and one fungal isolate of *A. muscarius* species (AGM5) (Broumandnia and Rajabpour, 2020; Broumandnia et al., 2021). The isolates were cultured on potato dextrose agar (PDA)

medium in a germinator with a temperature of 23±1°C, relative humidity of 43%, and 16:8 light: dark hours.

Preparation of crude secondary metabolites

Mycelia from the two-week-old culture of the fungi were harvested and isolated using centrifugation at 1000 *g*. The bioactive compounds were isolated from the fungi after an incubation period of two weeks. Extracellular metabolites were isolated from the fungal cells using methanol:chloroform (1:2, v/v). Then, the mixture was centrifuged at 1000 *g* to remove fatty acids. The organic phase was separated and concentrated in a vacuum rotary evaporator. Relatively purified insecticidal toxic compounds were recovered and analyzed.

High-performance liquid chromatography (HPLC)

The crude extract of the fungal isolates was dissolved in methanol-acetonitrile solution. The mobile phase was run in a K-60 silica gel column (230–400 mesh, 325 240 mm, Darmstadt, Germany) of chromatography machine (KNAUER, Germany) and started step by step in 50-mm methylene. First, methylene:methanol-dichloride (95:5) was used, and the slope of this solvent was used to achieve the desired polarities. The prepared sample was diluted with methanol and analyzed using a reversed-phase HPLC column, and the peaks were detected by absorbing ultraviolet light at a wavelength of 240 nm. A typical slope of the thigh was used as follows: 0 minutes (0% acetonitrile), 30 minutes (40% acetonitrile), 40 minutes (50% acetonitrile), and 60 minutes (50% acetonitrile).

Results and discussion

Fungal metabolites were subjected to HPLC analysis by a reversed-phase C18 column, and the compounds were identified. The analysis report showed the presence of the insecticide's toxic cyclic peptide, e.g., bassianolide. Among the retention times of 10-13 minutes, four different peaks were observed for isolates PAL6, PAL7 and PAL8 (Figure 1).

In this way, these peaks in the sample obtained from the PAL6 isolate at retention times of 10.85, 11.97 and 12.35, the samples belonging to PAL7 at retention times of 11.12, 12.27 and 12.65, and samples related to PAL8 appeared at retention times of 10.53, 11.53 and 11.87. Three peaks with a new identity were detected within the metabolites from the fungal isolates of PAL6, PAL7 and PAL8 at a retention time of 5 minutes. In addition, other peaks were also observed in the chromatograms of these samples, which were not considered due to the time interval from the target intervals. In the case of the GMA5 isolate, however, one peak was observed at 11.53 retention time. In addition, a peak with high intensity also appeared at a retention time of 5.48. Comparison of the peaks with those of insecticidal compounds (Ravindran et al., 2018) revealed the presence of toxic compounds within the metabolites of tested isolates. However, the low intensity of the peaks reflects the relatively low concentration of these compounds. Furthermore, the peaks observed at 5 minutes, especially for the GMA5 isolate, can indicate the presence of a new compound in the fungal metabolites of these isolates, which requires additional tests in this regard.

EPF secrete various nonvital compounds that are key elements for infection to infect their hosts (Vey et al., 2001). Destruxins (A, B, C, and E) are a major group of compounds identified from *M. anisopliae* (Ravindran et al., 2016). *Akanthomyces* spp. are famous EPFs used for pest biocontrol, and their insecticidal metabolites have been applied against various insects (Butt et al., 1994; Isaka et al., 2005; Molnar et al., 2010). Among the genera, *A. lecanii* is an EPF that has been well studied as a microbial biocontrol agent, and the compounds derived from the fungus have appropriate toxic effects and have been applied against various arthropodan pests (Butt et al., 1994; Isaka et al., 2005; Molnar et al., 2010). Many compounds with insecticidal properties produced by the pathogen are cyclic peptides (Ravindran et al., 2018). Bassianolide is a toxic compound that has been identified within

A. lecanii metabolites (Ravindran et al., 2018). Bassianolide disrupts insect smooth muscle contraction by inhibiting the acetylcholine mechanism. Furthermore, the compound has cytotoxic, moderate antiplasmodial and antimycobacterial activities (Jirakkakul et al., 2008). In vitro studies indicated that bassianolide significantly induces the virulence of EPF, e.g., *B. bassiana*, to the larvae of *Helicoverpa zea* Boddie (Lepidoptera; Noctuidae) and *Bombyx mori* L. (Lepidoptera: Bombycidae). Therefore, the compound serves as a bona fide virulence factor of the EPF, significantly contributing to the commercial microbiological insecticide preparation containing the fungus conidia (Singh et al., 2015). Similarly, bassianolide was detected within metabolites of Iranian isolates of *A. lecanii* and *A. muscarius*. EPF usage against arthropodian hosts makes them more vulnerable to pathogen reinvasion. However, knowledge about the mechanisms of insect host defense and its specific bioresponse when it is exposed to metabolites is needed (Wu et al., 2016). The crude extract from the Iranian isolates of *A. lecanii* and *A. muscarius* showed the presence of two major and four minor peaks (Figures 1), which are characteristic of bassianolide. Previous studies have also characterized the presence of cyclic peptides such as beauvericin, bassianolide and DXTs from EPFs such as *B. bassiana* and *M. anisopliae* using HPLC analysis (Kershaw et al., 1999; Liu et al., 2009; Namara et al., 2017; Ravindran et al., 2016; Sowjanya Sree et al., 2008).

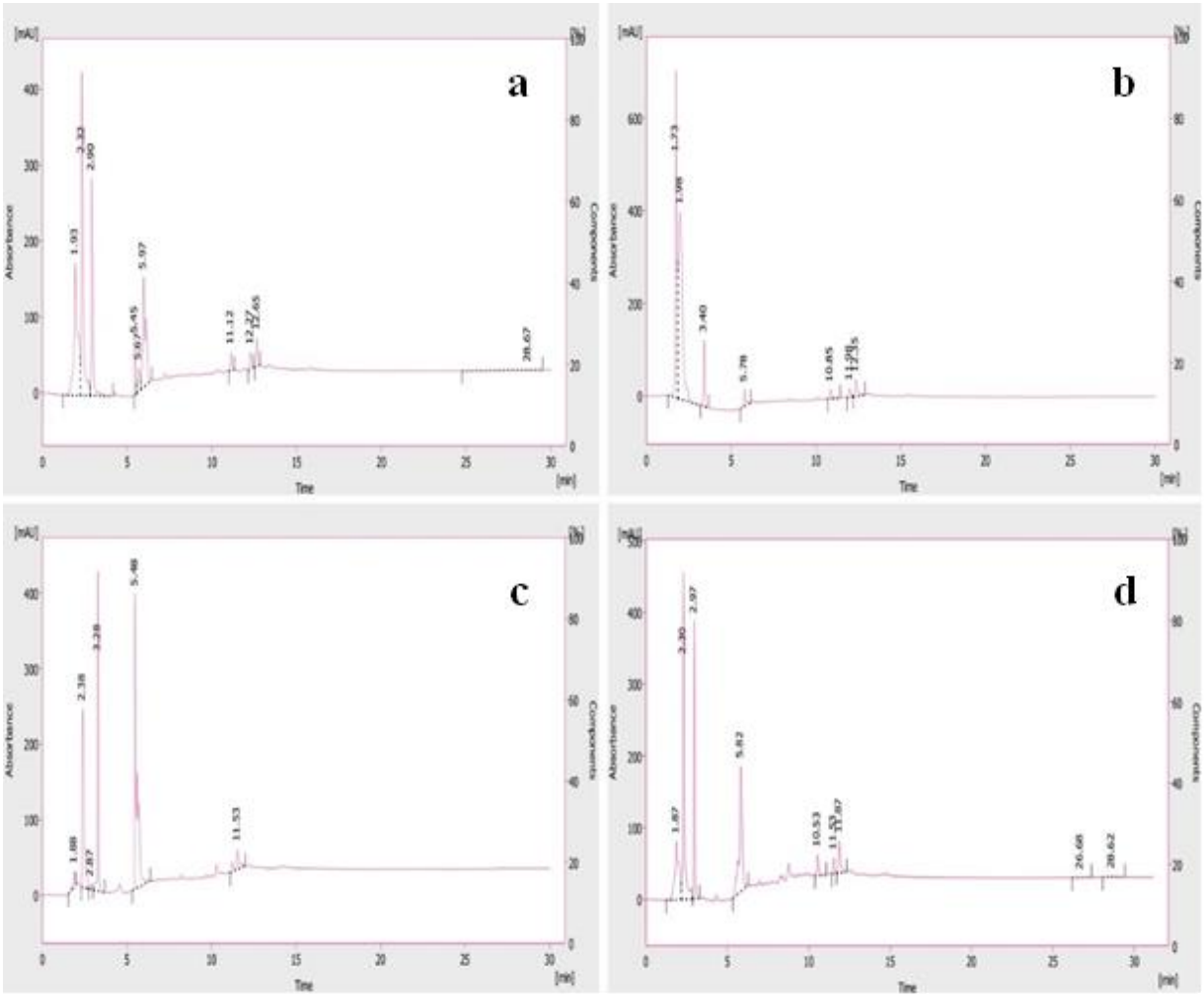

Figure 1. Chromatograms of metabolites of *Akanthomyces* isolates, including PAL7 (a), PAL6 (b) GMA5 (c) and PAL8 (d), using HPLC.

Our results demonstrated that bassianolide probably contributes to the insecticidal effects of *A. lecanii A. muscarius* against pests such as *T. castaneum* (Broumandnia & Rajabpour, 2020), *B. tabaci* (Broumandnia et al., 2021) and *A. fabae* (Soltani et al., 2022) and can be a promising source of toxic metabolites against other pests.

Conclusion

Both EPFs, *A. lecanii* and *A. muscarius*, are well-known EPFs that have been applied against several insect pests. They can produce secondary metabolites with toxic effects by which they fulfill their pathogenicity process. HPLC analysis of the metabolites of the EPF showed the presence of insecticidal toxic cyclic peptides such as bassianolide within the fungal metabolites. Moreover, one peak with a new identity was detected within the metabolites from the EPF. These toxic compounds are probably responsible for the insecticidal effects of the EPF.

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گزارش کوتاه انگلیسی

سموم حشره‌کش بالقوه مستخرج از برخی جدایه‌های قارچ‌های جنس *Akanthomyces*

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چکیده

قارچ‌های بیمارگر حشرات عوامل کلیدی برای مدیریت آفات در کشاورزی پایدار می‌باشند. بسیاری از قارچ‌های بیمارگر حشرات می‌توانند متابولیت‌های ثانویه‌ای را تولید نمایند که دارای اثرات سمی هستند و در بیماری‌زایی آن‌ها نقشی اساسی دارند. در این مطالعه، این متابولیت‌ها در قارچ‌های بیمارگر حشرات شامل گونه *Akanthomyces lecanii* (جدایه‌های PAL6، PAL7 و PAL8) و گونه *A. muscarius* (جدایه AGM5) از نظر بیوشیمیایی با استفاده از کروماتوگرافی مایع با کارایی بالا (HPLC) مورد تجزیه و تحلیل قرار گرفتند. در میان زمان‌های مختلف بازداری مورد ثبت بین ۱-۱۳ دقیقه، چهار پیک مختلف در بین جدایه‌های PAL6، PAL7 و PAL8 مشاهده شد. در مورد جدایه GMA5، تنها یک پیک در زمان بازدارندگی ۱۱/۵۳ ثانیه دیده شد. تحلیل مقایسه‌ای نتایج نشان‌دهنده حضور پپتیدهای حلقوی سمی مانند Bassianolide در متابولیت‌های جدایه‌های PAL6، PAL7 و PAL8 در زمان بازدارندگی 5 دقیقه و برای جدایه AGM5 در زمان بازدارندگی ۱۱/۵۳ دقیقه بود. این نتایج بیانگر تولید ترکیباتی سمی مانند Bassianolide توسط این قارچ‌های بیمارگر حشرات هستند که در ماهیت حشره‌کشی آن‌ها احتمالاً دخیل می‌باشند.

کلیدواژه‌ها: سموم قارچی، پپتیدهای حلقوی، حشره‌کش‌های زیستی، قارچ‌های بیمارگر حشرات

دبیر تخصصی: دکتر محسن یزدانیان

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