Jordan Journal of Pharmaceutical Sciences, Volume 16, No. 3, 2023

Assessment of the Fungicidal and Nematicidal Potential of *Reichardia tingitana* (L.) Roth on Phytopathogenic Fungi and Plant Nematode

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ABSTRACT

The primary concern was the removal of numerous soil fumigants and nematicides due to their potential risks to human and environmental safety. Fungal pathogens can cause serious diseases in humans and animals. Among these, root-knot nematodes such as Meloidogyne incognita and Tylenchulus semipenetrans pose a significant threat, leading to substantial damage and yield reduction in various economically important plants. Therefore, this study aimed to assess the fungicidal and nematicidal activities of the ethanol extract (EE) and lupeol (L), the major isolates from the aerial parts of Reichardia tingitana L. Roth (Asteraceae), against Aspergillus flavus and plant-parasitic nematodes. Antifungal actions of EE (10-120 ppm) and L (23.4-281.2 µM) were evaluated through in vitro and in vivo growth assays, spore germination inhibition assays, and the efficacy of inhibiting pod and kernel infection. Nematicidal activity of EE and L was tested by preparing cultures containing egg masses of nematode species M. incognita from infected eggplants and T. semipenetrans from infected citrus roots, using concentrations of 2.5, 5, 10, 20, 40, 80, and 120 ppm. Results showed that R. tingitana (EE) and (L) exhibited nematostatic or nematicidal effects on nematode viability, egg hatch in vitro, and development and reproduction in vivo. Lupeol was particularly effective in inhibiting the colonization of A. flavus in peanuts. EE and L demonstrated high toxicity against nematodes in laboratory exposure and were effective in controlling nematode infestation in eggplant roots for 45 days. Improvement in plant growth parameters, including shoot and root length and weights, varied and was proportional to the doses of EE and L treatments. The antifungal and bio-nematicide effects of the ethanol extract from the aerial parts of R. tingitana were superior to those of lupeol, which could be attributed to the synergistic effect of phytochemicals in the ethanol extract. Both EE and L have potential applications as antifungal and bio-nematicide agents.

Keywords: Aflatoxin, Aspergillus flavus, false sow-thistle, lupeol, plant nematode, triterpenes.

List of Abbreviations: EE: Ethanol extract, CC:Column chromatography. L:Lupeol, LC50: the median lethal concentration (LC50) values,TLC: thin-layer chromatography,S1:System 1, TMS : Tetramethylsilane as an internal standard , PDA: potato dextrose agar medium, DC: Diameter of the colony in the control (mm), DT: Diameter of the colony in the treatment (mm), NC: Number of the fungal colony in control, NT: Number of the fungal colony in treatment, PF: Final Population, PI: nematode initial Population.

HIGHLIGHTS

- The study investigates the fungicidal and nematicidal activities of Reichardia tingitana L. Roth (Asteraceae) against phytopathogenic fungi and plant-parasitic nematodes.
- The ethanol extract of the aerial parts of R. tingitana (EE) and lupeol, a major isolate (L), exhibited nematostatic or nematicidal effects on nematode viability, egg hatch in vitro, and development and

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Received: 8/10/2022 Accepted: 22/3/2023.
DOI: https://doi.org/10.35516/jjps.v16i3.529

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reproduction in in vivo applications.

- Lupeol effectively inhibits the colonization of A. flavus in peanuts.

1. INTRODUCTION

The genus Reichardia belongs to the dandelion family Asteraceae and is native to the Mediterranean and western Asia. Reichardia tingitana L. Roth, also known as false sow-thistle, has been reported to contain volatile oil, triterpenes, sterols, and sesquiterpenes. [1] This plant exhibits various beneficial properties, including antioxidative, anti-diabetic, anti-inflammatory, and antiviral activities. [2] Additionally, studies have investigated its insect deterrent and insecticidal properties, particularly regarding guaianolides. [3] Triterpenes were previously reported to exert an antifungal activity [4], and Lupeol was reported as inhibitors for both fungal growth and mycotoxin production of toxigenic *Fusarium* species. [5]

Peanut (Arachishypogaea) is a significant cash crop in tropical and subtropical countries. However, due to its pods being in contact with the soil, peanuts are vulnerable to fungal pathogens. One of the most common storage fungi that colonize peanuts is Aspergillus flavus. This fungus is capable of causing seed rots, mold growth on seeds, pre- and post-emergence damping-off, and reducing both seed viability and seedling growth in peanuts. [6] Mycotoxins are produced by Aspergilli and can contaminate not only food but also feedstuffs. It has been reported that at least 25% of the grain produced worldwide each year becomes contaminated with mycotoxins. Among these mycotoxins, aflatoxins, produced by A. flavus and A. parasiticus, are particularly notorious for their carcinogenic and immunosuppressive effects. Aflatoxins are toxic, low-molecular-weight metabolites that can harm plants, animals, and microorganisms. [7] In nature, at least 14 different aflatoxins are produced, with Aflatoxin B1 being the most toxic. Both A. flavus and A. parasiticus can produce Aflatoxin B1. [8,9] These fungi commonly infect various crops, including peanuts, tree nuts, and wheat. [10] Additionally, they pose a serious threat to the health of animals and humans, causing problems such as teratogenicity, immunotoxicity, and hepatotoxicity. [11] All animal species in addition to adult humans are resistant to the acute toxicity of aflatoxins also have a high tolerance for aflatoxin exposure and rarely yield acute aflatoxicosis. [12] However, children are particularly affected, and their exposure can lead to stunted growth and delayed development. [13]

Fungal pathogens have the potential to cause serious diseases in both humans and animals. [14] However, due to concerns regarding the toxicity of existing antifungal agents and the emergence of drug-resistant strains, there is growing interest in exploring alternative methods to control these pathogens, such as the use of plants. Many plants contain secondary compounds that have been found to have inhibitory effects on harmful bacterial and fungal pathogens that affect humans. [15,16]

Eggplant (Solanum melongena L.) is one of the most important and commonly cultivated vegetable crops worldwide. Belonging to the Solanaceae family, it is extensively grown in regions such as Asia, Egypt, and the Middle East. [17] Eggplants are a rich source of essential vitamins like C, K, B6, niacin, thiamin, as well as essential nutrients including magnesium, phosphorus, copper, dietary fiber, folic acid, potassium, and manganese. However, this crop is highly susceptible to pollution caused by root-knot nematodes, particularly those of the genus Meloidogyne, such as M. incognita. [18] While several nematicides are available for managing the root-knot nematode crisis affecting vegetables like eggplant, their use is expensive and poses environmental hazards. [19]

Traditionally, nematicides have been applied to control nematodes. However, there has been growing concern over the removal of numerous soil fumigants and nematicides due to their potential risks to human and environmental safety. [20] An alternative approach involves using plants as sources of compounds for sustainable management of plant-parasitic nematodes. This approach focuses on economically significant nematode species,

such as root-knot nematodes of the genus Meloidogyne, which have a wide range of hosts and pose threats to various annual and perennial crops. [21] In our study, we found that the roots of Pulsatilla Koreana exhibited strong nematicidal activity against Meloidogyne incognita after 48 hours, with an LC50 of 92.8µg/ml. This suggests that triterpenoid saponins from P. Koreana have the potential to be explored as natural nematicides for developing new agents to control root-knot nematodes. [22] Additionally, the citrus nematode T. semipenetrans is considered the most predominant and economically important pathogen causing significant damage to citrus trees in orchards and nurseries. [23] In the current study, we evaluated the effects of the ethanol extract (EE) and lupeol (L) isolated from R. tingitana on the colonization of Aspergillus flavus in peanuts. We also investigated their nematicidal effects on egg masses of nematode species Meloidogyne incognita, found in infected eggplants, and Tylenchulus semipenetrans, found in infected citrus roots.

2. MATERIAL & METHODS

2.1. Plant material

Aerial parts of *Reichardia tingitana* were obtained from Benghazi, Libya and identified by Dr. Reem Samir Hamdy, Professor of Plant Taxonomy, Botany Department, Faculty of Science, Cairo University, Giza, Egypt. At the Museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, https://goo.gl/maps/v6PsvJp6KJW52PkH8, the sample of the plant code 2015419 is deposited.

2.2. *Extraction*

The air-dried powdered aerial parts of R. tingitana (500g) were extracted by cold percolation with 95% ethanol (3L) until exhaustion. The ethanol extract was evaporated under reduced pressure, resulting in 120g of greenish-brown semi-solid residue. The dried residue (50g) was then suspended in distilled water and successively partitioned between *n*-hexane, chloroform, and *n*-butanol saturated with water, yielding 13g, 1g, and 7g, respectively.

2.3. Separation of the components of the *n*-hexane extractive

On a silica gel column, the n-hexane extract (10g) was chromatographed using *n*-hexane and *n*-hexane-ethyl acetate mixtures as the eluent. Twenty milliliters of each fraction were examined by TLC. Fractions that showed major spots were chromatographed on a silica gel column using n-hexane-ethyl acetate mixtures as the eluent. Lupeol was isolated as the major compound, identified by spectral data and comparison with previously reported data. [4] Both the ethanol extract of the aerial parts and the main isolate were subjected to tests to evaluate their antifungal and nematicidal effects.

2.4. Estimation of the antifungal effect

2.4.1. Isolation of fungal pathogen:

Peanut pods were cut into small pieces (2-5mm), and kernels were disinfected for three minutes using 2.5% sodium hypochlorite, rinsed three times in sterile distilled water, and then dried between layers of sterile filter paper (Whatman, No. 1). Ten groups of peanut pod pieces and kernels, each with five pieces of pods and kernels, were plated out on 15 ml of potato dextrose agar (PDA) medium (5 pieces of pod or kernel per plate) and then incubated at 25°C for 6 days. Subculturing was repeated several times to obtain pure cultures from mycelial tips, which were preserved on PDA slants until identification. The isolated A. flavus was identified based on colony and morphological characteristics. [24] The culture of the A. flavus strain was preserved on a PDA slant in the lab.

2.5. In vitro growth inhibitory assay

The stock solution of each R. tingitana sample (EE and L) was prepared by adding it to the PDA medium to achieve concentrations of 10-120 ppm. The medium was poured into Petri dishes and inoculated with equal discs (9mm in diameter) of the A. flavus pathogenic fungus. Plates containing mycelium discs without plant material were used as a negative control. All plates were incubated at 28 ± 2°C for 4 days. Fungal growth was measured as colony diameter, and the toxicity of plant materials against

A. flavus was expressed as the percentage of mycelia inhibition using the formula: Inhibition of Growth (%) = (DC – DT) / DC × 100, where DC represents the diameter of the colony in the control (mm) and DT represents the diameter of the colony in the treatment (mm), as defined by Ismail et al. [25].

2.6. Spore germination inhibition assay

Fungal spores were collected using sterile distilled water containing 0.1% Tween 80, from PDA plates after 7 days of growth at 28°C. Final concentrations of spores were adjusted to 1.45 × 106 spores/ml. for the assay; autoclaved PDA medium (20ml) was mixed with concentrations of (10 - 120 ppm) of samples of EE, L, and 1.45 × 106 spores and then poured into Petri plates (10cm diameter). After solidification, plates were incubated at 28 ± 2 °C for 4 days. The percent germination reduction of spores was determined by the next formula:

Inhibition of spore germination (%) = NC –NT/NC×100

Where, NC: Number of the fungal colony in control, NT: Number of the fungal colony in treatment.

In vivo efficacy inhibition of pod and kernel infection

Peanut pods and kernels (susceptible variety Giza 3) were surface sterilized by soaking them in a 0.1% aqueous solution of mercuric chloride for 3 minutes, rinsed with sterile distilled water, and then five pods and kernels each were placed separately in a sterile Petri dish (10 cm diameter) on filter paper. Six replicates with five pods and five kernels each were maintained. These peanut pods and kernels were treated with a requisite concentration of 120 ppm of EE and 281.2μM of L separately and inoculated with A. flavus by gently applying a conidial spore suspension (1×106 spores/ml) to the surfaces of the pods and kernels, followed by incubation at 25°C. To maintain high humidity, sterile distilled water (1-2 ml) was added every day during the first five days. After six days of incubation, the percentage incidence of A. flavus in the treated and untreated samples was determined by counting the number of kernels contaminated by A. flavus. The selection of concentrations was based on effectiveness, with a focus on choosing the lowest concentrations that could be used effectively, with their effects exceeding 50% on nematodes. This allowed them to be relied upon in establishing the toxicity threshold and calculating LC50.

2.7. Evaluation of the nematocidal activity

2.7.1. Nematode culture

Culture preparation of egg masses from each nematode species, root-knot nematode (M. incognita) from infected eggplant, and T. semipenetrans from infected citrus roots, was performed. Root tissues were placed in tap water for egg hatching. The egg suspension was poured onto cotton-wool paper and incubated at 28±2°C to obtain freshly hatched juveniles (J2). Juveniles were collected within 48 hours and used. [26]

2.7.2. Mortality test

A stock solution of each R. tingitana sample (EE) and (L) at concentrations of 2.5, 5, 10, 20, 40, 80, and 120 ppm was prepared by soaking the specified amount of each material (EE and L) in distilled water. One milliliter of nematode suspension containing 100 freshly hatched juveniles of Meloidogyne incognita and Tylenchulus semipenetrans was added to a fixed volume of the samples in Petri dishes (80mm). Additionally, 100 freshly hatched second-stage larvae of M. incognita were placed in 5ml of distilled water as a control. All dishes were incubated in an incubator at (25±2°C). After 24, 48, and 72 hours, the juveniles were counted for mortality and non-mortality under a stereoscope microscope. The death of nematodes was confirmed by keeping them in tap water for 24 hours. The percent mortality was calculated from an average of three replicates.

2.7.3. Greenhouse experiment

A greenhouse experiment was conducted to study the effect of R. tingitana on the nematode population of Meloidogyne incognita. One-month-old eggplant seedlings, Solanum melongena cv. White Balady, with uniform size, were transplanted singly into 15cm clay pots filled with sandy clay soil (2:1, v:v) at rates of 120, 80, and 40 ppm per pot. One week later, each pot was inoculated

with 2000 freshly hatched juveniles of Meloidogyne incognita. Each treatment was replicated three times.

All treatments were arranged in a completely randomized scheme under greenhouse conditions at a temperature of 35±2°C. Additionally, three control treatments were performed, including the bio-product (The tested Micronema (Bionematicide containing bacteria, supplied by the Agriculture Research Center, Giza, Egypt, from DOTRA Co, Haram, Giza, Egypt) applied at recommended rates as a soil drench by suspending the required quantity in 12ml. One week later, each pot was inoculated with 2000 freshly hatched juveniles of M. incognita. There were also untreated infected plants and untreated plants. Pots were watered periodically every two days, and the plants were harvested after 45 days from the time of inoculation.

2.7.4. Percentage reduction in nematode enumeration and plant growth parameters determination

The soil of each pot was processed for nematode extraction using the sieving and Baerman–pan technique (27). The count of second-stage juveniles (J2) within the soil of every pot was determined using a Hawksley counting slide and a stereoscopic microscope. Similarly, average records of eggs/egg masses were determined by washing four randomly selected egg masses per root system of every replicate in 1% sodium hypochlorite to release eggs from the egg matrix. The released eggs were then suspended in water and counted using a stereoscopic microscope. Galls and egg masses and their indices were evaluated, and collected juveniles were counted. The reduction percentage in gall formation, egg mass production, as well as female and juvenile numbers, were calculated using the following formula:

$$R\% = \frac{\text{Treatment - Control infected}}{\text{Control infected}} \times 100$$

The final population and nematode build-up were calculated for all treatments. The final population (PF) included the total number of juveniles in the soil, egg-masses, and females. The rate of build-up, denoted as (PF/PI), was determined by dividing the nematode final population (PF) by the nematode initial population (PI) (PI). [28] Plant growth responses, including shoot length, fresh and dry shoot weights, as well as root fresh weight and length, were measured and calculated for all treatments.

The data were subjected to analysis of variance (ANOVA), as outlined by Snedecor and Cochran. [29] Treatment means were compared using Duncan's Multiple Range Test at a 5% level of probability. [30] These analyses were conducted using SPSS Program version 16.

3. RESULTS

3.1. Isolation and identification of the major component of *n*-hexane extract

Lupeol was isolated as the major phytochemical in the hexane-soluble fraction of the ethanol extract from the aerial parts of R. tingitana. Its identity was confirmed based on spectral data; ^{1}HNMR and ^{13}CNMR (400MHz, and 100 MHz $CDCl_3$) which are presented in Table 1 and compared with previously reported data. [4] Lupeol was obtained as white needle crystals (50 mg) and crystallized from methanol with a melting point of 210-212°C. It gave a positive response in the Liebermann–Burchard test, indicating the presence of a triterpenoid skeleton. A violet color was observed with P-anisaldehyde / H_2SO_4 spray reagent. The IR spectrum exhibited absorption bands of hydroxyl group at 3415 cm^{-1} as well as olefinic bond absorption band at 1642, 880 cm^{-1} and 2945, 2869 cm^{-1} for (C-H). EI Mass (70eV) m/z: showed a molecular ion peak (M+) at 426.7 calculated for $C_{30}H_{50}O$ with characteristic fragment ions at 411 (M+- Me), 393 (M+-Me-H_2O), 365, 299, 297, 245, fragment ions at m/z 220, m/z 207 (allocate the hydroxyl group at C3 position), m/z 218, m/z 205 and m/z 189 all in accordance with the lupene skeleton[4]. The ^{1}HNMR spectrum (400 MHz in $CDCl_3$) in Table 1 exhibited seven tertiary methyl groups and the vinylic methyl at δ 1.69 ppm. In addition, a signal at δ 3.15 with a coupling of ~3 and 6 Hz is indicative of C3-H α-orientation. Two vinylic proton signals at δ 4.59 and 4.70

ppm are indicative of a terminal methylene group at C-20. ^{13}CNMR (100 MHz, in $CDCl_3$) in Table 1 confirmed the chemical shift assignments for lupeol, which were consistent with previously reported data. [4] The ethanol extract of the aerial parts and the main isolate were then tested for their antifungal and nematicidal effects.

Table (1): ^{1}HNMR and ^{13}CNMR (400MHz, and 100 MHz$CDCl_3$) of lupeol

Position	δH ppm	δC ppm
1		38.7
2		27.4
3	3.23 (1H,m)	79.0
4		38.8
5		55.3
6		18.3
7		34.6
8		40.8
9		50.4
10		37.1
11		21.1
12		25.1
13		38.0
14		42.8
15		27.6
16		35.5
17		43.0
18		47.9
19	2.36 (1H,t)	48.3
20		150.9
21		29.9
22		40.0
23	0.78 (3H, s)	28.0
24	0.81 (3H, s)	15.3
25	0.85 (3H, s)	16.1
26	0.96 (3H, s)	15.9
27	0.99 (3H, s)	14.5
28	1.05 (3H, s)	18.0
29	4.59 (1H, br.s, H-29a) 4.70 (1H, br.s, H-29b)	109.3
30	1.70 (3H,s)	19.3

3.2. Antifungal activity

3.2.1 Inhibition of mycelial growth

The results of antifungal screening with various concentrations of EE and L from R. tingitana are presented in Table 2. In this study, EE exhibited a greater reduction in mycelial growth compared to L. All concentrations showed inhibitory activity against the fungus A. flavus. Different concentrations of R. tingitana extract inhibited the growth of A. flavus in vitro, as shown in Table 2. Among them, the concentrations of 100, 110, and 120 ppm were the most effective in reducing the radial growth of the fungus, while the concentration of 10 ppm was the least effective in inhibiting the radial growth of this fungus.

Table (2): Effect of different concentration of crude extract (EE)and Lupeol (L) from *R. tingitana* on the percentage of reduction in fungal growth and spore germination of *Aspergillus flavus*

Plant materials	Concentration (ppm)	Diameter of colony (mm)	%of inhibition (%)	%of germination (%)	(%)Inhibition of spore germination
EE	10	77	7.2	100	0
	20	70	15.7	95	5
	30	65	21.7	87	13
	40	52	37.3	80	20
	50	50	39.8	78	22
	60	45	45.8	68	32
	70	41	50.6	53	47
	80	39	53.0	45	55
	90	37	55.4	37	63
	100	28	66.3	35	65
	110	25	69.9	28	72
	120	19	77.1	22	78
L	10	82	0.0	100	0
	20	81	1.2	100	0
	30	78	4.9	97	3
	40	75	8.5	86	14
	50	68	17.1	81	19
	60	57	30.5	74	26
	70	55	33.0	58	42
	80	53	35.4	48	52
	90	44	46.3	40	60
	100	41	50.0	38	62
	110	37	54.9	32	68
	120	29	64.6	27	73
Control	100	82	0.0	100	0.0

EE: Ethanol extract of the aerial parts of *R.tingitana*,L: Lupeol

3.2.2. On spore germination

The data presented in Table 2 demonstrate a reduction in spore germination of A. flavus due to the inhibitory effects of the two tested samples of R. tingitana (EE and L). The highest proportion of spore germination was observed at 30 ppm of EE and L from R. tingitana, with percentages of 87% and 97%, respectively. Conversely, the lowest percentage of spore germination was observed at 120 ppm of EE and L, with percentages of 22% and 27%, respectively. Lupeol (L) appears to be more effective in inhibiting spore germination than EE.

3.2.3. Inhibition of pod and kernel treatments

The in vivo inhibitory activities of active R. tingitana samples on A. flavus incidence in peanut pods and kernels of the susceptible variety (Giza 3) were determined and the results are presented in Table 3. Inoculation of A. flavus on pods and kernels resulted in 100% infection after 6 days in the absence of treatment. However, when peanut pods and kernels were treated with EE and L of R. tingitana at a concentration of 120 ppm immediately before, during, or after inoculation, A. flavus colonization was completely inhibited.

There was no significant difference observed between the three treatment timings with EE of R. tingitana in terms of the inhibition percentage of A. flavus (100%). On the contrary, the percentage of A. flavus incidence was significantly reduced when treated with lupeol (L) of R. tingitana immediately after inoculation (100%) for both pods and kernels. In the case of lupeol treatment two days before inoculation, the incidence of A. flavus decreased to 90% for both pods and kernels. Similarly, when treated two days after inoculation, the incidence decreased to 80% for pods and 90% for kernels.

Table (3): Effect of different concentrations of crude extract (EE) and Lupeol(L) from *R. tingitana* on the percentage of reduction in *A. flavus incidence*

***R. tingitana* treatments (120ppm)**	**Treatment time**	***A.flavus* incidence (%)**		**Inhibition over control (%)**	
		Pods	**Kernels**	**Pods**	**Kernels**
EE	Immediately after inoculation	0[d]	0[c]	100[a]	100[a]
	Two days before inoculation	0[d]	0[c]	100[a]	100[a]
	Two after before inoculation	0[d]	0[c]	100[a]	100[a]
L	Immediately after inoculation	0[d]	0[c]	100[a]	100[a]
	Two days before inoculation	10[c]	10[b]	90[b]	90[b]
	Two days after before inoculation	20[b]	10[b]	80[c]	90[b]
Without (control)		100[a]	100[a]	0[d]	0[c]

Means within the same column followed by the same letter are not significantly different according to Duncan's multiple range test ($P \geq 0.05$). EE: Ethanol extract of the aerial parts of *R.tingitana* , L: Lupeol

Plants produce a variety of compounds as a defense mechanism against various microorganisms, including plant pathogens and environmental organisms. These compounds are indicative of the effective protection mechanisms developed by plants. Consequently, plants and their secondary metabolites offer a promising source of structurally diverse active compounds, including antimicrobials. [31] Lupeol, a triterpene belonging to the lupane class, has been previously reported to exhibit potent antibacterial and antifungal properties. [4] The ethanol extracts of the aerial parts of R. tingitana showed superior inhibitory activity against the fungus Aspergillus flavus compared to lupeol. This difference in activity may be attributed to the synergistic effect of the secondary metabolite components present in the ethanol extract. In global peanut production, contamination with A. flavus and aflatoxins is a significant challenge, as these mycotoxins are of great concern due to their toxicological impact on humans and animals. [32] Raji and Raveendran [33] have reported that water extracts from Asteraceae members exhibit strong inhibitory effects on the growth of A. niger compared to species from other plant families. They suggest that specific compounds with unknown functional groups present in Asteraceae members may play a role in inhibiting fungal colonies. This observation may explain the fungal inhibitory properties observed in the Asteraceae family member, R. tingitana. In line with this, both EE and L at a concentration of 120 ppm from R. tingitana inhibited A. flavus incidence in peanut pods and kernels.

Extensive research has been conducted to investigate the antifungal activity and potential mechanisms, as well as the in vitro and in vivo anti-aflatoxigenic efficiency, of natural products derived from medicinal plants against A. flavus. [34,35]

3.3. Nematicidal activity

The impact of R. tingitana EE and L extracts at seven concentrations (2.5, 5, 10, 20, 40, 80, and 120 ppm) on the mortality percentage of newly hatched M. incognita and T. semipenetrans juveniles is presented in Table 4. In general, a positive relationship has been observed between nematode juveniles' mortality percentages and extract concentration at three different exposure times. Larval mortality percentages increased with the increase of R. tingitana extract concentration from 2.5 ppm up to 120 ppm. Table 4 also demonstrates that the tested concentrations of EE were superior to those of L in terms of larval mortality percentages at all exposure times, with mortality values of 98.2% and 91.8%, respectively, after 72 hours.

Furthermore, the records in Table 4 illustrate the efficacy of R. tingitana EE and L on the mortality percentage of T. semipenetrans juveniles at the seven concentrations (2.5, 5, 10, 20, 40, 80, and 120 ppm). Both EE and L reduced the activity and induced mortality in the nematode. It is also evident that the tested concentrations of R. tingitana EE were superior to those of L in terms of larval mortality percentages at all exposure times, with the highest mortality (93%) occurring at 120 ppm within 72 hours and the lowest (33.33%) at 2.5 ppm. Conversely, L recorded the highest mortality (88%) at 120 ppm within 72 hours and the lowest (24%) at 2.5 ppm.

Table (4): The nematicidal effects of *R.tingitana* (EE& L) on mortality percentage of *T. semipenetrans* and *M. incognita* juveniles under different concentrations at different exposure times.

Treatment	Conc.	*T. semipenetrans*			*M. incognita*		
		%Mortality(J2) / Time			% Mortality(J2) / Time		
		24h,	48h.	72h.	24h,	48h.	72h.
EE	2.5	29.77	32.22	33.33	31.33	35.33	38.12
	5.0	33.54	39.00	42.77	36.00	41.00	49.67
	10.0	41.66	43.32	49.99	43.77	46.65	58.00
	20.0	42.87	46.00	53.11	45.66	55.76	63.75
	40.0	50.66	53.33	89.65	66.53	72.54	83.87
	80.0	53.22	65.33	91.00	79.55	88.60	92.44
	120.0	69.00	84.33	93.00	80.88	89,92	98.22
L	2.5	20.00	23.07	24.00	25.30	28.62	33.97
	5.0	25.77	26.99	38.23	32.33	35.45	39.93
	10.0	30.76	31.22	40.34	35.43	41.66	55.21
	20.0	32.46	42.00	49.22	45.00	53.55	66.23
	40.0	35.76	45.77	65.33	65.43	69.00	84.00
	80.0	52.08	75.00	84.37	69.77	72.78	88.66
	120.0	63.33	79.34	88.00	75.87	85.55	91.89
Check	00	00	00	00	00	00	00

Conc.:concentration, EE: Ethanol extract of the aerial parts of *R.tingitana* ,L Lupeol.

3.3.1. Efficacy of R.tingitana application on M. incognita development infected eggplant (Solanum melongena) under greenhouse conditions.

Data in Table 5 reveal that R. tingitana EE and L, when applied at rates of 40, 80, and 120 ppm/pot, exhibit bio-nematicidal potential. This potential was compared with the bio-product Micronema, used as a standard bio-nematicide at a dose of 12 ml/pot against Meloidogyne incognita infecting eggplant. The results showed a significant reduction in gall formation, egg masses, the final population, nematode build-up, and egg production per egg mass compared to the untreated control.

Differences in nematode reduction were evident among the treatment doses. The higher the dose, the greater the decrease in nematode numbers, with the maximum dose of 120 ppm/pot yielding the best results. There was a noticeable decrease in nematode juveniles (J2) per soil across all treatments, regardless of the concentration levels.

Table (5): Effect of *R.tingitana* (EE and L) on *M. incognita* development infected eggplant(*Solanum melongena*) under greenhouse conditions.

Treated	Dose /pot	Galls/ root	%R	In soil	%R	Egg masses /root	%R	Eggs/ egg mass	%R	Females	%R	F.P	%R	R.B
	120ppm	69^{a}±4.10	88.24	479^{a}±3.48	87.95	18^{a}±1.15	92.82	95^{a}±2.08	80.80	48^{a}±2.08	86.47	640 ^{a}±5.49	87.39	0.32
	80ppm	96^{b}±5.51	83.64	1114^{e}±7.02	71.98	26^{b}±1.15	89.64	156^{c}±1,00	68.48	61^{b}±1.00	82.81	1357^{d}±12.35	73.27	0.63
EE	40ppm	157de±10.14	70.18	2117^{g}±16.67	46.67	68^{d}±3,00	72.90	242^{f}±1.45	51.11	98^{d}±1.45	72.39	2525^{f}±15.68	50.26	1.26
	120ppm	138^{c}±8.37	76.49	727^{b}±14.53	81.71	63^{d}±1.45	74.90	139^{b}±1.35	71.91	117^{e}±1.53	67.04	1045^{b}±18.01	79.41	0.52
L	80ppm	179^{e}±2.89	69.50	1312^{f}±6.11	67.01	101^{f}±2,08	59.76	167^{cd}±0.58	66.26	161^{f}±0.58	54.64	1741^{e}±11.46	65.70	0.87
	40ppm	235^{f}±2.79	59.96	2263^{h}±29.63	43.09	121^{g}±3.84	51.79	203^{e}±6.01	58.98	213^{g}±6.01	40.00	2810^{g}±24.53	44.65	1.40
Bio product	12ml	127^{c}±2.87	78.36	853^{d} ±4.26	80.08	76^{e}±186	69.72	175^{d}±2.19		81^{c}±0.88	77.18	1127^{c}±10.99	80.08	56.
Check	-----	587^{g}±18.02	-------	3977^{i} ±14.53	------	251^{h}±1053		495^{g}±3.18		355^{h}±0.88	----	5077^{h}±13.75	-----	2.53
F. value		389.20	------	3766.03	-----	1114.78	-----	805.33	---	526.70	-----	6965.75	------	-----
P. value		00		0.00		0.00		0.00		0.00		000		-----

* All values are the mean of three replicates. Numbers following "±" represent the standard errors (SE). Different letters in the same column indicate statistically significant differences at the 0.05 probability level according to the Duncan test.EE: Ethanol extract of the aerial parts of *R.tingitana* ,L Lupeol,R:reduction,R.B: rate of buildup, F.P:final population.

For instance, both EE and L at the highest concentration level of 120 ppm/pot significantly reduced the percentage of nematode juveniles, with reductions of 87.39% and 81.71%, respectively. The lowest reduction in juvenile count, 46.76% and 43.09%, was obtained at a concentration of 40 ppm, respectively. As the concentration increased, the number of galls progressively decreased. The highest reduction, 88.24% and 76.49%, was observed at a concentration of 120 ppm in both EE and L treatments, respectively, while the lowest reduction was 72.39% and 40.00% at a concentration of 40 ppm.

Counts of egg masses per plant in most treatments were significantly lower than those in the control. The highest percentage reductions in egg mass production, 92.82% and 74.90%, were achieved at a dose of 120 ppm/pot in both EE and L treatments, respectively. Counts of nematode females within the roots of eggplants treated with R. tingitana EE showed that the higher dose resulted in lower female counts. The highest percentage of reduction in females was 86.47%, while the lowest was 72.63%. In the case of the L treatment, the highest concentration resulted in a reduction of 67.04%, and the lowest concentration led to a reduction of 59.84%.

On the other hand, both EE and L suppressed the final nematode population values, with averages of 87.39% and 79.41% at a concentration of 120 ppm, respectively. In comparison, Micronema, used as a standard bio-nematicide at a dose of 12 cm/pot, suppressed the final nematode population values with an average of 77.80%. The calculated rates of build-up revealed a similar trend, with the higher-dose treatments (EE and L) resulting in nematode populations folding by 0.42 and 0.52, respectively, at the highest concentration of 120 ppm, compared to 1.26 and 2.53 folds in the control treatment.

3.3.2. Growth response of M. incognita-infected eggplant plants as influenced by of R.tingitana (EE and L) application

Improvement in plant growth parameters, such as shoot and root length, as well as weights, varied and correlated with R.tingitana EE and L treatments at different doses

(Table 6). The concentration of 120 ppm in the EE treatment resulted in the best outcomes, with significant increases in fresh weight (91.68%), dry weight (81.00%), shoot length (28.21%), and leaf count (40%). In comparison, the L treatment at the same concentration caused increases of 68.75%, 33.33%, 25.64%, and 20%, respectively. On the other hand, the lowest concentration of 40 ppm in the EE treatment led to percentage increases of 31.25%, 31.19%, 10.98%, and 20%. In contrast, the standard bio-nematicide, Micronema, at a dose of 12 cm/pot, showed significant percentage increments in fresh weight (68.75%), dry weight (57.43%), and shoot length (18.33%). Therefore, the EE of R.tingitana exhibited the most significant improvement in plant growth parameters.

Table (6): Plant growth parameters of eggplant (*Solanum melongena*) affected by *Meloidogyne incognita* and treated by *R.tingitana* (EE and L) in different concentration under greenhouse conditions

Treatments	Dose/pot	Shoot								Root			
		Length (cm)	%Increase	Fresh Weight g)	%Increase	Dry Weight (g)	%Increase	Leaves (no)	%Increase	Length(cm)	%Increase	Fresh Weight(g)	%Increase
EE	120ppm	$41.33^{d}\pm1.86$	28.21	$30.67^{d}\pm1.86$	91.68	$6.23^{d}\pm0.37$	81.63	$7.00^{c}\pm0.33$	40.00	$22.67^{abc}\pm1.20$	22.05	$20.60^{d}\pm1.40$	31.57
	80ppm	$35.67^{abcd}\pm0.33$	16.82	$25.67^{c}\pm2.40$	60.43	$5.50^{cd}\pm0.35$	60.34	$6.00^{bc}\pm0.33$	20.00	$21.33^{abc}\pm0.88$	17.15	$16.67^{c}\pm0.33$	23.99
	40ppm	$33.33^{abc}\pm0.33$	10.98	$25.00^{c}\pm0.58$	31.25	$4.50^{b}\pm0.15$	31.19	$6.00^{bc}\pm0.33$	20.00	$21.00^{abc}\pm2.08$	15.85	$15.33^{c}\pm0.33$	17.35
L	120ppm	$39.33^{bcd}\pm0.67$	25.64	$27.00^{cd}\pm2.00$	68.75	$4.57^{b}\pm0.47$	33.23	$6.00^{bc}\pm0.58$	20.00	$25.67^{c}\pm0.67$	31,16	$16.00^{c}\pm0.58$	20.81
	80ppm	$37.67^{bcd}\pm1.45$	21.23	$21.00^{b}\pm0.58$	31.25	$4.13^{ab}\pm0.12$	20.40	$5.00^{a}\pm0.58$	--	$21.33^{abc}\pm0.67$	17.15	$15.33^{c}\pm0.33$	17.35
	40ppm	$33.33^{abc}\pm5.51$	10.98	$19.33^{ab}\pm0.33$	20.81	$3.87^{ab}\pm0.03$	12.82	$5.00^{a}\pm0.00$	--	$19.67^{ab}\pm3.84$	10.16	$14.33^{bc}\pm0.33$	11.58
Bio product	12ml	$36.33^{abcd}\pm2.03$	18.33	$27.00^{cd}\pm0.58$	68.75	$5.40^{c}\pm0.32$	57.43	$6.00^{ab}\pm0.00$	20.00	$24.00^{bc}\pm1.15$	26.37	$15.33^{c}\pm 1.33$	17.35
Healthy		$30.67^{ab}\pm2.87$	3.26	$28.00^{cd}\pm1.15$	75.00	$6.07^{cd}\pm0.12$	76.96	$7.00^{c}\pm00$	40.00	$19.67^{abc}\pm3.84$	10.16	$16.00^{c}\pm.58$	20.81
Check	-	$29.67^{a}\pm0.33$	-----	$16.00^{a}\pm1.15$	----	$3.43^{a}\pm0.07$	------	$5.00^{ab}\pm0.33$	------	$17.67^{a}\pm0.33$	----	$12.67^{ab}\pm0$- 63 ---	--
F. value	-		-----	35.65	-----	20.62	-----			54.20	-----	46.06	
P. value		0.00	-----	0.00	-----	0.00	-----			0.00	----	0.00	----

All values are the mean of three replicates. Numbers following "±" represent the standard errors (SE). Different letters in the same column indicate statistically significant differences at the 0.05 probability level according to the Duncan test. EE: Ethanol extract of the aerial parts of *R.tingitana* ,L Lupeol.

Additionally, R.tingitana (EE and L) exhibited high toxicity against nematodes in laboratory exposure tests and proved effective in controlling nematode infestations in eggplant roots over a 45-day period. Furthermore, improvements in plant growth parameters, such as shoot and root length and weight, were observed and varied depending on the R.tingitana (EE and L) treatments at different doses. The induction of resistance in susceptible host plants against invading nematodes has been a significant goal in developing nematode management programs. The application of amino acids to infected plants may elevate protein, lipid, and phenol levels [36] thereby activating physiological processes in plants to resist and overcome invading pathogens.

Many plant products associated with resistance responses to nematodes belong to classes of compounds such as alkaloids, glucosides, and organic acids. [37] Some metabolites found in several plant species have been tested for their nematicidal activity. For instance, Tithonia diversifolia (Hemsl.) A. Gray, which is rich in alkaloids, can suppress egg hatching of M.incognita by up to 98% starting from 2 days after incubation. The ethanol extract

of Alstonia scholaris flowers, when subjected to chromatographic examination, yielded several triterpenoid compounds that demonstrated nematicidal effects. [38] Numerous plant species worldwide are known to possess pesticidal properties. The application of botanicals, biological control, and soil amendment approaches is widespread, primarily due to their environmental safety. Several tested plants and phytochemical isolates with reported in vitro or in vivo nematicidal properties have been documented. [39] The mechanisms of action of plant extracts may include denaturing and degrading proteins, inhibiting enzymes, and interfering with electron flow in the respiratory chain or ADP phosphorylation. [40]

4. DISCUSSION

A wide variety of compounds are known to be produced by plants to defend themselves against various microorganisms, including plant pathogens and environmental organisms. These compounds are indicative of the effective protective mechanisms developed by plants. Consequently, plants and their secondary metabolites offer a promising source of structurally diverse active compounds, including potential antimicrobials. [30] Lupeol, as a lupane class triterpene, has been reported to exert potent antibacterial and antifungal properties. [4]

The aerial part ethanol extracts of R. tingitana demonstrated superior inhibitory activity against the fungus Aspergillus flavus compared to lupeol. This difference may be explained by the synergistic effects of the secondary metabolite components present in the ethanol extract. Worldwide peanut production faces a significant challenge due to contamination with A. flavus and aflatoxins, which are of great concern due to their toxicological effects on humans and animals. [31] Raji and Raveendran [32] reported that water extracts of Asteraceae members exhibited the strongest effect in reducing the growth of A. niger compared to species from other families. They suggested that specific compounds with unknown functional groups present in Asteraceae members may play a role in inhibiting fungal colonies. This may explain the antifungal properties observed in the study species of the Asteraceae family, H. radicata. Consistent with these findings, both EE and L at concentrations of 120 ppm from R. tingitana inhibited A. flavus incidence in peanut pods and kernels. The antifungal activity and potential mechanisms, both in vitro and in vivo, of natural products derived from medicinal plants against A. flavus have been extensively investigated. [33,34]

R. tingitana (EE and L) exhibited high toxicity against nematodes in laboratory exposure experiments. Furthermore, they proved effective in controlling nematode infestations in the roots of eggplants over 45 days. The improvement in plant growth parameters, such as shoot and root length and weight, varied and correlated with the doses of R. tingitana (EE and L) treatments. The induction of resistance in susceptible host plants against invading nematodes has been a major goal in the development of nematode management programs. The application of amino acids to infected plants may lead to elevated levels of proteins, lipids, and phenols. [35] These compounds, in turn, activate physiological processes in plants to help them respond to and overcome invading pathogens.

Many plant products associated with resistance responses to nematodes belong to classes of compounds like alkaloids, glucosides, and organic acids. [36] Some of the metabolites found in various plant species have already been tested for their nematicidal activity. For example, Tithonia diversifolia (Hemsl.) A. Gray, which is rich in alkaloids, can suppress egg hatching of M. incognita by 98% within 2 days after incubation. Chromatographic examination of the ethanol extract derived from the flowers of Alstonia scholaris revealed several triterpenoid compounds that demonstrated nematicidal effects. [37] Numerous plant species worldwide are known for their pesticidal properties. The application of botanicals,

biological control methods, and soil amendments, among other strategies, is considered environmentally safe. [41-43] Several tested plants and phytochemical isolates with reported in vitro or in vivo nematicidal properties have been documented. [38] The mechanisms of action of plant extracts may include protein denaturation and degradation, enzyme inhibition, and interference with electron flow in the respiratory chain or with ADP phosphorylation. [39]

5. CONCLUSION

The antifungal and bio-nematicidal effects of the ethanol extract (EE) from the aerial parts of R. tingitana are greater than those of lupeol (L). This difference can be attributed to the synergistic interactions among the various secondary metabolites present in the ethanol extract, which are responsible for the observed activities. Both EE and L have the potential to be used as antifungal and bio-nematicidal agents.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding was available.

Authors' contributions

Plant material supplement: OS. Design of the experiment and writing of the article: A M S, S G, GM, AH. Performing the chemical study: A MS. Performing the biological study: G M and AH. All authors have read and approved the manuscript.

ACKNOWLEDGEMENTS

Not applicable

REFERENCES

1. Alfy TE., Tantawy MEE., Motaal A A., Metwally FG. Pharmacological, biological study and GC/MS analysis of the essential oil of the aerial parts and the alcohol soluble fraction of the n. hexane extract of the flowers of Reichardia tingitana L. *Can J Pure Appl Sci*. 2015; 9: 3167-3175.
2. Sassi AB., Harzallah-Skhiri F., Bourgougnon N., Aouni M. Antiviral activity of some Tunisian medicinal plants against Herpes simplex virus type 1. *Nat Prod Res*. 2008; 22: 53-65.
3. Boussada O., Kamel MBH., Ammar S., Haouas D., Mighri Z., Helal AN. Insecticidal activity of some Asteraceae plant extracts against Tribolium confusum. *B Insectol*. 2008; 61: 283-289.
4. El Sayed A. M., Ezzat S. M., Sabry O. M. A. New anti-bacterial lupane ester from the seeds of *Acokanthera oppositifolia* Lam., *Nat Prod Res*.2016; 4: 1-6.
5. Aristimuño Ficoseco ME., Vattuone MA., Audenaert K., Catalan CA., Sampietro DA. Antifungal and antimycotoxigenic metabolites in Anacardiaceae species from northwest Argentina: isolation, identification, and potential for control of Fusarium species. *J Appl Microbiol*. 2014; 116(5): 1262-1273.
6. Horn BW., Dorner JW. Effect of nontoxigenic *Aspergillus flavus* and *A. parasiticuson* aflatoxin contamination of wounded peanut seeds inoculated with agricultural soil containing natural fungal populations. *Biocont Sci Techno*. 2009; 119: 249–262.
7. Perrone G., Gallo A*Aspergillus* Species and Their Associated Mycotoxins. *Mycotoxigenic fungi*. 2016; 1542: 33-49.
8. Boutrif E. Prevention of aflatoxin in pistachios. Food *Nutr Agric*. 1998; 21: 32-38.
9. Romani L. Immunity to fungal infections. *Nat. Rev. Immunol*. 2004; 4: 1-23.
10. Severns D. E., Clements M. J., Lambert R. J., White D. G. Comparison of Aspergillus ear rot and aflatoxin contamination in grain of high-oil and normal oil corn hybrids. J Food Prot. 2003; 66: 637-643.

11. Roze L. V., Hong S. Y., Linz J. E. Aflatoxin biosynthesis: current frontiers. *Annu Rev Food Sci T*. 2013; 4: 293-311.
12. Williams JH., Phillips TD., Jolly PE., Stiles JK., Jolly CM., Aggarwal D. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am. J. Clin. Nutr*. 2004; 80 (5): 1106-1122.
13. Abbas HK. Aflatoxin and Food Safety. *CRC Press*. 2005. ISBN 978-0-8247-2303-3.
14. Van Burik A. and Magee PT. Aspects of fungal pathogenesis in humans. *Annu Rev Microbiol*. 2001; 55: 743-772.
15. Lall N., Meyer JJM. Antibacterial activity of water and acetone extracts of the roots of Euclea natalensis. J *Ethnopharmacol*. 2000; 72: 313-316.
16. Magama S., Pretorius JC., Zietsman PC. Antimicrobial properties of extracts from *Euclea crispa* subsp. *crispa* (Ebenaceae) towards human, *S Afr J Bot*. 2002; 69: 199-203.
17. Anonymous, Agriculture Statistics of Pakistan, Govt. of Pakistan, Ministry of Food and Agriculture, Food and Agriculture Division. (Economic wing). Islamabad, Pakistan. 2010.
18. Khan MW., Khan MR., Khan AA. Identity of root-knot nematodes on certain vegetables of Aligarh district in northern India. *International Nematological Network News*. 1984; 1: 6-7.
19. Khan MR. Biological Control of Fusarial Wilt and Root-Knot of Legumes. Government of India Publication, Department of Biotechnology, Ministry of Science and Technology. New Delhi, India. 2005.
20. ElGayed SH., El Sayed A. M., Al-Ghonaimy AM., Abdel Wahab SM. High performance liquid chromatography-Ultraviolet (HPLC-UV) fingerprint profile and bioactivity of *Citrus aurantium* var. deliciosa fruits: peel and seeds on certain plant-parasitic nematodes. *J Med plants Res*. 2017; 11(15): 284-295.
21. Lee MD In: Nematodes Chapter 5Marek Renčo, Nicola Sasanelli and Lara MaistrelloPlants as Natural Sources of Nematicides ISNB: 978-1-62948-764-9© 2014 NOVA Science publisher, Inc. (2014).
22. Li G., Lu H., Zhang K. Volatile compound methyl thiobutyrate and application Faming Zhuanli Shenqing Gongkai Shuomingshu, CN103439426A. (in Chinese) 2013.
23. Nandwana RP, Varma MK, Arjun LAssociation of Tylenchulus semipenetrans with slow decline of citrus in humid South eastern plains of Rajasthan. *Ind. J. Nematol*. 2005; 35: 222-224.
24. Raper KB. and Fennell DI. The Genus *Aspergillus*. Robert EK Publish. Co., *Huntington*, New York, USA: 1977; 1-68.
25. Ismail IMK., Salanta A., Ali M., Cuf SA. Bioassay of Eucalyptus rostrata leaf extractives on *Sclerotium cepivorum Berk*. Egypt J Bot. 1989; 32: 109-26.
26. Nazli R., Akhter M., Ambreen S., Solangi AH., Sultana N. Insecticidal, nematicidal and antibacterial activities of *Gliricidia sepium*. Pak J Bot. 2008; 40(6): 2625-2629
27. Southey JF. Laboratory methods for work with plant and soil nematodes ministry of Agricultural Fish and Food Tech. Bulletin ed.2. Her Majesty`s Stationery Office London. 1970; 148.
28. Norton DC. Ecollogy of plant parasitic nematode.JonWilleg and Soms, New York. 1978; 238.
29. Sendecor GW., Cochran WG. Statistical methods. Oxford & J.BH Publishing com.7th edition; 1980.
30. Duncan D B. Multiple range and multiple F tests. *Biometrics*. 1955; 11: 1-42.
31. Chung PY., Navaratnam P., Chung LY. Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against *Staphylococcus aureus* strains. *Ann Clin Microbiol Antimicrob*. 2011; 9:10-25.
32. Torres AM., Barros GG., Palacios SA., Chulze SN., Battilani P. Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Res Int*. 2014; 62: 11-19.

33. Raji R., Raveendran K. Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger*. *Asian J Plant Sci Res*. 2013; 3(1): 13-15.
34. Hu Y., Zhang J., Weijun K., Gang Z., Meihua Y. Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on *Aspergillus flavus*. Food Chem. 2017; 220: 1- 8.
35. Tarazona A., Gómez JV., Gavara R., Mateo-Castro R., Gimeno-Adelantado JV., Jiménez M., Mateo E. M. Risk management of ochratoxigenic fungi and ochratoxinA in maize grains by bioactive EVOH films containing individual components of some essential oils. *Int J Food Microbiol*. 2018; 269: 107-119.
36. Vaitheeswaran M., Ibrahim SM., Selvapriya P., Kumar JV. Chemotherapeutic action of alanine as soil drench against root –knot nematode, Meloidogyne incognita on Vignaunguiculata. *Indian J. Nematol*. 2003; 33(2): 136-142.
37. Kaplan DT., Keen NT., Thomason IJ. Studies on the mode of action of glyceollin in soybean incompatibility to the root knot nematode, Meloidogyne incognita. Physiol. *Plant Pathol*. 1980; 16: 319-325.
38. Sultana N., Akhte M., Saify ZS., Khatoon Z., Ul-Hasan M., Qazi M. S., Ali Y. Isolation and structure determination of nematicidal iridoid sweroside from *Alstonia scholaris. J. Entomol. Nematol*. 2013; 5(2): 19-23.
39. Atolani O., Fabiyi OA. Plant Parasitic Nematodes Management Through Natural Products: Current Progress and Challenges. In: Ansari R., Rizvi R., Mahmood I. (eds) Management of Phytonematodes: Recent Advances and Future Challenges. 2020 Springer, Singapore. https://doi.org/10.1007/978-981-15-4087-5_13
40. Konstantopoulou I., Vassilopoulou L., Mawogantisi PP., Scouras G. Insecticidal effect of essential oils: a study of essential oils extracted from eleven Greek aromatic plants on Drosophila auroria. *Experientia* 1994; 48: 616-619.
41. Napolitano G., Fasciolo G., Venditti P., Mitochondrial Management of Reactive Oxygen Species Antioxidants (Basel, Switzerland). 2021; 10(11): 1824. https://doi.org/10.3390/antiox10111824.
42. ElSayed A.M., El ghwaji W., Youseif Z. M., El-Deeb K.S., ElSayed A.M. Fertility control impact of the aerial parts *Ferula tingitana L.* via alteration of hypothalamic-pituitary-gonadal axis responses of female Wistar rats. *Jordan J. Pharm. Sci*. 2022; 15(1).
43. Nour S., Salama M., Mahrous E, El-Askary H., Hifnawy M., Abdel Kawy M.A. *Severinia buxifolia* Leaves: Isolation, Characterization of Major Metabolites from the Bioactive Fractions and their Antiprotozoal Activity. *Jordan J. Pharm. Sci*. 2023; 16(1): 18-29.

تقييم إمكانات مبيدات الفطريات والنيماتودا في والديدان الخيطية النباتية على الفطريات الممرضة للنبات Reichardia tingitana (L.) Roth

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[4] قسم العقاقير، كلية الصيدلة، جامعة هليوبوليس للتنمية المستدامة، مصر.

ملخص

كانت إزالة العديد من مبخرات التربة ومبيدات النيماتودا هي الشغل الشاغل لسلامة الإنسان والبيئة. يمكن أن تسبب مسببات الأمراض الفطرية أمراضًا خطيرة للإنسان والحيوان. تتسبب العقدة العقدية الجذرية، و *Meloidogyne incognita* و *Tylenchulus semipenetrans* في حدوث ضرر كبير وانخفاض في الغلة للعديد من النباتات الاقتصادية. لذلك، كان الهدف من الدراسة هو تقييم أنشطة مبيدات الفطريات ومبيدات النيماتودا لمستخلص الإيثانول (EE) و lupeol (L)؛ عزلة رئيسية للأجزاء الهوائية من *Reichardia tingitana* L. Roth (Asteraceae) على *Aspergillus flavus* والنيماتودا الطفيلية النباتية. تم اختبار التأثيرات المضادة للفطريات لـ EE(10–120 جزء في المليون) و L(23.4– 281.2 ميكرومتر) من خلال مقايسات في المختبر والنمو في الجسم الحي ومقايسات تثبيط إنبات الجراثيم وفعالية تثبيط عدوى القرون والنواة، على التوالي. في حين تم اختبار نشاط مبيد النيماتودا من EEو Lخلال تحضير كتل بيض من أنواع النيماتودا *M.incognita* من الباذنجان المصاب و *T. semipenetrans* من جذور الحمضيات المصابة بتركيز 80،40،20،10،5،2.5 و 120 جزء في المليون (EE) *R. tingitana*. و (L)كانا نيماتوستاتيًا أو مبيدًا للنيماتودا لحيوية الديدان الخيطية، وفقس البيض في المختبر، والتطور والتكاثر في الجسم الحي. يلعب Lupeol دورًا فعالاً في تثبيط استعمار A. flavus في الفول السوداني. كان EEو Lشديد السمية ضد الديدان الخيطية في التعرض المختبري، كما أنه فعال في السيطرة على إصابة جذور الباذنجان بالديدان الخيطية لمدة 45 يومًا. كان التحسن في معاملات نمو النبات من حيث الفروع وطول الجذور والأوزان متغيرًا ومتناسبًا مع معاملتي EE و L بجرعات مختلفة. تتفوق التأثيرات المضادة للفطريات والمبيدات الحيوية لمستخلص الإيثانول في الجزء الجوي من يمكن استخدام *R.tingitana* على تأثير اللوبيول الذي يمكن تفسيره على أنه التأثير التآزري للمواد الكيميائية النباتية في مستخلص الإيثانول. كعوامل مضادة للفطريات ومبيدات حيوية.

الكلمات الدالة: الأفلاتوكسين، الرشاشيات فلافوس، الشوك الكاذب، اللوبيول، النيماتودا النباتية، الترايتيربين.

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تاريخ استلام البحث 2022/10/8 وتاريخ قبوله للنشر 2023/3/22.