International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 20-11-2021, Accepted: 06-12-2021, Published: 21-12-2021
Volume 6, Issue 6, 2021, Page No. 275-281

The endophytic fungus, *Alternaria tenuissima* isolated from the leaves of common castor bean *Ricinus communis* L. as biocontrol tool against *Locusta migratoria* L

Laib Djamel Eddine[1, 2*], Benzara Abdelmadjid[2], Boulaouad belkacem Aimene[3], Akkal Salah[4]

[1] Department of Agronomy, University of 20 august 1955, Skikda, Algeria

[2] Department of Agriculture and Forestry Zoology, National Agronomic Institute, El-Harrach Algiers, Algeria

[3] University of Bordj Bou Arreridj, El Anceur, Algeria

[4] Department of Chemistry, Valorization of Natural Resources, Bioactive Molecules and Biological Analysis Unit, University of Mentouri Constantine 1, Constantine, Algeria

Abstract

The present study aimed to investigate the insecticidal activity of the endophytic fungus, *A. tenuissima*, isolated from the leaves of common castor bean *R. communis*. To evaluate the insecticidal activity, ten concentrations (0. 2 to 2 g/L) of the fungal extract were applied by contact and ingestion against *L. migratoria.*

The results showed that the fungal extract had variable insecticidal activities that depended on the concentration used and time after treatment. The most effective concentration against the insects was 2 g/L, with a maximum mortality rates of 73, 91 % and 100 % for *L. migratoria* upon contact and ingestion treatments, respectively.

The LC50 values indicated that the fungal extract was toxic against the insects with LC50 values ranging from 0, 56 to 1, 39 for *L. migratoria* contact treatment and from 0, 803 to1, 321 for *L. migratoria* ingestion treatment.

Chemical analysis of the fungal extract revealed the presence of polyphenols, alkaloids, terpenoids**,** saponins, and tannins as the metabolites responsible for these activities. Attenuated total reflection-Fourier transform infrared analysis of the fungal extract revealed the presence of the presence of sulfone, nitro, imine, enol ether, alkyne, and hydroxyl groups.

Specifically, GC-MS-MS analysis revealed the presence of many insecticidal volatiles for both fungal extract and colony.

Keywords: insecticidal activity, *L. migratoria*, endophytic fungi, *Alternaria tenuissima*

Introduction

Many insects are responsible for significant crop losses worldwide (Rahman *et al*., 2018) [20]. Among them, the migratory locust, *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae), whose gregarious phase is under the influence of population density, is a bio-aggressor with a polyphagous diet (Le Gall *et al*., 2019) [15] ample ecological plasticity, and a large geographical distribution, all of which lead to the formation of spectacular swarms responsible for considerable crop damage (Sangbaramou *et al*., 2018). Conventional control methods against the aforementioned pest predominantly consist of the application of considerable amounts of chemical pesticides (Shuping & Eloff, 2017; Zhang & lecoq, 2021) [26, 35]. These chemicals are cost-effective and efficient, but their excessive use has created severe problems, such as resistance, environmental pollution, and adverse effects on human health (Shuping & Eloff, 2017; Berestetskiy & Hu, 2021) [26, 35]. Therefore, finding alternatives that show an acceptable degree of effectiveness, given the requirements dictated by the market and consumer preference as well as government regulations for crop protection without disregarding environmental and human health hazards while ensuring economically efficient agriculture, has become an issue of predominant importance (Zhang & Lecoq, 2021) [35]. Among such alternatives, endophytic fungi that colonize the tissues of their host plants without apparent symptoms of infection provide an attractive and promising option for plant protection against insect pests and phytopathogenic fungi due to their production of bioactive secondary metabolites belonging to various structural groups with unusual chemical diversity and a broad spectrum of biological activities (Yan *et al*., 2018; Toghueo, 2019;Kaur, 2020) [31, 29, 12]. Accordingly, the present study aimed to investigate the insecticidal activity of the extract obtained from the endophytic fungus, *A. tenuissima*, isolated from the leaves of common castor bean *R. communis* against *L. migratoria*.

Material and Methods

Biological material

Leaves of *R. communis* were collected with a scalpel from five healthy plants within a natural vegetation community in the region of Beni Bechir, Skikda, Algeria (36º46'20"N; 6º57'30"E) and transported to the laboratory in February 2019.

L. migratoria is a potential pest of many crops in Algeria, and these insects were collected in August 2019 from the same region as was *R. communis.*

Insects breeding

Insects were bred under laboratory conditions to maintain a sufficient number of *L. migratoria* individuals to conduct our experiments. As described by Ouedraogo *et al.* (2002) [19], *L. Migratoria* individuals were bred in a wooden breeding cage (50 cm^3) covered with a tulle to ensure aeration and avoid moisture condensation (maximum 50–60% humidity), which causes epizootics in *L. migratoria*, and provided a sliding door to allow the various necessary

manipulations. The bottom of the cage consisted of nesting boxes filled with peat (~66%) and river sand (~33%); these materials were sterilized and humidified to 10% moisture content to allow the recovery of oothecae. *L. migratoria* individuals were fed fresh wheat leaves. Lighting and heat (30±1°C) in the cage were provided continuously for 12 h/day using a 60-watt incandescent bulb, which allowed the insects to thermoregulate and attain their optimal temperature for molting and laying eggs.

Submerged fermentation and production of the fungal extract.

This step was conducted following a previous method (Dolatabad *et al*, 2017) [5] with slight modifications. Agar discs with associated mycelium of the endophytic fungus were placed in 250 ml flasks containing 50 ml of PDB medium and closed to disallow contamination. The cultures were incubated for 21 days at 25±2°C and shaken back and forth each hour on a mechanical shaker. After 21 days, the mycelia of the endophytic fungus were separated from the medium by filtration through Whatman No. 1 filter paper. The filtrate was mixed for 15 minutes with an equal volume of ethyl acetate and let to stand for 10 minutes to separate it into the two immiscible organic and aqueous layers. Then the ethyl acetate was evaporated employing a rotary evaporator. The concentrated fungal extract was subjected to freeze-drying in a lyophilizer (Virtis) till dry powder was acquired. Each 1 mg of the fungal extract was dissolved in 1 ml of methanol followed by filtration through Varian Bond Elute C18 solid-phase extraction to remove impurities.

Insecticidal activity of the fungal extract

Direct spray and forced ingestion treatments were applied to adults of *L. migratoria* at a rate of five insects per plastic box. Ten doses of fungal extracts (0. 2;0. 3;0. 4;0, 5;0, 6;0, 7;0, 8;1;1, 2;1, 4;1, 6;1, 8 and 2g/L) were employed, including five replicates per concentration and five replicates that were treated with distilled water, as controls. The mortality rate (in percentage) was calculated for each treatment after during 7 days and corrected using Abbott's formula Abbott (1925) [1].

$$\text{Corrected Mortality (\%)} = \frac{\text{Test mortality (\%)} - \text{Control mortality(\%)}}{100 - \text{Control mortality (\%)}}$$

Chemical analyses of the fungal extract

Detection of the major classic of metabolites was conducted using previously described techniques by Harborne (1998) [10]. Extract was dissolved individually in dilute HCL and filtered then treated with K_2HgI_4. The formation of a yellow colored precipitate demonstrated the presence of alkaloids. For the detection of flavonoids, the extract was treated with a few drops of NaOH solution. The formation of intense yellow color, which became colorless upon the addition of dilute HCL, demonstrated the presence of flavonoids. For the detection of phenols the fungal extract was dissolved in 5 ml of distilled H_2O and a few drops of 5% $FeCl_3$ solution were added. A dark green color indicated the presence of phenolic compounds. For the saponins the fungal extract was vigorously shaken with distilled H_2O and was then allowed to stand for 10 minutes, formation of a fairly stable emulsion indicated the presence of saponins. For detection of tannins, the crude fungal extract was treated with an alcoholic $FeCl_3$ reagent, and a bluish-black color that disappeared upon the addition of a little dilute H_2SO_4 followed by the formation of a yellowish-brown precipitate indicated the presence of tannins.

Attenuated total reflection (ATR)-Fourier transform infrared (FTIR) spectroscopy analysis of the fungal extract samples.

Samples (1mg) of the fungal extract were analyzed by ATR-FTIR spectroscopy using a Cary-630 series FTIR spectrometer (Agilent Technologies, Santa Clara, California) with an ATR (MIRacle™ single reflection ATR) configured with a diamond (Di) IRE plate (PIKE Technologies, Fitchburg, Wisconsin). For data acquisition, eight scans were performed between 4000 and 650cm^{-1}, with a resolution of 4 cm^{-1}. The background spectrum was automatically subtracted using the Resolution Pro version 5. 0 software (Agilent Technologies) for each sample spectrum (Sala *et al.*, 2020) [21].

Analysis of volatile components of the fungal extract

GC-MS analysis of the volatile components of the fungal extract was carried out using a Shimadzu GC-MS-QP2020 NX apparatus coupled with a fused silica capillary column. The GC-MS instrument was employed as previously illustrated by Senthilkumar *et al*. (2014) [23].

The instrument was put at an initial temperature of 110 ºC and held at this temperature for 2 minutes. Next, the temperature was gradually increased to 280 °C at a rate of 5 °C/min and kept at that point for 9 min; the temperature of the injection port was kept at 250 °C and the helium carrier gas flow rate was set at 1 mL/min. Finally, the ionization voltage was set to 70 eV before 1 µL of each sample/1mL of methanol was injected, and the mass spectrum scan range was set to 45-450 (m/z). The generated GC-MS mass spectrum data were interpreted using the National Institute of Standards and Technology (NIST) database. The name, molecular weight, and molecular formula of the volatile components were determined.

Analysis of volatile components of the fungal colony

The analysis of the volatile components produced by the fungal hyphae of the endophytic fungus *A. tenuissima* was carried out using a previously described technique by Fiers *et al*. (2013) [9]. 50µL of a fungal spores solution ($2X10^7$ spores / mL) was placed in 20 mL sloped solid -phase micro-extraction (SPME) vials containing 7 mL of PDA medium, closed to disallow contamination and incubated in an oven at 23 ° C. Each micro-extraction vial was placed before collection in a 25 ° C water bath for 10 minutes to concentrate the volatile compounds in the headspace of the vial, Then SPME fibers were set inside the micro-extraction vials for 20 minutes to extract the volatile compounds produced by the fungal hyphes. The instrument (GC-MS-MS) was put to an initial temperature of 100 º C and held at this temperature for 1 minute, then the temperature was gradually increased to 250 ° C (35 ° C for 2 minutes, 5 ° C / min up to 155 ° C, 20 ° C / min up to 250 ° C and 250 ° C for 10 minutes). The temperature of the injection port was kept at 250 ° C and the helium flow rate at 1. 5 mL / min, the ionization voltage was at 70 eV. Finally SPME fibers were put in the injection port of the GC-MS instrument and the mass spectrum scan range was set to 45–450 (m/z). The generated GC-MS mass spectrum data were interpreted using the National Institute of Standards and Technology

(NIST) database. The name, molecular weight, and molecular formula of the volatile components were determined.

Statistical data analysis

An analysis of variance (ANOVA) and Tukey's (HSD) test (with a confidence interval of 95%) were performed to compare corrected mortality means and ranks these results into homogeneous groups. The corrected mortality was subjected to probit analysis to obtain the (LC50) values, which were estimated using a binomial model with a complementary log-log link function. The data from the time-mortality (survival) bioassays were subjected to a non-parametric survival analysis using Kaplan-Meier estimators to obtain the median survival time (LT 50). Similarly, the statistical heat maps of recorded mortality (insecticidal activity) under different concentrations were carried out. All statistical data analyzes were carried out using the XLSTAT 2019 software (Addinsoft, 2019. XLSTAT Statistical Data Analysis Solution, Paris, France).

Results and Discussion

Results

Insecticidal activity of the fungal extract

The fungal extract showed an insecticidal activity that varied with the concentration used and the time after treatment. Thus, the most effective concentration against the insects was 2 g/L, The maximum mortality rates were73, 91 % and 100 % after 7 days of treatment for *L. migratoria* upon contact and ingestion treatments, respectively figures (1, 2). The capital letters indicate a significant difference among insecticidal activity of different concentrations of the extract on different days (at 0. 05 significance level). The lowercase letters indicate significant differences among insecticidal activity at different concentrations of the extract on the same day (at 0. 05 significance level).

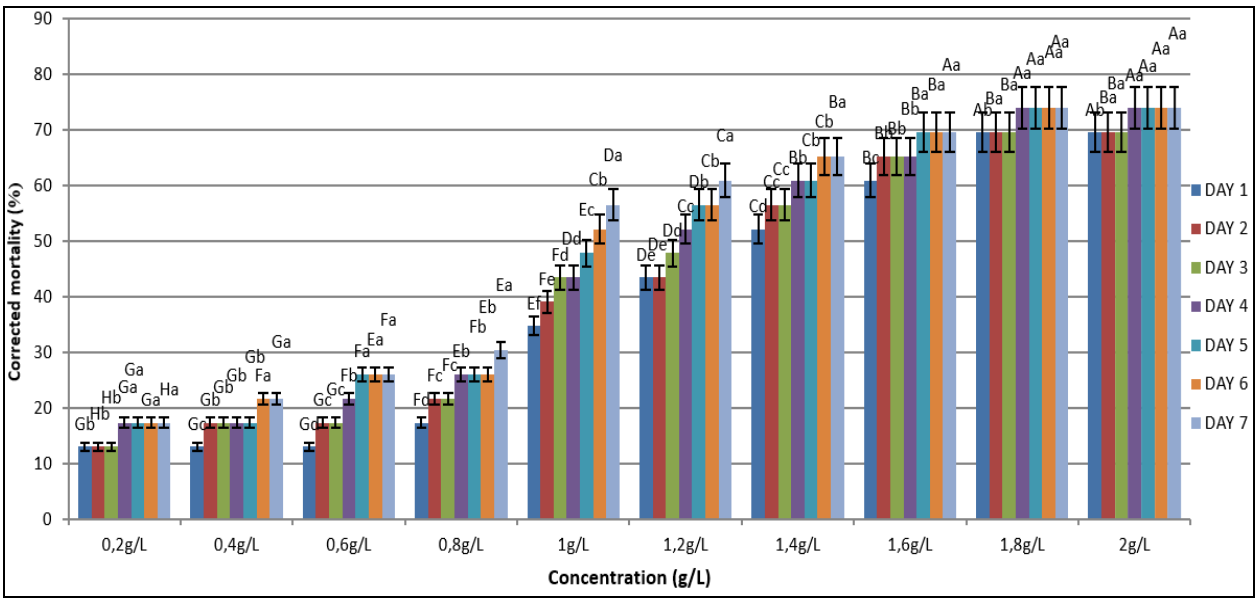


Fig 1: Corrected mortality recorded after contact treatment of *L. migratoria* with *Alternaria tenuissima* extract.

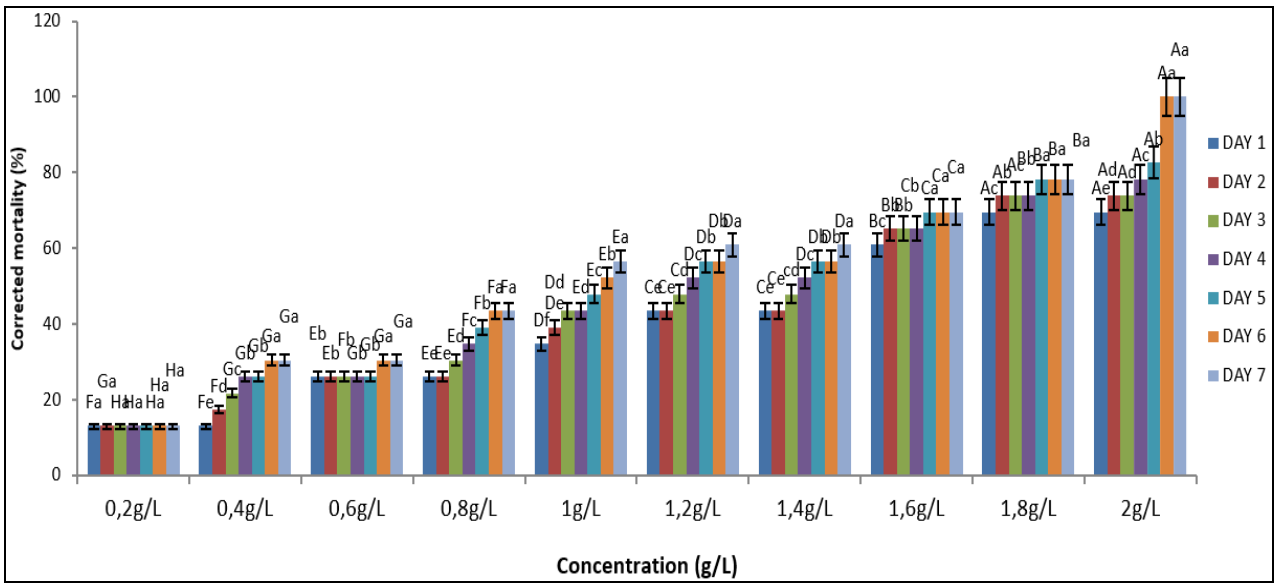


Fig 2: Corrected mortality recorded after ingestion treatment of *L. migratoria* with *Alternaria tenuissima* extract.

The gradual transition of the heat map from green to red correlates with an increase in the mortality of insects from low to high. The most effective concentration against the insects was 2 g/L. figures (3, 4).

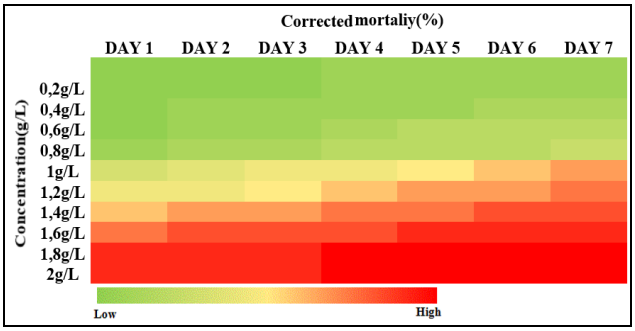


Fig 3: Statistical heat map of insecticidal activity under different concentrations of the fungal extract for various exposure duration (contact treatment).

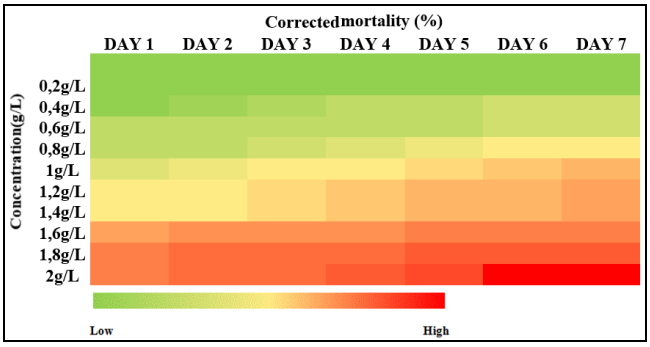


Fig 4: Statistical heat map of insecticidal activity under different concentrations of the fungal extract for various exposure duration (ingestion treatment).

In addition to the mortalities recorded in *L. migratoria* after the application of the fungal extract, a reduction in motor activity, intense defecation, and unusual water loss in the form of diarrhea were observed in the treated locusts. The application of these extracts also caused a change in the pigmentation of the locust bodies to a red color after the contact treatment and the appearance of black color in the abdomen upon the ingestion treatment (figure. 5)

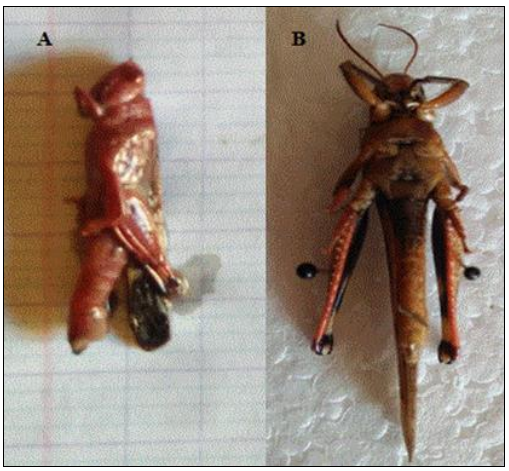


Fig 5: Effect of extract obtained from the endophytic fungus *Alternaria tenuissima*. A. Contact treatment against *L. migratoria.* B. Ingestion treatment against *L. migratoria*.

The LC50 values indicated that the fungal extract was toxic against the insects with LC50 values ranging from 0, 56 to 1, 39 for *L. migratoria* contact treatment and from 0, 803to1, 321for *L. migratoria* ingestion treatment (Table. 1).

Table 1: Lethal concentration 50 of the fungal extract under different exposure times of *L. migratoria*

Day	LC_{50} Contact treatment of *L.migratoria*. (g/L)	LC_{50} Ingestion treatment of *L.migratoria*. (g/L)
1	1,39±0,03	1,321±0,03
2	1,27±0,08	1,202±0,08
3	1,23±0,04	1,174±0,04
4	1,10±0,02	1,047±0,02
5	1,04±0,07	0,9±0,05
6	0,99±0,03	0,812±0,09
7	0,562±0,03	0,803±0,06

The mean survival times (LT50) were ranging from 4, 914 to 6, 187 days for *L. migratoria* contact treatment and from 6, 187 to 6, 818 days for *L. migratoria* ingestion treatment (Table. 2)

Table 2: Lethal time 50 of *L. migratoria* treatments.

Concentration (g/L)	LT_{50} Contact treatment of *L.migratoria*. (days)	LT_{50} ingestion treatment(g/L) of *L.migratoria* (days)
0,2	6,187±0,329	6,818±0,329
0,4	6,187±0,329	6,187±0,329
0,6	6,187±0,329	5,889±0,333
0,8	6,187±0,329	5,889±0,333
1	5,352±0,361	5,435±0,365
1,2	5,036±0,368	5,352±0,361
1,4	4,914±0,377	4,799±0,363
1,6	4,914±0,377	4,799±0,363
1,8	4,914±0,377	4,720±0,358
2	4,914±0,377	4,678±0,350

Chemical analysis of the fungal extract

Chemical analysis of the fungal extract revealed the presence of polyphenols**,** alkaloids, terpenoids**,** saponins, and tannins.

ATR-FTIR spectroscopy-based analyses of the extract

The results of FTIR-ATR analysis of the *A. tenuissima* extract revealed the presence of sulfone (1100-1150 cm^{-1}), nitro (1250-1400 cm^{-1}), imine (1480-1690 cm^{-1}), enol ether (1600-1660 cm^{-1}), alkyne (2100–2300 cm^{-1}), and hydroxyl (3300–3500 cm^{-1}) groups (Figure. 6).

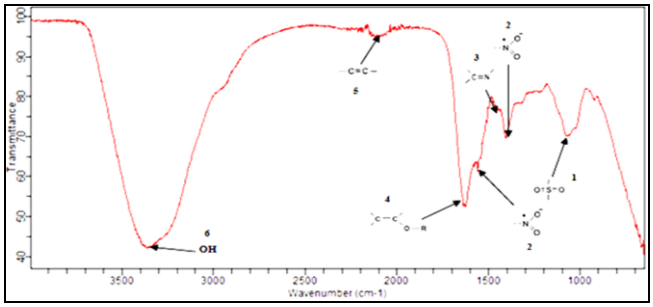
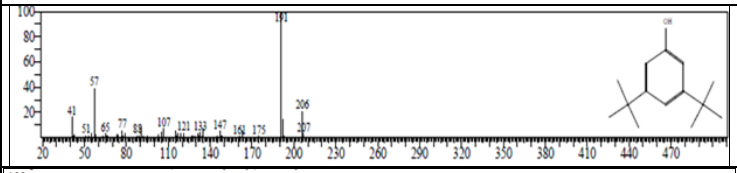
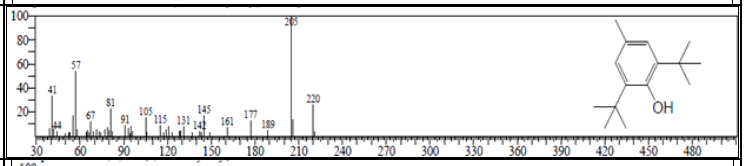
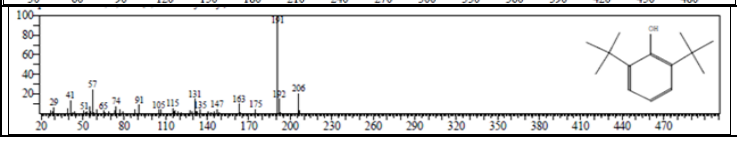


Fig 6: FTIR-ATR analysis of the extract obtained from the endophytic fungus *Alternaria tenuissima*. 1. sulfone group, 2. Nitro group, 3. Imine group, 4. enol ether group, 5. Alkyne group, 6 hydroxyl group.

Analysis of volatile components found in fungal extract and produced by fungal hyphes of *A. tenuissima.*

GC-MS-MS analysis results indicated the presence of different volatile secondary metabolites with insecticidal activities (Table. 3).

Table 3: Volatile secondary metabolites of *A. tenuissima* extract with insecticidal activities.

Volatil compound	Formula	Molecular weight (g/Mol)	Obtained Chromatogram	Activity	References
Phénol, 3,5-bis (1,1-diméthyléthyl) -	$C_{14}H_{22}O$	206		Insecticidal	(Zhao *et al.*,2020) [36].
Phénol, 2,6-bis (1,1-diméthyléthyl) - 4-méthyl-	$C_{15}H_{24}O$	220			
2,6-Di-tert-butyl-4-méthyl-phénol	$C_{15}H_{24}O$	220			

Discussion

Endophytic fungi occupied the internal tissues of their host plants without any apparent symptoms of infection (Kaur, 2020). They confer host resistance against insect pests by producing specific bioactive volatile and non-volatile secondary metabolites (El-Sayed *et al*., 2020) [7].

Among non-volatile secondary metabolites, many alkaloids are toxic and appear to serve primarily as a component of the plant defense mechanism against various herbivorous insects through their deterrent and anti-food or toxicity effects (Taye & Borkataki, 2020) [28].

Phenolic compounds are defensive compounds that exert a toxic effect on insects upon ingestion (Kortbeek *et al.*, 2019) [19].

Saponins are known insecticides, deterrents, molt disruptors, and regulators of insect growth. Their mechanism of action involves interaction with cholesterol, which disrupts the ecdysteroid synthesis. Additionally, they act as protease inhibitors and are reportedly cytotoxic to some insects (Hussain *et al*., 2019) [11].

Tannins are toxins that affect insect growth and development by acting as food deterrents against many insect pests (Taye & Borkataki, 2020) [28].

And terpenoids which are involved in inhibiting insect steroid–receptor activity, which allows the cell to sense the presence of hormones and generally show insect-repellent activity, preventing or reducing plant-insect contact as well as the transmission of viral diseases and reportedly involved in plant defense responses as toxins and deterrents against numerous insect pests (Kortbeek *et al.*, 2019;Clouse, 2011) [14, 4].

Detection of hydroxyl groups by FTIR-ATR has confirmed the presence of phenols and tannins in fungal extracts (Sharaf *et al*., 2013) [25].

Imines and their derivatives have long been acknowledged as key intermediates in the synthesis of nitrogenous heterocyclic compounds, especially in the field of alkaloid synthesis (Lv *et al.*, 2021) [17].

Therefore, the detection of these compounds by FTIR-ATR confirmed the presence of alkaloids in the fungal extract. Similarly, the detection of alcohol groups by FTIR-ATR confirmed the presence of saponins in the fungal extract, as reported previously by (Veiga *et al.*, 2018) [30].

Sulfones are organosulfur compounds that have been previously investigated due to their importance as synthetic intermediates for the production of a wide range of chemically and biologically active molecules with insecticidal properties (Yu *et al*., 2016) [33].

Many alkyne derivatives are key intermediates in the synthesis of numerous insecticides, Among them neurotoxic pyrethroids act at low doses on the central nervous system of insects by blocking their sodium channels and causing a lack of nervous tone and ultimately the death of the insects (Soderlund, 2012) [27].

The nitro group is considered a exceptional functional group in chemistry, and it is commonly used in the production of agricultural chemicals such as fungicides, insecticides (Nishiwaki, 2020) [18].

Enol ethers are also intermediates in the synthesis of many insecticidal products (Liu *et al*., 2015) [16].

In addition to the mortalities recorded in *L. migratoria* after the application of the fungal extract, a reduction in motor activity, intense defecation, and unusual water loss in the form of diarrhea were observed in the treated locusts. These observed signs of intoxication are certainly attributable to the fungal extract treatment (Kemassi *et al*., 2018) [13].

The application of these extracts also caused a change in the pigmentation of the locust bodies to a red color after the contact treatment and the appearance of a black color in the abdomen (location of the digestive tract) upon the ingestion treatment. The pigment responsible for the red color in the cuticle of locusts is β-carotene; thus, the red color observed after treatment is due to excess production of this pigment (Yang *et al*., 2019) [32]. The black pigmentation of the digestive tract is due to the presence of specific melanin (Chauvin, 1956) [3].

However, the dark black color of the abdomen observed after treatment is due to excess melanization caused by intestinal toxicity, lack of nervous tone due to the harmful effects of the fungal extract on the central nervous system, distension, and cellular necrosis, and a physiological reaction of the insects whereby they attempt to heal the wounds in the intestine, which can no longer fulfill its role of absorption and assimilation of the nutrients, causing protein and lipid deficiency and ultimately the death of the insects (Shamim *et al*., 2014) [24].

In comparison with studies on the insecticidal activity of secondary metabolites produced by endophytic fungi. The compound 6, 8-di-O-methylbipolarin produced by *Acremonium vitellinum* exhibited a good larvicidal activity

against the third instar larvae of H. *armigera* with an LC50 value of 0. 72 mg/mL (Yuan *et al*., 2020) [34].

The endophytic fungi *C. lunata* MRDS 302 showed excellent insecticidal activity against *S. cretica* first instar larvae with a maximum mortality rate of 100 % (EL-Lebody *et al*., 2021) [8].

Among the volatile substances with insecticidal activity, 2, 4-di-tert-butylphenol analogs are highly important (Zhao *et al.,* 2020) [36].

These results show that the endophytic fungus *A. tenuissima* is an excellent source of volatile and non-volatile secondary metabolites with insecticidal activity against *L. migratoria*.

Conclusion

The present study aimed to investigate the insecticidal activity of the endophytic fungus, *Alternaria tenuissima*, isolated from the leaves of common castor bean *Ricinus communis.* To evaluate the insecticidal activity, ten concentrations of the fungal extract were applied by contact and ingestion against *Locusta migratoria*.

The results showed that the fungal extract had variable insecticidal activities that depended on the concentration used and time after treatment. Chemical analysis of the fungal extract revealed the presence of polyphenols that exert a toxic effect on insects upon ingestion**,** alkaloids with deterrent and anti-food or toxicity effects, terpenoids with well known insecticidal activity**,** saponins as insecticides, deterrents, molt disruptors, and regulators of insect growth, and tannins that affect insect growth and development by acting as food deterrents against many insect pests. Attenuated total reflection-Fourier transform infrared analysis of the fungal extract revealed the presence of many groups commonly used in the production of insecticides.

Specifically, GC-MS analysis revealed the presence of volatile substances with insecticidal activities. These results show that the fungal extract of *A. tenuissima* is an excellent source of volatile and non-volatile secondary metabolites with insecticidal activity.

References

1. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology,1925:18:265-267. https://doi.org/10. 1093/ jee/18.2.265a.
2. Berestetskiy A, Hu Q. The Chemical Ecology Approach to Reveal Fungal Metabolites for Arthropod Pest Management. *Microorganisms*,2021:9:1379. https://doi.org/10.3390/ microorganisms 9071379.
3. Chauvin R. Physiologie des insectes. Le comportement, les grandes fonctions, écophysiologie. Paris, Fr:Edition INRA, 1956.
4. Clouse SD. Brassinosteroid signal transduction:From receptor kinase activation to transcriptional networks regulating plant development *The Plant Cell*,2011:23(4):1219-1230. doi: 10.1105/tpc.111. 084475.
5. Dolatabad HK, Nikkhah MJ, Shier WT. Evaluation of antifungal, phosphate solubilisation, and siderophore and chitinase release activities of endophytic fungi from *Pistacia vera. Mycology Progress*,2017:16(8):777-790. https://doi. org/10. 1007/s11557-017-1315-z
6. EL Lebody KA, Maali SS, EL-Metwally FE, Moustafa HZ, Abd-Elaziz MAA. Isolation and pathogenicity of endophytic fungi associated with some maize hybrids against certain Lepidoptera pests. Egyptian Journal of Agricultural Research,2021:99(1):49-60. Doi:10.21608 /ejar.2021.58352.1073
7. El-Sayed ASA, Moustafa AH, El-Sheikh AA, Rady AM, Fathy NAM, Hussein *et al*. Efficient biocontrol of *Spodoptera littoralis* by *Aspergillus nidulans*, an endophyte of *Lantana camara*. International journal of pest management, 2020, 1-8. https://doi.org/10.1080/ 09670874.2020.1771472.
8. EL Lebody KA, Maali SS, EL-Metwally FE, Moustafa HZ, Abd-Elaziz MAA. Isolation and pathogenicity of endophytic fungi associated with some maize hybrids against certain Lepidoptera pests. Egyptian Journal of Agricultural Research,2021:99(1):49-60. Doi:10.21608/ ejar.2021.58352. 1073
9. Fiers M, Lognay G, Fauconnier ML, Jijakli H. Volatile Compound-Mediated Interactions between Barley and Pathogenic Fungi in the Soil, PLoS ONE,2013:8(6):e66805.
10. Harborne JB. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. 3th ed, Netherlands: Springer Netherlands, 1998.
11. Hussain M, Debnath B, Qasim M, Bamisile BS, Hameed MS, Wang L *et al*. Role of Saponins in Plant Defense Against Specialist Herbivores, *Molecule*s,2019:24:2067 doi:10.3390/ molecules24112067.
12. Kaur T. Fungal Endophyte-Host Plant Interactions: Role in Sustainable Agriculture Fungal. *Sustainable Crop Production*, 2020, 1-10. doi: http://dx.doi.org/10. 5772/intechopen.92367.
13. Kemassi A, Boual Z, Hadjseyd A, Bouziane Nawal, Herouini Amel, Mensouri K *et al*. Effets biotoxiques des extraits de *cleome arabica* l. (capparidaceae) sur le criquet pelrin *schistocerca gregaria* (forskål, 1775) (orthoptera, acrididae). Algerian journal of arid environment,2018:8(2):79-98.
14. Kortbeek RWJ, Van der Gragt M, Bleeker PM. Endogenous plant metabolites against insects. European Journal of Plant Pathology,2019:154:67-90. https://doi. org/10.1007/s10658-018-1540-6.
15. Le Gall M, Overson R, Cease A. A Global Review on Locusts (Orthoptera: Acrididae) and Their Interactions With Livestock Grazing Practices. Frontiers in Ecology and Evolution2019:7:263. doi:10.3389/fevo.2019. 00263.
16. Liu A, Yu W, Liu M, Bai J, Liu W, Liu X *et al*. Synthesis and Insecticidal Activity of Novel Nitropyridyl-Based Dichloropropene Ethers. Journal of Agricultural and Food Chemistry,2015:63:7469-7475. doi:10.1021/acs.jafc.5b02279.
17. Lv M, Ma Q, Zhang S, Xu H. Agrochemical properties evaluation of some imines alkaloids of matrine/ oxymatrine. Bioorganic & Medicinal Chemistry Letters,2021:48:128246. https://doi.org/10.1016/j. bmcl.2021.128246.
18. Nishiwaki N. A Walk through Recent Nitro Chemistry Advances. Molecules,2020:25:3680. doi:10.3390/ molecules25163680.
19. Ouedraogo RM, Kamp A, Goettel MS, Brodeur J, Bidochka MJ. Attenuation of fungal infection in thermoregulating *Locusta migratoria* is accompanied by changes in hemolymph proteins. Journal of Invertebrate Pathology,2002:81:19-24. doi:10.1016/

s0022-2011(02)00117-9.
20. Rahman SFSA, Singh E, Pieterse CMJ, Schenk PM. Emerging microbial biocontrol strategies for plant pathogens. Plant Science,2018:267:102-111. doi:10.1016/j.plantsci.2017.11.012.
21. Sala A, Spalding K, Ashton K, Board R, Butler H, Dawson T Harris DA *et al*. Rapid Analysis of Disease State in Liquid Human Serum combining Infrared Spectroscopy and "Digital Drying". Journal of Biophotonics,2020:13(9):e202000118. https://doi.org/10.1002/jbio.202000118.
22. Sangbaramou R, Camara I, Xin-zheng H, Shen J, Tan S, Shi W. Behavioral thermoregulation in *Locusta migratoria manilensis* (Orthoptera: Acrididae) in response to the entomopathogenic fungus *Beauveria bassiana*. Plos One,2020:13(11):1-19. https://doi.org/10.1371/journal.pone.0206816
23. Senthilkumar N, Murugesan S, Suresh Babu D, Rajeshkannan C. GC-MS analysis of the extract of endophytic fungus, *Phomopsis* sp. isolated from tropical tree species of India, *Tectona grandis* L. International Journal of Innovative Research in Science, Engineering and Technology,2014:3(3):10176-10179.
24. Shamim G, Ranjan SK, Pandey DM, Ramani R. Biochemistry and biosynthesis of insect pigments. European Journal of Entomology,2014:111(2):149-164. doi:10.14411/eje.2014.021.
25. Sharaf S, Higazy A, Hebeish A. Propolis induced antibacterial activity and other technical properties of cotton textiles. International Journal of Biological Macromolecules,2013:59:408-416. https://doi.org/10.1016/j.ijbiomac.2013.04.030.
26. Shuping DSS, Eloff JN. The use of plants to protect plants and food against fungal pathogens: a review. African Journal of Traditional, Complementary and Alternative Medicines,2017:4(14):120-127.
27. Soderlund DM. Molecular mechanisms of pyrethroid insecticide neurotoxicity: Recent advances. Archives of Toxicology,2012:86:165-181. doi:10.1007/s00204-011-0726-x.
28. Taye RR, Borkataki S. Role of secondary metabolites in plant defense against insect herbivores. International Journal of Chemical Studies,2020:8(1):139-142. doi:10.22271/chemi.2020.v8.i1b. 8245
29. Toghueo KRM. Bioprospecting endophytic fungi from *Fusarium* genus as sources of bioactive metabolites *Mycology*,2019:11(1):1-21. doi:10.1080/21501203.2019.1645053.
30. Veiga FF, De Castro HLV, Sato F, Bombassaro A, Vicente VA, Mendes V *et al*. *Fusarium oxysporum* is an onychomycosis etiopathogenic agent. Future Microbiology,2018:13:1745-1756. https://doi.org/10.2217/fmb-2018-0245.
31. Yan C, Liu W, Li J, Deng Y, Chen S, Liu H. Bioactive terpenoids from *Santalum album* derived endophytic fungus *Fusarium* sp. YD-2. RSC Advances,2018:8:14823-14828. https://doi.org/10.1039/C8RA02430H.
32. Yang M, Wang Y, Liu Q, Liu Z, Jiang F, Wang H *et al*. A β-carotene-binding protein carrying a red pigment regulates body-color transition between green and black in locusts. eLife,2019:8:e41362. doi:10.7554/eLife.41362.
33. Yu X, Liu YX, Li Y, Wang Q. Design, Synthesis, Acaricidal/Insecticidal Activity, and Structure-Activity Relationship (SAR) Studies of Novel Oxazolines Containing Sulfone/ Sulfoxide Groups Based on the Sulfonylurea Receptor Protein Binding Site. Journal of Agricultural and Food Chemistry,2016:64(15):3034-3040. doi:10.1021/acs.jafc.6b00645.
34. Yuan XL, Wang XF, Xu K, Wei L, Dan C, Zhang P. Characterization of a New Insecticidal Anthraquinone Derivative from an Endophyte of *Acremonium vitellinum* against *Helicoverpa armigera*. Journal of Agricultural and Food Chemistry,2020:68(41):11480-11487. https://dx.doi.org/10.1021/acs.jafc.0c05680
35. Zhang L, Lecoq M. *Nosema locustae* (Protozoa, Microsporidia), a Biological Agent for Locust and Grasshopper Control. Agronomy,2021:11:711. https://doi.org/10.3390/agronomy11040711.
36. Zhao F, Wang P, Lucardi RD, Su Z, Li S. Natural Sources and Bioactivities of 2, 4-Di-Tert-Butylphenol and Its Analogs. *Toxins* (Basel),2020:12(1):35. doi:10.3390/toxins12010035.