

RESEARCH

Open Access



Insecticidal activity and possible modes of action of secondary metabolites of some fungal strains and wild plants as natural pesticides against *Spodoptera frugiperda*

Reda R. H. Abdullah^{1*}, A. H. Abd El-Wahab² and Samira A. Abd El-Salam³

Abstract

Background The fall armyworm (FAW), *Spodoptera frugiperda*, is an invasive and destructive pest to certain strategic crops, especially maize in Egypt. This research was conducted mainly to obtain secondary metabolites from some wild plants and fungal strains, use them in controlling FAW, and investigate their mode of action.

Results The ethyl acetate extracts of *Cladosporium cladosporioides* and *Verticillium lecanii*, as well as the alkaloid extracts of *Ricinus communis* and *Nicotiana glauca*, were extracted to obtain their secondary metabolites. The secondary metabolite contents were identified by gas chromatography mass spectrometry and NMR. The toxicity of all extracts against the 3rd-instar larvae of FAW was evaluated. The possible mode of action of the extracts was studied via their effects on larval enzyme activities and larval tissue. The toxicity results illustrated that, the extract of *C. cladosporioides* was more effective with LC₅₀ 229 ppm, followed by the extract of *V. lecanii* with LC₅₀ 341 ppm and *N. glauca* with LC₅₀ 404 ppm, while the least effective extract was *R. communis* with LC₅₀ 1110 ppm after 72 h of treatment. While, the results of larval enzyme activities showed that *C. cladosporioides*, *V. lecanii*, and *R. communis* extracts led to significant activity of AST, ALT, ACP, and ALP enzymes, but GST and AchE were inhibited in treated larvae compared with control. While *N. glauca* alkaloid extract caused significant inhibition of AST, ALT, ACP, AchE, and GST enzymes, ALP was activated in the treated larvae compared with control. The results of larval tissue slides indicated that the most affected tissues were the cuticle layer and the membrane lining of the midgut, in addition to the fatty bodies.

Conclusion Thus, natural pesticides would have a promising role in terms of controlling the FAW and according to this study, it was recommended that, alkaloid extracts of tested wild plants and ethyl acetate extracts of fungal strains be used as natural pesticides to control the fall armyworm, *S. frugiperda*.

Keywords Fall armyworm, *Cladosporium cladosporioides*, *Verticillium lecanii*, *Ricinus communis*, *Nicotiana glauca*, Natural pesticides

*Correspondence:

Reda R. H. Abdullah
redakenany@yahoo.com

¹ Cotton Pesticides Evaluation Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

² Piercing and Sucking Insect Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

³ Horticultural Insects Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

1 Background

Fall armyworm (FAW), *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), a destructive pest to certain strategic crops [1, 2]. It is native to North and South America and since 2016 started to invade the African continent causing a significant damage to certain cultivated crops particularly maize crops [3, 4]. Then, it has

subsequently spread throughout the continent and across Asia. However, fall armyworm had a wide range of plants hosts and could feed on over 350 plant species. Damage of FAW has been estimated to cause up to 13 billion US dollars per annum in crop losses throughout sub-Saharan Africa. According to CIMMYT Annual Report [5], FAW had devastated almost 1.5 million hectares of cultivated corn crops in six African countries. In Egypt, FAW was firstly recorded in maize field in a village in Kom Ombo city of Aswan governorate, Upper Egypt 2019 season according to the Agricultural Pesticide Committee (APC) and Ministry of Agriculture and Land Reclamation. As the north coast of Africa are environmentally suitable for FAW infestations besides its wide range of host plants, destructive damage would be expected, and it is contingent to harm other strategic crops such as cotton, soybean, and rice. Therefore, the population of FAW *S. frugiperda* should be under control.

So, many interested in the plant protection field in Egypt started to study several methods to suppress the FAW *S. frugiperda* population and reduce its damages. Among of these methods, using entomopathogenic fungi and plant extracts. Many previous studies reported that the mode of action of most entomopathogenic fungi refer to their production from bioactive secondary metabolites or enzymes and toxic proteins [6, 7]. In this study, authors used secondary metabolites of entomopathogenic fungi *Cladosporium sp.* and *Verticillium sp.* as well as alkaloid extract of wild plants castor bean, *Ricinus communis* and tobacco, *Nicotiana glauca*.

The fungus of *Cladosporium sp.* belongs to Ascomycota division. It was used as an entomopathogenic fungus to control several of insects in many previous studies. Shaker et al. [8] and Elbanhawey et al. [9] evaluated the efficiency of secondary metabolites which were extracted from *Cladosporium cladosporioides* against cotton aphids, *Aphis gossypii*. They found high toxicity effect where LC_{50} values were 24 and 36 ppm against nymphs and adults of aphids, respectively. Also, Habashy et al. [10] mentioned that spores suspension of *C. cladosporioides* caused high mortality percentage of spotted spider mites, *Tetranychus urticae*. Abdullah [11] isolated *Cladosporium sp.*, *Verticillium sp.*, *Epicoccum sp.*, *Alternaria sp.*, *Fusarium sp.*, *Penicillium sp.*, and *Aspergillus sp.* during his M.S. thesis from aphid and whitefly. The toxicity of isolated fungi was tested against aphids and whitefly. He found that *Cladosporium sp.*, *Verticillium sp.*, and *Epicoccum sp.* were more toxic fungi compared with other tested fungi. Also, Bahar et al. [12] isolated *Cladosporium sp.* from eggs of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and tested its pathogenicity against eggs and larvae of *H. armigera*, cotton aphids, *Aphis gossypii* and whitefly, *Bemisia tabaci*. *Verticillium lecanii* is

an opportunistic Ascomycete's fungus and widely distributed. *V. lecanii* (Zimm.) is commonly known as the "white holo.," and it causes mycosis of numerous insects belonging to the orders Lepidoptera, Coleoptera, and Homoptera [13]. *Lecanicillium lecanii*, *L. longisporum*, *L. attenuatum*, *L. nodulosum*, and *L. muscarium* are among the new taxonomic entities divided from the species *V. lecanii*. There are number of important species from the *Verticillium spp.* group, which are used as biocontrol agents to control insect pests and diseases in agriculture [14]. There are 15 productions of bioinsecticides belonged to *Verticillium spp.* commercialized worldwide [15, 16].

Castor bean (*Ricinus communis* L) is a species of perennial flowering plant that belongs to family Euphorbiaceae [17]. The phytochemical screening of *R. communis* indicated the presence of flavones, isoflavones, flavonols, chalcones, aurones, sterols, saponins, and leucoanthocyanidins [18]. In addition, six flavonol glycosides; kaempferol-3-O- β -D-xylopyranoside, kaempferol-3-O- β -D-glucopyranoside, quercetin-3-O- β -D-xylopyranoside, quercetin-3-O- β -D-glucopyranoside, kaempferol-3-O- β -rutinoside, and quercetin-3-O- β -rutinoside as well as two alkaloids; ricinine and N-demethylricinine were screened by Kang et al. [19].

Several previous studies reported that the crude extract of secondary metabolites of castor plant *R. communis* found to have insecticidal activity against pests such as sugarcane aphid, *Melanaphis sacchari* [20], acaricidal and insecticidal activities against *Haemaphysalis bispinosa* Neumann adult and hematophagous fly *Hippobosca maculata* Leach [21], malaria vector *Anopheles gambiae* [22] and in controlling the termites which damage the wood of *Mangifera indica* and *Pinus longifolia* [23].

Tobacco plant (*Nicotiana glauca*) is a flowering plant in the tobacco genus *Nicotiana* of family Solanaceae. The alkaloid fraction of *N. glauca* was analyzed by UPLC/MS and GC/MS, anabasine was found to be the major constituent (60%). In addition to, five compounds were identified as nicotine, nornicotine, ammodendrine, chlorogenic acid, and rutin [24]. The acute toxicity of *N. glauca* was due to the highly percentage of anabasine compound [25]. It was found that *N. glauca* plant had antioxidant activity as a result of its content of flavonoids and phenolic compounds, which recorded their highest values in the leaves and flowers [26]. As a result of the presence of a high percentage of alkaloids in *N. glauca*, great importance was given to its use as antimicrobial agents [27] and insecticides to control aphids [28]. The aim of this study is to extract the secondary metabolites from entomopathogenic fungi, *C. cladosporioides* and *V. lecanii*, as well as from wild plants, *R. communis* and *N. glauca*. In addition to evaluating the influence of their

crud extracts on larval mortality, enzyme activity, and larval tissue of the fall armyworm, *S. frugiperda*.

2 Methods

2.1 Insect rearing procedure

The colony of fall armyworm was established initially from larvae collected during the summer season of 2022 from corn field located in Dakahliah governorate. Larvae were reared individually to avoid cannibalism and were fed on fresh leaves of castor oil plants *Ricinus communis* L. replaced every 2 days, depending on how long they remain green and fresh. Larvae were kept in climatic control chamber ($24 \pm 1^\circ\text{C}$, 70% RH, 14L: 10D photoperiod) till pupation. Pupae were collected and placed in PVC container filled with sand under same condition until adult emergence. Following emerging, moths were collected and held in 1 L. glass mason jars, 20–40 moth per jar were kept together to encourage mating and were fed on honey bee solution from a cotton wick hanged from the jar top. Jars were kept in a growth chamber under same previous conditions with pieces of zigzag shaped papers to provide dark arena for eggs oviposition. Once mated adult started oviposition, egg mass were collected and held in plastic containers 200 ml in climatic control chamber until hatching. In order to obtain larvae for the experiment, a number of five neonate larvae were kept in plastic container 100 ml and were provided with small pieces of corn cob. Once larvae reached third instar, we isolate and count the proper number of larvae for the experiment then they were subjected to treatments.

2.2 Fungal strains

Fungal strains *Cladosporium cladosporioides* and *Verticillium lecanii* were kindly obtained from the Microbiology Department, Faculty of Agriculture, Mansoura University, Egypt. Both fungal strains were cultured on potato dextrose agar medium (PDA) made from potato infusion 200 ml, dextrose 20 g, and agar 15 g per liter, then incubated at 25°C . Figure 1 shows the colonies growth on potato dextrose agar medium and spore shapes under light microscope of both fungal strains.

2.3 Extraction of fungal metabolites from their broth cultures

One hundred ml of potato dextrose broth medium (PDB) was prepared in a 250-ml conical flask and sterilized in an autoclave. One disk of solid fungal culture was used to inoculate 100 ml of sterilized PDB medium. The inoculated medium was incubated to 7 days at 25°C . After incubation period, one litter of sterilized PDB medium was inoculated by 100 ml broth culture in 2 litter conical flask and incubated to 21 days at 25°C . After incubation period, the broth culture was filtered to remove

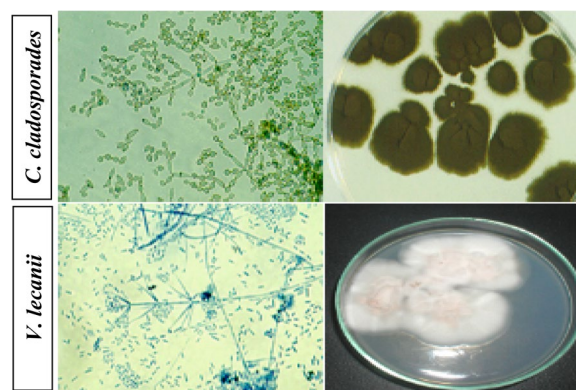


Fig. 1 The colonies growth of *C. cladosporioides* and *V. lecanii* on potato dextrose agar medium and their spore shapes under a light microscope

the mycelium and obtain to the filtrate by Whatman no1 filter paper, Sigma-Aldrich. The volume of filtrate was measured then the equal volume from ethyl acetate was added. The mixture was poured in separating funnel and moved well for homogenate then left to 2 h. The upper layer was taken then ethyl acetate was evaporated by rotary evaporator system to obtain the crud extract of fungal metabolites. This method was conducted as described by Abdullah [29].

2.4 Gas chromatography–mass spectrometry (GC–MS) analysis

Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m \times 0.25 mm \times 0.25 μm film thickness) was used to perform the chemical composition of fungal strains extracts. The temperature of the column oven was initially maintained at 50°C then raised by $5^\circ\text{C}/\text{min}$ to 250°C and maintained for two minutes then raised to final temperature 300°C by $30^\circ\text{C}/\text{min}$, where it was held for two minutes. The temperatures of injector and MS transfer line were maintained at 270°C , 260°C , respectively. The carrier gas was Helium at a constant flow rate of 1 ml/min. Electron impact (EI) mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200°C . By comparing the components' retention times and mass spectra to those in the WILEY 09 and NIST 14 mass spectral databases, the components were identified [29].

2.5 Plant material

Ricinus communis L "Euphorbiaceae" seeds were collected from plants in the villages of Shoha and Salamoun, beside to Mansoura city (31.065745° N–31.453642° E),

Table 1 Toxicity of secondary metabolites of tested fungal strains and wild plants against *S. frugiperda*

No.	Treatment	After 48 h of treatment				After 72 h of treatment				Toxicity index % (72h)
		LC ₅₀ (ppm)	95% Confidence limit (ppm)		Slope ± SE	LC ₅₀ (ppm)	95% Confidence limit (ppm)		Slope ± SE	
			Lower	Upper			Lower	Upper		
1	<i>C. cladosporioides</i>	886	674	1115	1.48 ± 0.30	229	86	346	2.01 ± 0.44	100
2	<i>V. lecanii</i>	1960	1610	2674	2.20 ± 0.34	341	154	485	1.54 ± 0.33	67
3	<i>N. glauca</i>	788	669	895	2.51 ± 0.30	404	250	527	1.89 ± 0.31	56
4	<i>R. communis</i>	2241	1773	3476	1.65 ± 0.31	1110	895	1373	1.46 ± 0.28	20



Fig. 2 Abnormal symptoms of *S. frugiperda* larvae after treatment by crude extracts of fungal strains and wild plants under laboratory conditions

Egypt in Mars 2022. *Nicotiana glauca* "Solanaceae" aerial parts were collected from Mansoura city (31.067849° N–31.415351° E), Egypt in June 2022. The collected plants were identified by prof. Ibrahim Mashaly, Botany department, Faculty of science, Mansoura University according to Boulos [30].

2.6 Extraction of secondary metabolites from plants

2.6.1 Processing of *Ricinus communis* L. (Seeds)

The dried seeds of *R. communis* L. (1 kg) were grinded and extracted with methanol (2L × 5). The

methanol extract was concentrated by rotary evaporator to obtained 300 ml oil extract. A certain amount of oil was exposed to PTLC (Preparative Thin Layer Chromatography, silica gel Merck GF 254 precoated plates 20 × 20 cm on aluminum sheets) with eluent system CHCl₃: MeOH (6:1) to isolated major compound (1), (25 mg, R_f 0.44), which gave positive result (orange color) with Dragendroff’s reagent.

2.6.1.1 Characterization of isolated compound (1) Compound (1): Slightly yellow to white needle crystal; ¹H NMR (Bruker 400 MHz with tetramethylsilane, CD₃OD/drops CD₃Cl, δ, ppm, J, Hz): 7.85 (d, J = 7.7 Hz, H-6), 6.33 (d, J = 7.7 Hz, H-5), 4.02 (s, 3H-9), and 3.53 (s, 3H-7). ¹³C NMR (100 MHz, CD₃OD/drops CD₃Cl): 37.8 (N-CH₃), 57.9 (O-CH₃), 88.2 (C-3), 95.2 (C-5), 114.4 (CN), 145.9 (C-6), 163.0 (C-2), 173.9 (C-4).

2.6.2 Processing of *Nicotiana glauca*

The dried powder aerial parts of *N. glauca* (1 kg) extracted with methanol (1.8L × 5). The methanol extract was concentrated by rotary evaporator till dryness (83 gm). The methanol extract was dissolved in small amount of methanol and then added distilled water acidified by 2N HCl till pH 3. The acidified mixture was extracted by separating funnel with chloroform three times. The acidified aqueous extract was basified using ammonia solution

Table 2 Changes in the enzyme activities in the treated larvae of *S. frugiperda* caused by the extracts of fungal strains and plants

Treatments	Insect enzymes activities					
	AST U/ml ± SE	ALT U/ml ± SE	ACP U/ml ± SE	ALP U/ml ± SE	AchE µg AchBr /min/gm body weight ± SE	GST Mmol sub. conjugated/min. mg protein ± SE
Control	2.82 ^c ± 0.09	2.56 ^d ± 0.11	0.055 ^c ± 0.001	0.71 ^d ± 0.020	786 ^a ± 94	1.32 ^a ± 0.10
<i>C. cladosporioides</i>	3.83 ^b ± 0.11	3.46 ^b ± 0.17	0.081 ^a ± 0.002	1.28 ^b ± 0.040	353 ^c ± 23	0.67 ^c ± 0.02
<i>V. lecanii</i>	4.34 ^a ± 0.17	3.99 ^a ± 0.06	0.058 ^c ± 0.001	1.42 ^a ± 0.015	576 ^b ± 5	0.96 ^b ± 0.06
<i>N. glauca</i>	2.32 ^d ± 0.15	1.21 ^e ± 0.03	0.040 ^d ± 0.003	1.24 ^b ± 0.058	282 ^c ± 14	1.04 ^b ± 0.03
<i>R. communis</i>	3.69 ^b ± 0.16	2.83 ^c ± 0.08	0.067 ^b ± 0.003	0.88 ^c ± 0.017	616 ^b ± 5	1.31 ^a ± 0.04
LSD (5%)	0.294	0.194	0.0045	0.069	160	0.197

ab,c,d,e The same letter in the same column means it is not significant at P 0.05. LSD The least significant difference

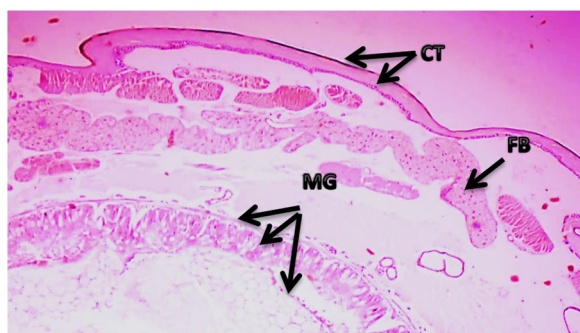


Fig. 3 Cross section through untreated 4th-instar larvae of *S. frugiperda*, stained using hematoxyline, Eosin (H, E X200), showing normal fibrous and cellular layer of cuticle (CT) also normal basement and peritrophic membranes in the midgut (MG) as well as normal cells of fat bodies (FB)

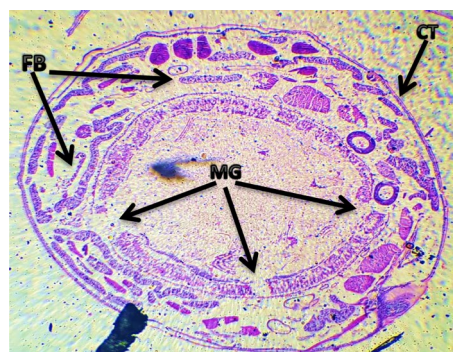


Fig. 6 Cross section through treated 4th-instar larvae of *S. frugiperda* by ethyl acetate extracts of *N. glauca* and stained using hematoxyline, Eosin (H,E X200), showing no effects in cells of cuticle layers (CT), severe necrosis and dissolved cells of fat bodies (FB), severe necrosis, dissolved and ruptured columnar cells and destroyed basement membrane of midgut (MG)

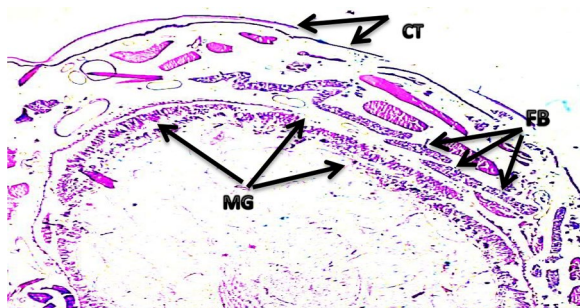


Fig. 4 Cross section through treated 4th-instar larvae of *S. frugiperda* by ethyl acetate extract of *C. cladosporioides* and stained using hematoxyline, Eosin (H, E X200), showing weak and necrosis of cuticle layers (CT), severe necrosis and dissolved cells of fat bodies (FB) and ruptured columnar cells and destroyed basement membrane of midgut (MG)

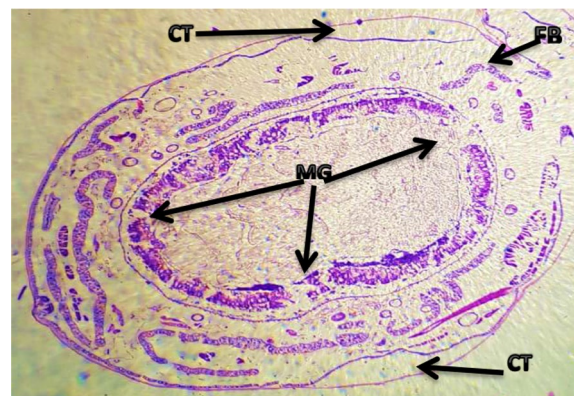


Fig. 7 Cross section through treated 4th-instar larvae of *S. frugiperda* by ethyl acetate extract of *R. communis* and stained using hematoxyline, Eosin (H,E X200), showing dissolved cells in fat bodies (FB), severe necrosis, dissolved and ruptured columnar cells and destroyed basement membrane of midgut (MG), necrosis and separating in some parts of cuticle layers (CT)



Fig. 5 Cross section through treated 4th-instar larvae of *S. frugiperda* by ethyl acetate extract of *V. lecanii* and stained using hematoxyline, Eosin (H,E X200), showing necrosis of some parts in cuticle layers (CT), no effects in cells of fat bodies (FB) and severe necrosis, dissolved and ruptured columnar cells and destroyed basement membrane of midgut (MG)

33% till pH 11 then extracted with chloroform (Alkaloid fraction 1.85 gm).

2.6.2.1 Characterization of identified compound (2) Compound (2) pale yellow oil. GC/MS, m/z (rel. int.): 162 (49%) [C₁₀H₁₄N]⁺, 133 (70%) [C₉H₁₁N]⁺, 119 (58%) [C₈H₉N]⁺, 105 (75%) [C₇H₇N]⁺, 92 (20%) [C₆H₆N]⁺, 84 (100%) [C₅H₁₀N]⁺.

2.7 Bioassay experiment

Spray bioassay method was used to evaluate the toxicity of four crud extracts as natural pesticides against *S. frugiperda* under laboratory conditions. Five serial concentrations of each crud extract were prepared (500,

1000, 1500, 2000, 2500 ppm). Ten third-instar larvae of *S. frugiperda* were transferred to each jar (10D * 25H cm). Pieces of castor leaves were used to feed larvae. Two milliliter of each concentrate were sprayed on larvae and castor leaves in each jar. Two milliliter of water were used to treat control group. The number of live larvae was recorded every day. Mortality percentages were calculated and corrected by Abbot's formula [31]. LC₅₀, confidence limits and slope values were calculated as described by Finney method [32]. Also, toxicity index was calculated as described by Sun [33].

2.8 Estimation of the changes in insect enzyme activities

Fourth-instar larvae of *S. frugiperda* were used to investigate the effect of tested secondary metabolites on insect enzymes activities. The same method of bioassay experiment was repeated but LC₅₀ of each crude extract was used to spraying the larvae. Also, water was used to spray control group. After four days, the live larvae were weighted and frozen in Eppendorf tube until analysis. All enzyme activities were estimated colorimetrically by UV-visible spectrophotometer, model V1200, China. The estimated insect enzymes were aspartate transaminase (AST), alanine transaminase (ALT) according to Reitman and Frankel [34] at 520 nm, acid phosphatase

Table 3 GC-MS analysis of ethyl acetate extract of the liquid culture of *C. cladosporioides*

No.	R.t min	Compounds	Peak area (%)	Molecular weight	Molecular formula
1	4.53	2,3-Butanediol	1.21	90.12	C ₄ H ₁₀ O ₂
2	10.35	4-Hydroxy-2-hexenoic acid -lactone	2.14	112.00	C ₆ H ₈ O ₂
3	15.21	Benzeneacetic acid	1.56	136.14	C ₈ H ₈ O ₂
4	19.34	Tetradecanoic acid	1.45	228.37	C ₁₄ H ₂₈ O ₂
5	21.81	Tetradecanoic acid, 12-methyl-, methyl ester	1.34	256.42	C ₁₆ H ₃₂ O ₂
6	23.65	n-Hexadecanoic acid (Palmitic acid)	31.63	256.42	C ₁₆ H ₃₂ O ₂
7	25.77	Hexadecanoic acid, methyl ester	14.74	270.45	C ₁₇ H ₃₄ O ₂
8	26.99	9,12-Octadecadienoic acid (Z,Z)- (Linoleic acid)	17.97	280.44	C ₁₈ H ₃₂ O ₂
9	29.20	9-Octadecenoic acid (Z)- (Oleic acid)	0.84	282.46	C ₁₈ H ₃₄ O ₂
10	31.93	Octadecanoic acid (Stearic acid)	5.07	284.47	C ₁₈ H ₃₆ O ₂
11	33.06	Hexadecanoic acid, 15-methyl-, methyl ester	5.87	284.47	C ₁₈ H ₃₆ O ₂
12	35.40	9,12-Octadecadienoic acid, methyl ester, (E,E)-	13.14	294.47	C ₁₉ H ₃₄ O ₂
13	37.74	Octadecanoic acid, methyl ester (Methyl stearate)	3.04	298.50	C ₁₉ H ₃₈ O ₂

R.t.: Retention time (min)

Table 4 GC-MS analysis of ethyl acetate extract of the liquid culture of *V. lecanii*

No	R.t min	Compounds	Area (%)	Molecular weight	Molecular formula
1	4	Tetramic acid	13.22	99.09	C ₄ H ₅ NO ₂
2	5.25	3-Methylazepin-2-one	9.20	121.14	C ₇ H ₇ NO
3	6.52	4-Acetoxy-3-methylbut-2-enoic acid. methyl ester	4.97	172.18	C ₈ H ₁₂ O ₄
4	7.45	7-Isopropyl-1,4a-dimethyldecahydronaphthalene # (Selinane)	16.09	208.38	C ₁₅ H ₂₈
5	8.5	Sandaracopimar-7.15-dien-6-one	4.81	286.50	C ₂₀ H ₃₀ O
6	10.32	5-(4-Chlorobenzoyl)-1,4-dimethyl-1H-pyrrole-2-acetic acid (Zomepirac)	6.35	291.73	C ₁₅ H ₁₄ ClNO ₃
7	11.52	Methyl ester of ricinoleic acid	14.9	312.5	C ₁₉ H ₃₆ O ₃
8	12.15	1-pyrazineacetamide. n-(2-cyano-4,5-dimethoxyphenyl)hexahydro-4-(2-hydroxyethyl)-	9.12	348.4	C ₁₇ H ₂₄ N ₄ O ₄
9	13.29	Ergost-5-ene-3,25-diol	5.02	416.70	C ₂₈ H ₄₈ O ₂
10	15.35	12-Deoxyphorbol-13-(2-methylpropionate)	5.44	418.5	C ₂₄ H ₃₄ O ₆
11	16.36	{[4-(tert-Butyldimethylsiloxy)-2,6-dimethylphenyl] (methoxy)methylene} pentacarbonylchromium(0)	4.42	470.51	C ₂₁ H ₂₆ CrO ₇ Si
12	17.38	(14.beta.20R)9,19-Cyclo-6,7-epoxylanostan-3-ol. acetate	6.46	484.8	C ₃₂ H ₅₂ O ₃

R.t.: Retention time (min)

(ACP), alkaline phosphatase (ALP) according to Powell and Smith [35] at 510 nm, acetyl choline esterase (AChE) according to Simpson et al. [36] at 515 nm, and glutathione S-transferase (GST) according to Pan et al. [37] at 540 nm.

2.9 Determination of histological changes in insect tissue

Also the fourth-instar larvae of *S. frugiperda* were treated by LC₅₀ of each extract using spray technique. Two live larvae of treated and untreated larvae were taken after five days of treatment and saved in 10% formalin solution. Tissue processing, sectioning, and staining were conducted according to Gaaboub et al. [38] in Faculty of Medicine, Mansoura University, Egypt.

3 Results

3.1 Toxicity effect of fungal and wild plants extracts against *S. frugiperda*

Table 1 shows the toxicity effect of ethyl acetate extracts of two fungal strains (*C. cladosporioides* & *V. lecanii*) and alkaloid fraction of *N. glauca* as well as methanol extract of *R. communis* against *S. frugiperda*. The more effective extract after 48 h of treatment was the alkaloid extract of *N. glauca* (LC₅₀=788 ppm) followed by the ethyl acetate extract of *C. cladosporioides* (LC₅₀=886 ppm), while there were different results after 72 h of treatment where the extract of *C. cladosporioides* was more effective extract (LC₅₀=229 ppm) followed by the extract of *V. lecanii* (LC₅₀=341 ppm) and *N. glauca* (LC₅₀=404 ppm). Also, there were different values of toxicity line's slope between all tested extracts. This illustrate that could be there were different mode of actions of tested extracts against *S. frugiperda*. In addition, toxicity index showed that *S. frugiperda* was more susceptible to *C. cladosporioides* extract than other tested extracts. Figure 2 shows some

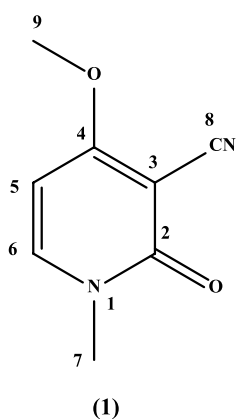


Fig. 8 Chemical structure of ricinine (compound 1)

Table 5 ¹H NMR data for ricinine (compound 1) in CH₃OD/CD₃Cl (δ) [ppm] (Multiplicity, J [Hz])

Position	(δ _H) Compound 1	(δ _C) Compound 1
1	–	–
2	–	163.0
3	–	88.2
4	–	173.9
5	6.33 (d, J=7.7 Hz, 1H)	95.2
6	7.85 (d, J=7.7 Hz, 1H)	145.9
7	3.53 (s, 3H)	37.8
8	–	114.4
9	4.02 (s, 3H)	57.9

abnormal symptoms of treated *S. frugiperda* larvae as necrosis and analysis of larvae body also the larva could not get rid of some parts of the cuticle layer, which led to the larva not growing normally.

3.2 The changes in insect enzymes activity

In this study, the changes of five insect enzymes activities were estimated in the treated larvae of *S. frugiperda*. The results in Table 2 show that *C. cladosporioides*, *V. lecanii*, and *R. communis* extracts led to significant activity of AST, ALT, ACP, and ALP enzymes, but GST and AChE were inhibited in treated larvae compared with control, while *N. glauca* alkaloid extract caused significant inhibition of AST, ALT, ACP, AChE, and GST enzymes, but ALP was activated in the treated larvae compared with control.

3.3 Histological changes of treated larvae

Figures 3, 4, 5, 6, and 7 show the histological changes of treated 4th-instar larvae by *C. cladosporioides*, *V. lecanii*, *N. glauca*, and *R. communis* extracts as well as control treatment. The description of histological changes is under each cross section's photo. The most affected tissues were the cuticle layer and the membrane lining of the midgut, in addition to the fatty bodies.

3.4 Discussion

The entomopathogenic fungi *C. cladosporioides* and *V. lecanii* will be active and promising agents to control the fall army worm (FAW) in Egypt. In this study, *C. cladosporioides* and *V. lecanii* were more toxic against FAW larvae with LC₅₀s of 229 and 341 ppm after 72 h, respectively. On the other hand, both fungi were used as entomopathogens against many insect pests in other studies, such as *Aphis gossypii* [8, 9, 11, 42], *Helicoverpa armigera*, *Bemisia argentifolii* [12], *Sitophilus*

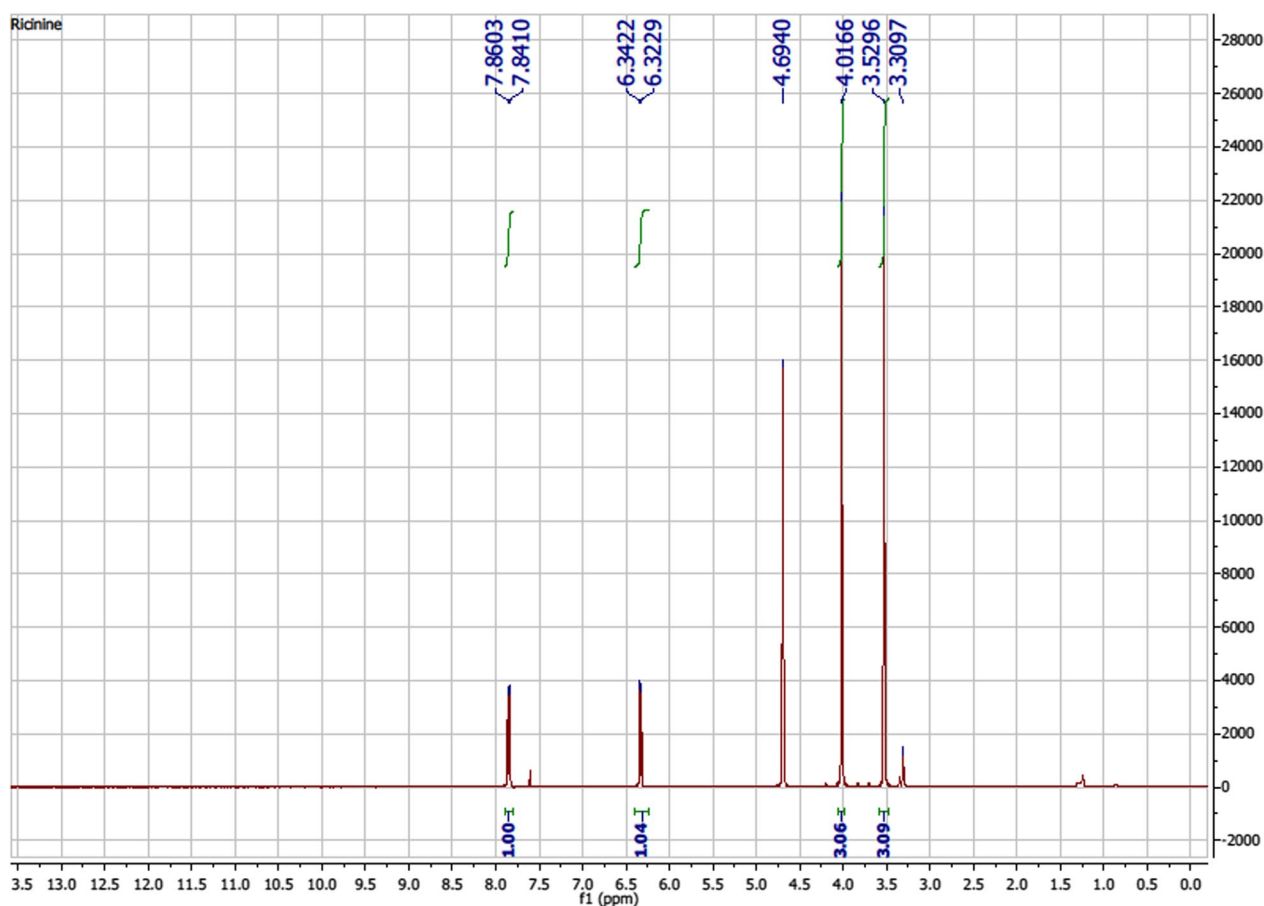


Fig. 9 ¹H NMR (400 MHz) spectrum of ricinine (compound 1) in CD₃OD/CD₃Cl

granarius, *S. oryzae*, *S. zeamais*, *Rhizopertha dominica*, and *Trogoderma granarium* [43], *Plutella xylostella* [44], *Nilaparvata lugens* [45, 46].

However, using entomopathogenic fungi to manage insect pests still has several flaws when it comes to field application. Entomopathogenic fungi are exposed to different weather stresses after being used in the field, including temperature [47, 48], humidity [48–50], and UV rays [51]. The entomopathogenic fungi produce a large number of bioactive secondary metabolites that have insecticidal properties [52]. Additionally, the entomopathogenic fungi can kill the pest more rapidly by producing some mycotoxins like beauvericin, cyclodepsipeptide, destruxin, and desmethyldestruxin in the early stages of infestation [53]. In this study, the tested fungi produced some compounds in the liquid culture that have insecticidal activities, like n-hexadecanoic acid, octadecadienoic acid, tetramic acid, selinane, and methyl ester of ricinoleic acid, as shown in Tables 1 and 2. Also, previous studies reported that

these compounds had insecticidal activity: n-hexadecanoic acid and octadecadienoic acid [29, 54]; methyl ester of ricinoleic acid [55]; selinane [56]; and tetramic [57, 58].

Tables 3 and 4 show the identified compounds in the ethyl acetate extract of *C. cladosporioides* and *V. lecanii* broth cultures by GC–MS analysis. Thirteen compounds were identified in *C. cladosporioides* extract. The major compounds were n-hexadecanoic acid (Palmitic acid), hexadecanoic acid, methyl ester, 9, 12-Octadecadienoic acid (Z, Z)- (Linoleic acid) and 9,12-octadecadienoic acid, methyl ester, (E,E)- where their peak area percentages were 31.63%, 14.74%, 17.97%, and 13.14%, respectively. On the other hand twelve compounds were identified in *V. lecanii* extract. Three compounds had the more percentages of peak area compared with other compounds. These compounds were tetramic acid (13.22%), 7-Iso-propyl-1,4a-dimethyldecahydronaphthalene # (Selinane) (16.09%) and methyl ester of ricinoleic acid (14.9%).

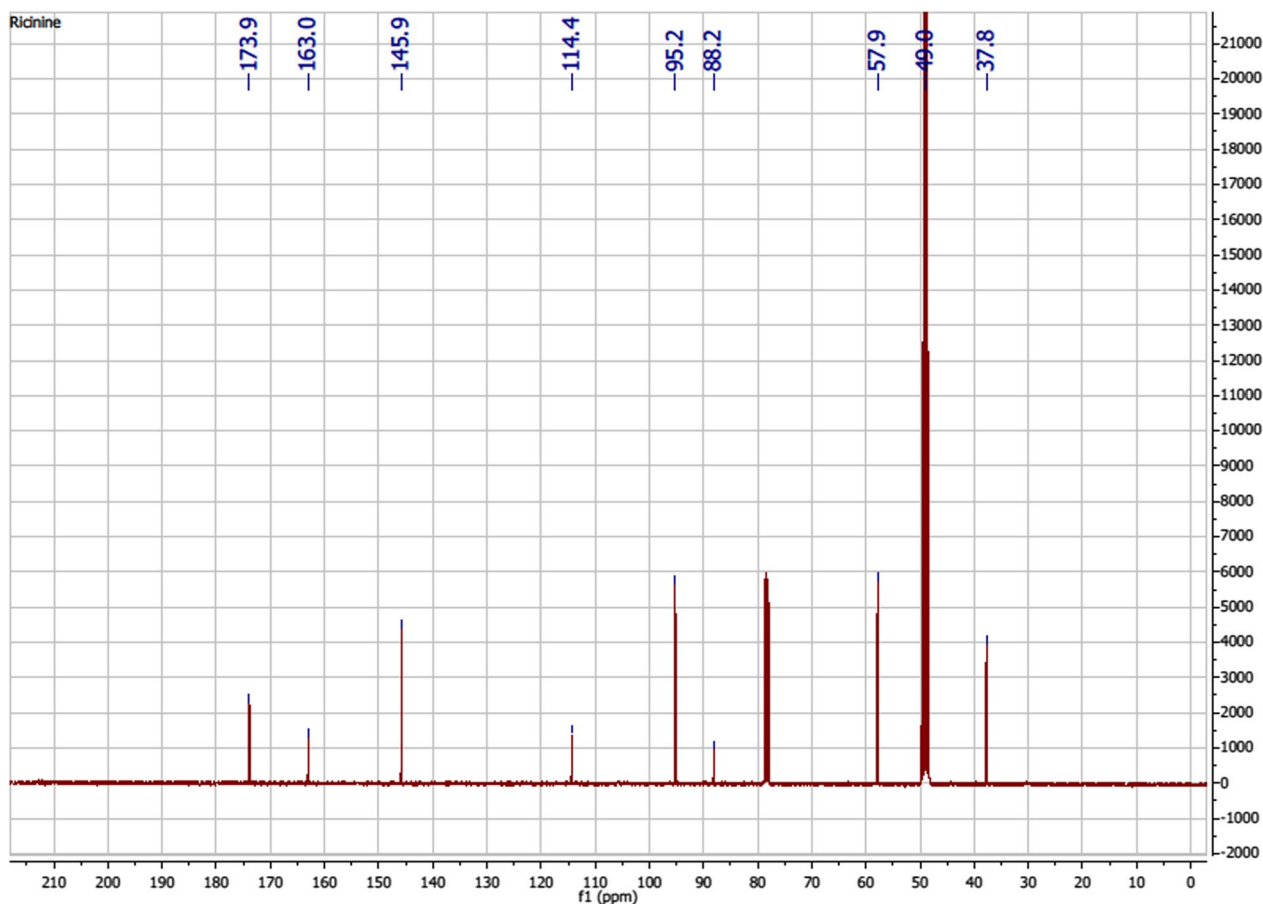


Fig. 10 ¹³C NMR (100 MHz) spectrum of ricinine (compound 1) in CD₃OD/CD₃Cl

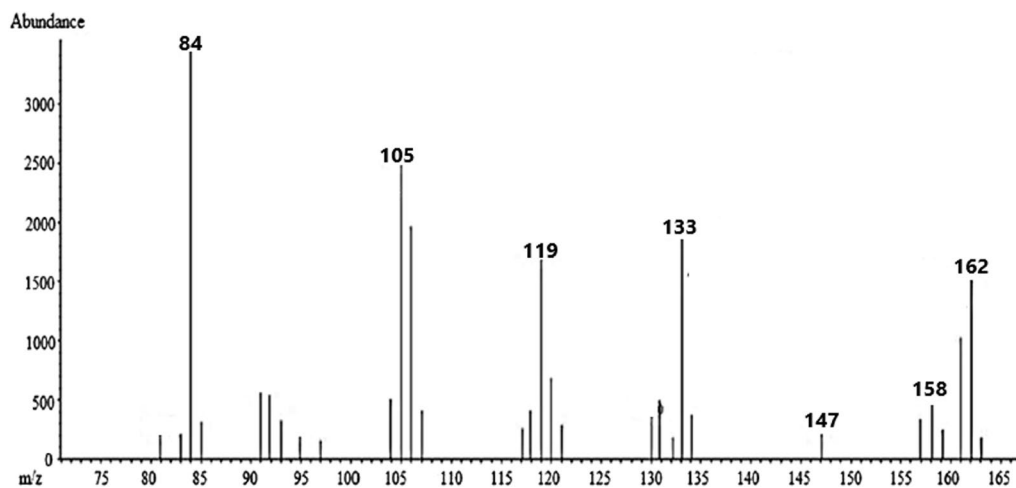


Fig. 11 GC/MS analysis of anabasine (compound 2)

On the other hand, two wild plants (*R. communis* and *N. glauca*) were selected according to their alkaloids, which have a wide range of uses in many fields, especially

as insecticides. Alkaloids are among the most important compounds that have a toxic effect on insects [59]. The analysis of *R. communis* extract indicated that ricinine

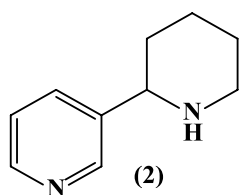


Fig. 12 Chemical structure of anabasine (compound 2)

(**Compound 1**) was the major constituent in the extract [39]. Ricinine alkaloid was used as a natural pesticide against *Tetranychus urticae* and two predatory phytoseiid mites [40]. As well, ricinine can be used as an insecticide against leaf-cutting ants [60]. The extracts of *R. communis* were used by [20] as botanical insecticides to control the sugarcane aphid, *Melanaphis sacchari*.

Compound (1) was isolated as a needle crystal and appeared as orange color with Dragendroff's reagent. Figure 8 shows the chemical structure of compound (1). ^1H NMR and ^{13}C NMR spectra (Table 5 and Fig. 9 and 10) showed two aromatic doublet signals at δ_{H} 6.33 (*d*, $J=7.7$ Hz, H-5), δ_{C} 95.2 ppm and δ_{H} 7.85 (*d*, $J=7.7$ Hz, 1H), δ_{C} 145.9 ppm. As well as, two singlet signals at δ_{H} 3.53 (s, N-CH₃), δ_{C} 37.8 ppm and δ_{H} 4.02 (s, O-CH₃), δ_{C} 57.9 ppm. Also, ^{13}C NMR spectrum indicated four quaternary carbon atoms at δ_{C} 88.2 (C-3), 114.4 (CN), 163.0 (C-2), 173.9 (C-4). Compound (1) was identified as ricinine by comparing its data with literature, [39, 40].

In addition, the anabasine (Compound 2) was found to be the major constituent of the alkaloid fraction of *N. glauca* [24]. The anabasine alkaloid has been widely used as a pesticide. Its insecticidal effect is due to interaction with nicotinic acetylcholine receptors [61, 62]. Alkaloid fraction of *N. glauca* was analyzed by GC/MS technique. Compound (2) was identified as the major constituent in the fraction (Rt = 17.34, 65.52%) by comparing their mass spectra with those of their analogous reported by NIST library (Fig. 11). The GC/MS analysis indicated that the molecular ion peak at m/z 162 as $[\text{M}]^+$ ($[\text{C}_{10}\text{H}_{14}\text{N}_2]^+$) and base peak at m/z 84 for piperidine ring $[\text{C}_5\text{H}_{10}\text{N}]^+$. The fragment ion peaks at m/z 133, 119, 105, and 92 due to fragment ions $[\text{C}_9\text{H}_{11}\text{N}]^+$, $[\text{C}_8\text{H}_9\text{N}]^+$, $[\text{C}_7\text{H}_7\text{N}]^+$, and $[\text{C}_6\text{H}_6\text{N}]^+$, respectively, were in agreement with anabasine [24, 41]. Figure 12 shows the chemical structure of anabasine (compound 2).

4 Conclusion

In conclusion, natural pesticides would have a promising role in terms of controlling the FAW where they avoid environmental damage, reduce synthetic pesticides, and overall reduce control cost. Thus according to this study, it was recommended that, alkaloid extracts of tested wild

plants and ethyl acetate extracts of fungal strains be used as natural pesticides to control the fall armyworm, *S. frugiperda*.

Abbreviations

FAW	Fall armyworm
AST	Aspartate transaminase
ALT	Alanine transaminase
ACP	Acid phosphatase
ALP	Alkaline phosphatase
AchE	Acetyl choline esterase
GST	Glutathione S-transferase
GC-MS	Gas chromatography-mass spectrometry
LC ₅₀	Median lethal concentration
Ppm	Part per million
NMR	Nuclear Magnetic Resonance

Acknowledgements

The authors thank Dr. Ahmed Ramadan El-Rokh, Plant Protection Research Institute, Mansoura Branch, Agriculture Research Center, for his help to the identification of extracted secondary metabolites from wild plants.

Author contributions

RRHA performed the culturing of the fungal strains, extracted the ethyl acetate extract of the tested fungal cultures, performed the estimation of insect enzyme experiment, and prepared the larvae for histological processing. AHA performed the rearing of the fall armyworm. SAA performed by collecting the wild plants and extracting the alkaloid extract of the tested wild plants. All authors detected toxic effects from the extracts, wrote, read, and approved the final manuscript.

Funding

There is no fund for this research.

Availability of data and materials

All data and materials are available if requested.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have approved the manuscript and agree with its submission to Beni-Suef University Journal of Basic and Applied Sciences.

Competing interests

The authors declare that they have no competing interests.

Received: 1 August 2023 Accepted: 11 January 2024

Published online: 20 January 2024

References

- Nagoshi RN, Adamczyk JJ, Meagher J, Gore RL, Jackson R (2007) Using stable isotope analysis to examine fall armyworm (Lepidoptera: Noctuidae) host strains in a cotton habitat. *J Econ Entomol* 100(5):1569–1576. <https://doi.org/10.1093/jee/100.5.1569>
- Bueno RCOF, Carneiro TR, Bueno AF, Pratisoli D, Fernandes OA, Vieira SS (2010) Parasitism capacity of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) on *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) eggs. *Braz Arch Biol Technol* 53(1):133–139. <https://doi.org/10.1590/S1516-89132010000100017>
- Goergen G, Kumar PL, Sankung SB, Togola A, Tamo M (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith)

- (Lepidoptera: Noctuidae), a new alien invasive pest in west and Central Africa. *PLoS ONE* 11(10):e0165632
4. Kumela T, Simiyu J, Sisay B, Likhayo P, Gohole L, Tefera T (2018) Farmers' knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. *International Journal of Pest Management* 65(1):1–9. <https://doi.org/10.1080/09670874.2017.1423129>
 5. CIMMYT Annual Report (2017) International Maize and Wheat Improvement Center. One of fifteen CGIAR research centers helps to improve the lives in developing countries by encouraging more productive, sustainable maize and wheat farming. <https://www.cimmyt.org/annual-reports/cimmyt-annual-report-2017/>
 6. Isaka M, Kittakoop P, Kirtikara K, Hywel-Jones NL, Thebtaranonth Y (2005) Bioactive substances from insect pathogenic fungi. *Acc Chem Res* 38:813–823. <https://doi.org/10.1021/ar040247r>
 7. Ortiz-Urquiza A, Keyhani NO (2013) Action on the surface: entomopathogenic fungi versus the insect cuticle. *Insects* 4(3):357–374. <https://doi.org/10.3390/insects4030357>
 8. Shaker NO, Ahmed GMM, Ibrahim HYE, El-Sawy MM, Mostafa ME, Ismail HNA (2019) Secondary metabolites of the entomopathogenic fungus, *cladosporium cladosporioides* and its relation to toxicity of cotton aphid, *aphis gossypii* (Glov). *Int J Entomol Nematol* 5(1):115–120
 9. Elbanhawey AA, Elsherbiny AE, Abd El-Mageed AE, Abdel-Fattah GM (2019) Potential of fungal metabolites as a biocontrol agent against cotton aphid, *Aphis gossypii* Glover and the possible mechanisms of action. *Pestic Biochem Physiol* 159:34–40. <https://doi.org/10.1016/j.pestbp.2019.05.013>
 10. Habashy MG, Al-Akhdar HH, Elsherbiny EA, Nofal AM (2016) Efficacy of entomopathogenic fungi *Metarhizium anisopliae* and *Cladosporium cladosporioides* as biocontrol agents against two Tetranychid mites (Acari: Tetranychidae). *Egypt J Biol Pest Control* 26:197–201
 11. Abdullah RRH (2004) Studies on some bioagents against certain sucking insect pests. Thesis (M.S.) – Mansoura University, Faculty of Agriculture, Department of Microbiology, 2004, p. 142
 12. Bahar MH, Backhouse D, Gregg PC, Mensah R (2011) Efficacy of a *Cladosporium* sp fungus against *Helicoverpa armigera* (Lepidoptera: Noctuidae), other insect pests and beneficial insects of cotton. *Biocontrol Sci Technol* 21(12):1387–1397. <https://doi.org/10.1080/09583157.2011.622036>
 13. Upadhyay V, Rai D, Rana M, Mehra P, Pandey AK (2014) *Verticillium lecanii* (Zimm.): a potential entomopathogenic fungus. *Int J Agric Environ Biotechnol* 7(4):719–727. <https://doi.org/10.5958/2230-732X.2014.01380.1>
 14. Goettel MS, Koike M, Kim JJ, Aiuchi D, Shinya R, Brodeur J (2008) Potential of *Lecanicillium* spp. For management of insects, nematodes and plant diseases. *J Invertebr Pathol* 98:256–261. <https://doi.org/10.1016/j.jip.2008.01.009>
 15. Goettel MS, Eilenberg J, Glare TR (2005) Entomopathogenic fungi and their role in regulation of insect populations. In: Gilbert LI, latrou K, Gill S (eds) *Comprehensive Molecular Insect Science* 6. Elsevier, Oxford, pp 361–406
 16. Faria MR, Wraight SP (2007) Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biol Control* 43:237–256. <https://doi.org/10.1016/j.biocontrol.2007.08.001>
 17. Wedin GP, Neal JS, Everson GW, Krenzok EP (1986) Castor bean poisoning. *Am J Emerg Med* 4(3):259–261. [https://doi.org/10.1016/0735-6757\(86\)90080-x](https://doi.org/10.1016/0735-6757(86)90080-x)
 18. Barrales-Cureño HJ, Ruiz JEZ, Reyes CR, Hoyos PA, Cruz AL, Herrera LMS, Ruiz JAC, Caballero MCC, Salinas SC, Valdez LGL (2016) Chemical bio-directed determination of *Ricinus communis* and *Argemone mexicana* extracts. *IOSR J Pharm* 6(9):01–06
 19. Kang SS, Cordell GA, Soejarto DD, Fong HHS (1985) Alkaloids and Flavonoids From *Ricinus communis*. *J Nat Prod* 48(1):155–156. <https://doi.org/10.1021/np50037a041>
 20. Sotelo-Leyva C, Salinas-Sánchez DO, Peña-Chora G, Trejo-Loyo AG, González-Cortázar M, Zamilpa A (2020) Insecticidal compounds in *Ricinus communis* L (Euphorbiaceae) to control *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae). *Florida Entomologist* 103(1):91–95. <https://doi.org/10.1653/024.103.0415>
 21. Zahir AA, Rahuman AA, Bagavan A, Santhosh KT, Mohamed RR, Kamaraj C, Rajakumar G, Elango G, Jayaseelan C, Marimuthu S (2010) Evaluation of botanical extracts against *Haemaphysalis bispinosa* Neumann and *Hippobosca maculata* Leach. *Parasitol Res* 107(3):585–592. <https://doi.org/10.1007/s00436-010-1898-7>
 22. Wachira SW, Omar S, Jacob JW, Wahome M, Alborn HT, Spring DR, Masiga DK, Torto B (2014) Toxicity of six plant extracts and two pyridine alkaloids from *Ricinus communis* against the malaria vector *Anopheles gambiae*. *Parasit Vectors* 7(312):1–8. <https://doi.org/10.1186/1756-3305-7-312>
 23. Sharma S, Vasudevan P, Madan M (1991) Insecticidal Value of Castor (*Ricinus communis*) against Termites. *Int Biodeterior* 27(3):249–254. [https://doi.org/10.1016/0265-3036\(91\)90053-T](https://doi.org/10.1016/0265-3036(91)90053-T)
 24. Massadeh RK, El-Elimat T, Al-Gharaibeh M, Tawaha K, Alali FQ (2022) UPLC-HRESI-MS and GC-MS analysis of the leaves of *Nicotiana glauca*. *Acta Pharm* 72:97–108. <https://doi.org/10.2478/acph-2022-0001>
 25. Schep LJ, Slaughter RJ, Beasley DMG (2009) Nicotinic plant poisoning. *Clin Toxicol* 47(8):771–781. <https://doi.org/10.1080/15563650903252186>
 26. Hassan HE, Abd El-Hameed TZ, Nasr EA (2014) Ecological and phytochemical studies on *nicotiana glauca* from Egypt. *Egypt Soc Exp Biol* 10(1):87–95
 27. Alghamdi AA (2021) Phytoconstituents screening and antimicrobial activity of the invasive species *Nicotiana glauca* collected from Al-Baha region of Saudi Arabia. *Saudi J Biol Sci* 28:1544–1547. <https://doi.org/10.1016/j.sjbs.2020.12.034>
 28. El-Rokh AR (2007) Chemical Studies on Some Natural Extracts and Their Constituents to Control Some Aphid Species. M. Sc. Thesis, Faculty of Science, Mansoura University, Egypt
 29. Abdullah RRH (2019) Insecticidal activity of secondary metabolites of locally isolated fungal strains against some cotton insect pests. *J Plant Prot Pathol Mansoura Univ* 10(12):647–653. <https://doi.org/10.21608/jppp.2019.79456>
 30. Boulos L (2000) *Flora of Egypt*. Al Hadara Publishing Cairo, Egypt 2, 288. ISBN: 9789775429223, 9775429226.
 31. Abbott WS (1925) A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–267. <https://doi.org/10.1093/jee/18.2.265a>
 32. Finney DJ (1971) *Probit analysis*. Cambridge University Press
 33. Sun YP (1950) Toxicity index on improved method of comparing the relative toxicity of insecticides. *J Econ Entomol* 43(1):45–53. <https://doi.org/10.1093/jee/43.1.45>
 34. Reitman S, Frankel S (1957) A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. *Am J Clin Pathol* 28(1):56–63. <https://doi.org/10.1093/ajcp/28.1.56>
 35. Powell MEA, Smith MJH (1954) The determination of serum acid and alkaline phosphatase activity with 4- amino antipyrine (AAP). *J Clin Pathol* 7(3):245–248. <https://doi.org/10.1136/jcp.7.3.245>
 36. Simpson DR, Bulland DL, Linquist DA (1964) A semi microtechnique for estimation of cholinesterase activity in boll weevils. *Ann Entomol Soc Am* 57:367–371
 37. Pan L, Ren L, Chen F, Feng Y, Luo Y (2016) Antifeedant activity of *Ginkgo biloba* secondary metabolites against *Hyphantria cunea* larvae: Mechanisms and applications. *PLoS ONE* 11(5):e0155682. <https://doi.org/10.1371/journal.pone.0155682>
 38. Gaaboub IA, El-Kady HA, El-Khayat EF, El-Shewy AM (2012) Biochemical and histological effect of some plant extracts, insecticide (methomyl) and bio insecticide (protecto) against cotton leafworm, *Spodoptera littoralis* (Boisd.). In: 1st International Conference On Biotechnology Applications In Agriculture. Benha University, Moshtohor and Hurghada, 18–22, February 2012, Egypt
 39. El-Naggar MH, Elgaml A, Abdel Bar FM, Badr FA (2018) Antimicrobial and anti-quorum-sensing activity of *Ricinus communis* extracts and ricinine derivatives. *Nat Prod Res* 33(11):1–7. <https://doi.org/10.1080/14786419.2017.1423306>
 40. Ellaithy AYM, Abdel-Khalek AA, Mohammed MA (2022) The potency of ricinine biopesticide from *Ricinus communis* leaves as an alternative host for mass rearing process of *tetranychus urticae* and two predatory phytoseiid mites. *Egypt J Chem* 65(6):535–549. <https://doi.org/10.21608/EJCHEM.2021.107114.4922>
 41. Ali EA (2004) Isolation, Purification and Biochemical Characterization of Acetyl cholinesterase (Ach E) Inhibitor Anabasine alkaloid toxin from *Anabasis setifera* Toxicity to rats. Ph.D. Thesis, Faculty of Science, Mansoura University

42. Ebadollahi A, Davari M, Razmjou J, Naseri B (2017) Separate and combined effects of mentha piperata and mentha pulegium essential oils and a pathogenic fungus *Lecanicillium muscarium* against *Aphis gossypii* (hemiptera: aphididae). *J Econ Entomol* 110(3):1025–1030. <https://doi.org/10.1093/jee/tox065>
43. Mantzoukas S, Lagogiannis I, Kitsiou F, Eliopoulos PA (2023) Entomopathogenic action of wild fungal strains against stored product beetle pests. *Insects* 14(1):91. <https://doi.org/10.3390/insects14010091>
44. Keppanan R, Sivaperumal S, Hussain M, Dash CK, Bamisile BS, Qasim M, Wang L (2018) Investigation and molecular docking studies of Bassianolide from *Lecanicillium lecanii* against *Plutella xylostella* (Lepidoptera: Plutellidae). *Compar Biochem Physiol Part C* 206–207:65–72. <https://doi.org/10.1016/j.cbpc.2018.03.004>
45. Minarni EW, Soesanto L, Suyanto A, Rostaman R (2021) Effectiveness of Secondary Metabolites From Entomopathogenic Fungi For Control *Nilaparvata lugens* Stål. in the laboratory scale. *J Perlindungan Tanaman Indonesia* 25(1):86–97. <https://doi.org/10.22146/jpti.62116>
46. Atta B, Rizwan M, Sabir AM, Golgi MD, Farooq MA, Batta YA (2020) Efficacy of Entomopathogenic Fungi against Brown Planthopper *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) Under Controlled Conditions. *Gesunde Pflanzen* 72(2):101–112. <https://doi.org/10.1007/s10343-019-00490-6>
47. Saldarriaga AJJ, D'Alessandro CP, Conceschi MR, Mascarin GM, Delalibera JI (2017) Efficacy of entomopathogenic fungi against adult *Diaphorina citri* from laboratory to field applications. *J Pest Sci* 90(3):947–960. <https://doi.org/10.1007/s10340-017-0846-z>
48. Zaman S, Hasan M, Ahmad F, Javed N (2020) Pathogenicity of Entomopathogenic Fungi against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) under Abiotic Factors. *Pak J Agric Sci* 57(1):79–86. <https://doi.org/10.21162/PAKJAS/20.8661>
49. Hsia ICC, Islam MT, Ibrahim Y, How TY, Omar D (2014) Evaluation of conidial viability of entomopathogenic fungi as influenced by temperature and additive. *Int J Agric Biol* 16(1):146–152
50. Rai D, Updhyay V, Mehra P, Rana M, Pandey AK (2014) Potential of entomopathogenic fungi as biopesticides. *Indian J Sci Res Technol* 2(5):7–13
51. Kaiser D, Bacher S, Mène-Saffrané L, Grabenweger G (2018) Efficiency of Natural Substances to Protect *Beauveria bassiana* Conidia from UV Radiation. *Pest Manag Sci* 75(2):556–563. <https://doi.org/10.1002/ps.5209>
52. Molnar I, Gibson DM, Krasnoff SB (2010) Secondary metabolites from entomopathogenic Hypocrealean fungi. *Nat Prod Rep* 27(9):1241–1275. <https://doi.org/10.1039/C001459C>
53. Wang X, Gong X, Li P, Lai D, Zhou L (2018) Structural diversity and biological activities of cyclic depsipeptides from fungi. *Molecules* 23(1):169. <https://doi.org/10.3390/molecules23010169>
54. Yokeswari NP, Mary JKS, Mohan VR (2018) Assessment of bio-active components present in the whole plant extract of *Catharanthus pusillus*. *Int J Creat Res Thoughts (IJCRT)* 6(1):406–612
55. Sogan N, Kapoor N, Kala S, Patanjali PK, Nagpal BN, Vikram K, Valecha N (2018) Larvicidal activity of castor oil nanoemulsion against malaria vector *Anopheles culicifacies*. *Int J Mosq Res* 5(3):1–6
56. Sosa A, Costa M, Salvatore A, Bardon A, Borkosky S, Vera N (2017) Insecticidal Effects of Eudesmanes from *Pluchea sagittalis* (Asteraceae) on *Spodoptera frugiperda* and *Ceratitis capitata*. *Int J Environ Agric Biotechnol* 2(1):361–369. <https://doi.org/10.22161/ijeab/2.1.45>
57. Weichen Y, Xiaoyu L, Xiankun L, Yuanqing C, Xiaofei W, Pei L, Rimao H (2022) Synthesis and insecticidal activity of tenuazonic acid and derivatives. *Chin J Pest Sci* 24(2):260–271. <https://doi.org/10.16801/j.issn.1008-7303.2022.0001>
58. Mikami Y, Nishijima Y, Suzuki A (1972) Tenuazonic acids, stereochemical properties and biological activities of tenuazonic acids (Note). *J Agric Chem Soc Jpn* 46:473–476
59. Yamamoto I, Casida JE (1999) Nicotinoid insecticides and the nicotinic acetylcholine receptor. Springer Japan. <https://doi.org/10.1007/978-4-431-67933-2>
60. Cazal CM, Batalhão JR, Domingues VC, Bueno OC, Filho ER, Forim MR, da Silva MFGF, Vieira PC, Fernandes JB (2009) High-speed counter-current chromatographic isolation of ricinine, an insecticide from *Ricinus communis*. *J Chromatogr A* 1216:4290–4294. <https://doi.org/10.1016/j.chroma.2009.02.008>
61. Mizrachi N, Levy S, Goren Z (2000) Fatal poisoning from nicotiana glauca leaves: identification of anabasine by gas-chromatography/mass spectrometry. *J Forensic Sci* 45(3):736–741. <https://doi.org/10.1520/JFS14761J>
62. Zammit M, Shoemaker C, Attard E, Azzopardi LM (2014) The Effects of Anabasine and the Alkaloid Extract of *Nicotiana glauca* on Lepidopterous Larvae. *International Journal of Biology* 6(3):46–53. <https://doi.org/10.5539/ijb.v6n3p46>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.