Efficiency of *Lecaniicillium lecanii* Crude Filtrate as a Bioinsecticide Against the Sunn Pest, *Eurygaster testudinaria* on Wheat Plants

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Abstract

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This study investigated extracellular crude secondary metabolites of the entomopathogenic fungus *Lecaniicillium lecanii* as bioinsecticide spray against nymphs and adults of the sunn pest, *Eurygaster testudinaria* (Hemiptera: Scutelleridae), on wheat crop. The results showed that the highest mortality rate of *E. testudinaria* adults 96.70% was obtained in response to the concentration 100%, 10 days post-treatment. Whereas the mortality rate of 5th instar nymphs reached 100% in response to the same concentration, 5 days post-treatment compared with 0.00 % in the control treatment, under laboratory conditions. In the field experiments, the 100% concentration was more effective compared with the rest of the concentrations. The study identified compounds in the crude secondary metabolites of *L. lecanii* which are effective in controlling the sunn pest *E. testudinaria* on wheat crops.

Keywords: Sunn pest, *Eurygaster testudinaria*, entomopathogenic fungi, *Lecaniicillium lecanii*, secondary metabolites, wheat.

Introduction

The sunn pest, *Eurygaster testudinaria* (Geoffroy) (Hemiptera: Scutelleridae) spreads in many countries of the world. The adult and nymph stages cause damage to wheat plants and grains by feeding on various plant and grain parts, which directly reduces the grain yield and quality (Fathi *et al.,* 2010; Hariri *et al.,* 2000). In recent years, new techniques were developed to control pest insects, including nanotechnology (Jabbar *et al*., 2023; Mohmed, 2020). In addition, entomopathogenic and the secondary metabolites of entomopathogenic fungus (EPF) were also reported as a safe alternative to synthetic chemical insecticides (Albehadli & Mohmed, 2022).

Verticillium lecanii, (Zimmerman), commonly known as *Lecanicillium lecanii*, is an EPF that has been widely used in biocontrol (Ali *et al*., 2020), and it proved to be an important biocontrol agent against pests of Orthoptera, Hemiptera, Lepidoptera, Thysanoptera, and Coleoptera (Lokma *et al.*, 2023; Ravindran *et al*., 2018; Saleh *et al*., 2023).

The success of using fungal entomopathogens as biocontrol agents is attributed to high host specificity, long shelf life and non-toxic effects to the environment. The entomopathogenic fungus *L. lecanii* was used as an effective bio-pesticide to manage insects (Ali *et al*., 2020; Vinodhini *et al.,* 2017).

The present study investigated the insecticidal activity of the crude filtrate of *L. lecanii,* against the sunn pest, *E. testudinaria*, under laboratory and field conditions and the identification of the compounds in the crude filtrate by using GC-Mass spectrometry.

Materials and Methods

Pest collection and rearing

From wheat felids in Diwaniyah district Qadisiyah Governorate, the *E. testudinaria* adults were manually collected from wheat fields in Diwaniyah district, Quadisiyah governorate and brought to the laboratory. The insects were kept for one week before the bioassays. Insects were placed in plastic boxes (25 ×35 × 18 cm) and covered with a muslin cloth, secured using a rubber band, and kept at 27±1°C, 60±10% RH and 16 h light: 8 h dark photoperiod with wheat seeds on wet cotton pieces placed in the rearing container, to provide a water source for the insects (Allahyari *et al*., 2010).

Entomopathogenic fungi

The EPF *L. lecanii*, was acquired from the Organic Agriculture Center-Ministry of Agriculture, Iraq. The isolate was transferred to potato dextrose agar (PDA) plates and incubated in the dark at 26±2°C for 10 days. The sample was then purified using the hyphal tip technique, as described by Fathy & Saad (2017).

Preparation of Fungal Crude Filtrate

Two discs with a diameter of (5 mm) of *L. lecanii* fungal colonies growing on solid media potato dextrose agar (PDA) were used for each beaker containing liquid media of potato dextrose broth (PDB). The beaker was stirred slightly and then incubated at the temperature of 28±2°C for 25 days. The biomass was harvested by filtration using Whatman No. 1, then the filtrate was sterilized using the Millipore sterile filter 0.45 µm. The resulting filtration was considered a stock

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solution (100%) and then diluted to prepare 75 and 50% concentrations with a sterile distilled water. The control treatment used sterile distilled water only (Mohammed *et al*, 2020).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis technique

To diagnose compounds of the crude filtrate *L. lecanii* used, the gas chromatography-mass spectrometer technique (GCMS) by Gas Chromatography–Mass spectrometry Shimadzu's in laboratories of the University of Tehran.

Laboratory experiment

Ten adults and a 5th instar nymph, both separately of the *E. testudinaria* were inserted without regard to their sexual orientation in a Petri dish (90 ×15 mm) included food and sterile filter paper at the bottom, as well as ventilation holes in the cover of the hand sprayer. Each Petri dish was treated with two ml of each concentration (50, 75 and 100%) while the control treatment was sprayed with sterile distilled water only. The experiment was carried out with three replicates for each concentration. Treated insects were placed under laboratory conditions and the mortality rate was recorded after 1, 3, 5, 7 and 10 days post-treatment.

Field experiment

Field experiments were conducted in a wheat field located in Al-Qadisiyah Governorate-Iraq in season 2021 to study the efficacy of the secondary metabolites of *L. lecanii* at three concentrations against adults of the *E. testudinaria*. The field was divided into plots beside untreated plots, were distributed randomly in a complete block randomized design (RCBD). Three replicates of the treatments and untreated plots were used. The wheat plants were sprayed once with concentrations (50, 75 and 100%) using a knapsack sprayer (18 L capacity). The numbers of adults present per 2m^2 were counted carefully directly on the wheat plants, were chosen randomly before application, and recorded after 1, 3, 5, 7 and 10 days of treatment.

Statistical analysis

With the Two-factor design, mortality rates in Laboratory conditions were calculated using the Abbot formula (Abbott, 1925) while the percentages of infestation under the field conditions were estimated according to Henderson & Tilton's equation (1955). The treatment means were compared by the least significant difference at a 5% level of significance ($P \leq 0.05$).

Results and Discussion

GC–Mass spectrometry analysis

Gas chromatography-mass spectrometry separated different compounds in the crude filtrate of *L. lecanii*, which included fatty acids, fatty acid methyl esters, amino acids, and several other organic compounds that were identified and quantified based on the retention time and area under curve (Table 1).

Table 1. Chemical compounds identified in the crude filtrate of *L. lecanii.*

Chemical Name	Retention time (min)	Molecular formula	Area %	Molecular weight	Compound nature and biological activity
Acetic acid, 6-morpholin-4-yl-9-oxobicyclo[3.3.1]non-3-yl ester	20.14	$C_{15}H_{23}NO_4$	1.05	281.35	Ester
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	23.34	$C_6H_8O_4$	4.26	144.12	Anthocyanin derivative, antioxidant
butyric acid	24.21	$C_4H_8O_2$	1.93	88.11	Saturated short-chain fatty acid
Chlororesorcinol	30.09	$C_6H_5ClO_2$	2.29	144.55	Used as pharmaceutical, pesticide intermediates
Nonadecane	53.93	$C_{19}H_{40}$	0.47	268.5	Alkane hydrocarbon
Palmitic acid methyl ester	54.73	$C_{17}H_{34}O_2$	0.66	270.5	Acid (fatty acid methyl ester), larvicidal activity
Ppalmitic acid	55.98	$C_{16}H_{32}O_2$	4.36	256.42	Saturated fatty acid, pesticide & nematicide
Linoleic acid methyl ester	60.09	$C_{19}H_{34}O_2$	0.61	294.5	Unsaturated fatty acid
Methyl Elaidate	60.31	$C_{19}H_{36}O_2$	1.36	296.5	Fatty acid, antibacterial, antifungal, antioxidant,
Cyclopropaneoctanal, 2-octyl	60.48	$C_{19}H_{36}O$	3.14	280.5	
alpha-Linoleic acid	61.36	$C_{18}H_{32}O_2$	1.45	280.4	Unsaturated fatty acid
9-Octadecenoic acid (Oleic Acid)	61.56	$C_{18}H_{34}O_2$	15.2	282.5	Fatty acid, antimicrobial pesticide
2-Mono palmitin	63.14	$C_{19}H_{38}O_4$	5.94	330.5	Acid (fatty acid ethyl ester)

Laboratory experiment

The results obtained (Table 2) demonstrated that when the concentration of crude filtrate of *L. lecanii* increased, the mortality rate of *E. testudinaria* adults and nymphs increased. The highest adult mortality rate of *E. testudinaria* was 96.70% at concentration of 100%, 10 days post-treatment, whereas the nymphs' mortality rate 5 days after treatment at the same concentration reached 100% compared to 0.00% in the control treatment, and these findings were consistent with those of Mourad (2010). Furthermore, the cumulative daily mortality rate increased as the period after treatment increased. Nymphs had a higher mortality rate than adults, because adults have thicker cuticle, which offer some protection against entomopathogenic filtrate.

Field experiment

The results obtained (Table 3) for the effectiveness of crude filtrate of *L. lecanii* at different concentrations on the *E. testudinaria* adults under field conditions are shown in Table 4. Mortality rate of *E. testudinaria* after one day of treatment reached 44% at the 100% concentration, and 86% 10 days after treatment compared with 76.7% at the 50% concentration, 10 days after treatment The results agree with those of Albehadli & Mohmed (2022) who reported the impact of EPF culture filtrates of *Metarhizium anisopliae* and *Cheatomium globosum* on adults and nymphs mortality rates of the aphid *Schizaphis graminum* on wheat crop.

The present study confirmed the insecticidal activity of secondary metabolites of the fungus *L. lecanii* as a biopesticide against nymphs and adults of the sunn pest, *E. testudinaria*, on wheat crops under laboratory and field conditions, and recommends its use as a safe alternative to chemical insecticides for controlling the sunn pest, *E. testudinaria*.

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Table 2. Insecticidal activity of crude filtrate of *L. lecanii* against adults of *E. testudinaria* in laboratory.

Conc. %	Mortality rate (%) at different periods after treatment										Mean mortality rate	
	1st day		3rd day		5th day		7th day		10th day			
	Adults	5th instar	Adults	5th instar	Adults	5th instar	Adults	5th instar	Adults	5th instar	Adults	5th instar
50	16.7	26.7	30.0	30	53.3	90.0	76.7	100	83.3	100	52.0	69.34
75	20.0	40.0	56.67	73.3	63.3	96.7	76.7	100	90.0	100	61.33	82.00
100	30.0	60.0	56.67	76.7	70.0	100	80.0	100	96.7	100	66.67	87.34
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.00	0.00
Mean	16.67	31.67	35.83	45.00	46.65	71.67	58.35	75.00	67.50	75.00	-	-

For adults: $LSD_{0.05}$ for concentration= 1.58, for time period= 1.81, for interference= 3.29
For 5th instar: $LSD_{0.05}$ for concentration= 1.28, for time period= 1.46, for interference=3.27

Table 3. Efficacy of crude filtrate of *L. lecanii* against *E. testudinaria* under field conditions.

Conc. (%)	Mortality rate (%)					Mortality mean
	1st day	3rd days	5th days	7thdays	10th days	
50	19.4	58.93	61.1	72	76.7	67.18
75	19.1	63.70	72.9	76.7	84.4	74.43
100	44	72	81.7	86	86	81.43

$LSD_{0.05}$ for concentration= 0.71, for time period= 0.92, for Interference= 2.76

الملخص

جبار، أحمد شمخي، أحمد سعيد محمد وأحمد محمد حسين. 2025. فعالية راشح الفطر *Lecaniicillium lecanii* كمبيد حشري حيوي ضدّ حشرة السونة (*Eurygaster testudinaria*) على محصول القمح. مجلة وقاية النبات العربية، 43(3):425-428.

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بحثت هذه الدراسة في فعالية رشّ منتجات الأيض الثانوية للفطر *Lecaniicillium lecanii* كمبيد حشري حيوي ضدّ حوريات وبالغات حشرة السونة (*Eurygaster testudinaria*) (Hemiptera: Scutelleridae) على محصول القمح. أظهرت النتائج أن أعلى معدل موت لبالغات حشرة السونة بلغ 96.70% للتركيز

100% بعد 10 أيام من المعاملة. في حين بلغ معدل موت حوريات العمر الخامس 100% عند التركيز نفسه بعد 5 أيام من المعاملة مقارنة بـ 0.00% في معاملة الشاهد تحت الظروف المختبرية. وفي التجارب الحقلية كان التركيز 100% أكثر فعالية مقارنة ببقية التراكيز. حددت الدراسة وجود مركبات في الأيض الثانوية للفطر *L. lecanii* فعالة في مكافحة حشرة السونة على محصول القمح.

كلمات مفتاحية: حشرة السونة، الفطور الممرضة للحشرات، منتجات الأيض الثانوية، القمح.

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