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Efficient biocontrol of *Spodoptera littoralis* by *Aspergillus nidulans*, an endophyte of *Lantana camara*

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ABSTRACT

Spodoptera littoralis has been recognized as a very destructive polyphagous insect, thus, searching for novel bioagents is the current challenge. Endophytic fungi have been recognized as repertoire for bioactive compounds. Thus, the objective of this work was to isolate endophytic fungi inhabiting *Lantana camara* and evaluating their insecticidal activity against *S. littoralis*. *Aspergillus nidulans,* an endophyte of *L. camara,* displayed a significant larvicidal activity towards 2nd larval instar of *S. littoralis*. The morphological identity of *A. nidulans* has been confirmed from the molecular sequence of ITS region, with genbank accession # MK367603 and deposition # AUMC 14095. The bioactive metabolites of *A. nidulans* were extracted with EtOAc and DCM and their bioactivity towards *S. littoralis* was assessed. The values of LC_{50} were 22.6 and 34.4 µg/mL for dichloromethane and ethylacetate extract, respectively after 18 days of treatment. The chemical identity of insecticidally active compounds was resolved by GC-MS analysis, revealing the presence of major compounds namely 1-docosanol and 1-octadecanol on the EtOAc extract, while cis-9-hexadecenoic acid, and 1-octadecanol were found in the DCM extract. These results indicate that this endophytic fungus could be a potential source for bioactive metabolites and can be used in integrated pest management and other important applications.

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Spodoptera littoralis; *Aspergillus nidulans*; *Lantana camara*; secondary metabolites

Introduction

Fungal endophytes are colonizing living, internal plant tissue without causing any apparent symptoms of infection or disease to the host, conferring the plant resistance to various pathogens. Endophytes protect their host plants from infection by phytopathogens and adverse conditions by secreting bioactive secondary metabolites. Insect pests are one of the main threats to agriculturalists. Currently, more than 20 percent of the world's crops are lost annually due to the insects threats, especially with the increase in global warming (Ali et al., 2016, Maxmen 2013).

The obvious ecological hazard due to the extensive usage of synthetic insecticides motivates the researchers to discover powerful, selective and safe natural alternatives (El-Sayed and Ali, 2020, Strobel and Daisy 2003). Currently, many synthetic agricultural agents have being targeted for removal from the market, due to their profound harmful effects on human health and environment. Thus, endophytic fungi could serve as a reservoir of untapped biologically active compounds that may present an alternative way to control farm pests and pathogens (El-Sayed and El-Sayed, 2020, Demain et al. 2000). Endophytic fungi are a ubiquitous group of microbial plant symbionts that spends the whole or part of its life cycle colonizing inter- and/or intra cellular healthy tissues of plants without causing visible disease symptoms (Sunitha et al. 2013; El-Sayed et al., 2017a), they reside inside the tissues of nearly all healthy plants. Endophytes are synergistic to their hosts and at least some of them are thought to be useful to the plant by producing special substances in the plant tissue such as secondary metabolites, that enhance the plant resistance against insect pests and pathogens (Faeth 2002; Hardoim et al. 2015). The antiherbivore properties of endophytic fungi have been attributed to their wide array of produced secondary metabolites such as alkaloids, terpenoids, phenolics produced (Edriss et al. 2012; El-Sayed et al. 2019a). Thus, the endophytic fungi have been accepted as an important source of novel bioactive secondary metabolites that can be excellent new platforms for the development of novel pharmaceuticals and/or agrochemicals.

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Lantana camara grows widely on the tropical, and sub-tropical parts of world. The leaves of *L. camara* have been used as source of various insecticidally active compounds (Dua et al. 2010; Rajashekar et al. 2014). Thus, the objective of the present study was to isolate the endophytic fungi inhabiting *L. camara* with potential insecticidally activity against the 2th larval instar of *S. littoralis*.

Materials and methods

Collection of plant samples and isolation of endophytic microbes

Fresh leaves of *L. camara* (collected from Al-Sharqia province, Egypt, during September/2017) were brought to lab in sterile bags and processed rapidly to reduce the chance of contamination. Fresh and healthy leaves of plant were washed thoroughly in running water and segments of 0.5–1 cm^2 were cut from the midrib portion of each leaf and surface sterilized by immersing in 70% ethanol for 1 min, followed by 4% sodium hypochlorite solution (v/v) for 2 min, and finally washed in sterile water for 1 min (Selvanathan et al. 2011; El-Sayed et al. 2018a,b, 2019a, 2017b). Sterilized samples were surface dried under sterile condition on placing over sterilized blotting paper. Leaves segments of *L. camara* were placed in petri dishes containing potato dextrose agar (with ampicillin 150 mg/ml), the plates were sealed with parafilm and incubated at 28 ± 2 °C for 15 days. The efficacy of sterilization procedure was ascertained from the mycelial growth of control plates. The developed fungal isolates were transferred onto fresh PDA plates to isolate pure colonies. The obtained fungal isolates were purified and maintained at 4 °C.

Identification of the potent insecticidally active fungal isolate

The isolated endophytic fungi were inoculated to plates of PDA media and allowed to grow at 28 °C for 10 days. The fungal isolates were identified according to their morphological features including colony, conidial morphology (El-Sayed et al. 2012, 2013, 2015, 2019b). For molecular identification, the fungal isolate was grown on PDB media for 5 days at 28 °C. The mycelium was harvested, and the genomic DNA was extracted. The fungal isolate was molecularly identified based on the entire sequence of internal transcribed spacer (ITS) flanking the 5.8S region (ITS1-5.8S-ITS2 rDNA) (Kebeish and El-Sayed 2012). The genomic DNA was extracted, used as a template for PCR with the primer set ITS4 5′-GGAAGTAAAAGTCGTAACAAG G-3′ and ITS5 5′-TCCTCCGCTTATTGATATGC-3′ (El-Sayed et al. 2015, 2019c). The PCR reaction contains 10 µl of 2× PCR master mixture (i-Taq^{TM}, Cat. No. 25027, iNTRON Biotech.), 2 µl of fungal gDNA, 1 µl of forward and reverse primers (10 pmol/µl), completed to 20 µl with sterile distilled water. The PCR was programmed to initial denaturation at 94 °C for 2 min followed by denaturation at 94 °C for 30 s, annealing at 55 °C for 10 s, extension at 72 °C for 30 s for 38 cycles, with final extension at 72 °C for 2 min. The PCR amplicon was analyzed by 1.5% agarose gel in 1× TBE buffer (Ambion Cat# AM9864) comparing to the DNA ladder (1 kb Nex-gene Ladder, Puregene, Cat.# PG010-55DI). Negative control PCR reactions (without fungal gDNA) was used. The amplicons were visualized by gel documentation system, purified and sequenced by Applied Biosystems Sequencer, HiSQV Bases, Version 6.0 using the same primer sets. The obtained ITS sequences were BLAST searched with non-redundant sequences on the NCBI database. The quality of retrieved sequences was visually inspected from the sequence chromatograms. For the multiple sequences alignment, FASTA sequences were imported into MEGA 6.0 software, aligned with ClustalW muscle algorithm (Edgar 2004). The phylogenetic tree of the target sequences was constructed with neighbor-joining method of MEGA 6.0 with 1000 bootstrap replication (Tamura et al. 2011).

Growing of the fungal culture and extraction of active compounds

The endophytic fungal isolates were grown on PDB medium (El-Sayed et al. 2012, 2013). Plugs from the periphery of 5 days old fungal cultures grown were inoculated to each 50 ml of PDB, and the cultures were incubated for 20 days at 28 °C under stationary conditions. After incubation, the cultures were filtered, and the filtrate was extracted with organic solvents; ethylacetate and dichloromethane. Liquid-liquid extraction was carried out three times for each solvent (1%V/V). To the filtrate, equal volume of ethylacetate was added, mixed well for 10 min, the two clear immiscible layers were developed. The upper layer of ethylacetate containing the extracted compounds was separated with separating funnel. The water phase was subjected to 2nd extraction by the same method with dichloromethane, and the lower organic layer was collected (El-Sayed et al. 2019b). Both ethylacetate and dichloromethane extracts were concentrated and evaporated under reduced pressure at 35 °C using rotary vacuum evaporator. The yields were weighed, stored as a stock in glass vials in freezer for further studies.

Insect rearing and bioassay studies

Cultures of cotton leaf worm, *S. littoralis* was obtained from the Cotton Pest Research Department, Agriculture Research Center, Giza, Egypt, without any

insecticidal pressure. The insects were reared on castor-bean leaves, *Ricinus communis*, under laboratory conditions at 25 ± 2 °C and 60 ± 5% R.H (Eldefrawi et al., 1964).

The pesticidal activity the recovered fungal extracts against 2nd instar larvae of *S. littoralis* (survival and development) has been evaluated by leaf dipping technique (Eldefrawi et al., 1964), with slight modifications. Briefly, different concentrations (0, 12.5, 25, 50 mg/ml) of each fungal extract were prepared and sprayed to both surfaces of the same-sized fresh castor bean leaves, air dried at room temperature. Treated dry leaves were put singly in plastic cups, 2nd instar larvae of *S. littoralis* (starved for 2 hours) were reared on treated and untreated (control) caster bean leaves in plastic containers (4 × 6 cm) (avoiding cannibalism) at 28 ± 2 °C and 65 ± 5% R.H. Castor bean leaves were refreshed regularly after two days. The experiment was repeated three times with twenty-seven larvae per treatment (9 larvae/replication). Three replicates of nine larvae were fed on ethylacetate and dichloromethane treated leaves (separately) for 48 h as control. Larval mortality was recorded after 48 h till pupation. Mortality percentage were calculated and subjected to probit analysis (Finney, 1952). LC-P lines were established and LC_{50} values were determined according to Abbott's formula

$$\frac{\textit{Corrected mortality } \% \;=\; \textit{Observed mortality} - \textit{control mortality } X\ 100}{100 \;-\; \textit{control mortality}}$$

The toxicity lines were statistically analyzed (Finney, 1952). The relative efficiency of tested extracts was determined (Sun, 1950) as follow:

$$\text{Toxicity index } = \frac{\text{LC50 of the highest efficient treatment (A)}}{\text{LC50 of the other compound (B)}} \times 100$$

A, is the most effective compound, and B, is the other effective compound.

Chemical characterization of bioactive compounds of potent fungal extracts

The chemical identity of the biologically active compounds on the ethylacetate and dichloromethane extracts from the fungal cultures were resolved by GC-MS/MS analyses (El-Sayed et al. 2019a, El-Sayed et al., 2020). The GC-MS/MS analysis was carried out with GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 μm film thickness), with injected volume 1 μl. The carrier gas was helium with the linear velocity of 1 ml/min. The injector and detector temperature were 200 °C and 250 °C, respectively. The MS operating parameters were ionization potential 70 eV, interface temperature 250° C, and acquisition mass range 50-800.The identification of bioactive compounds present in the extracts was based on a comparison of their mass spectra and retention time with those of the authentic compounds on NIST (National Institute of Standards and Technology, US) and WILEY libraries, as well as by comparing the fragmentation pattern of the mass spectral data with those reported in the literature. The name, molecular weight and structure of the components of the extract were ascertained.

Results and discussion

Isolation and identification of different fungal isolates from Lantana camara

Four endophytic fungal isolates were isolated from *Lantana camara* (Table 1), allocating to the different plant parts. Fungi were morphologically identified to the species level according to the universal keys (El-Sayed et al. 2015). These isolates were *Helminthosporium* sp and *Fusarium oxysporum, Alternaria alternata* and *Aspergillus nidulans*. Similar paradigm for endophytic fungal flora was reported from *Lantana camara* (Desire et al. 2014). The insecticidal activity of these fungi towards the 2nd larval instar of *S. littoralis* was evaluated. Among these fungal isolates, the significant insecticidal activity against *S. littoralis* 4th instar and 2nd instar larvae were detected on the filtrate of *A. nidulans*. Depending on morphological criteria, *A. nidulans* was appeared on potato dextrose agar as dark green with orange to yellow, reverse is purplish to olive, with moderate growth. Hyphae are septate and hyaline, conidial heads are columnar (Figure 1). From these morphological features, this isolate is very close

Table 1. Larvicidal activity of selected endophytic fungal isolates against 4th instar larvae of *S. littoralis*.

			Mortality %	
No.	Fungal Isolate	Isolate lab cod	1 day post-treatment	3 days post-treatment
1	*Aspergillus nidulans*	42	12.2 ± 2.2[a]	25 ± 2.9[a]
2	*Helminthosporium* sp	84	3.33 ± 3.3ab	13.33 ± 3.3abcd
3	*Helminthosporium* sp	84-1	6.6 ± 3.3ab	10.00 ± 0.0bcd
4	*Fusarium oxysporum*	67	0.00 ± 0.0[b]	11.1 ± 0.0bcd

The mean values followed by different letters a, b, c with in the same column are significantly different (ONE Way ANOVA, Tukey's test, $p < 0.05$).

to *A. nidulans*. Further molecular analyses to approve the morphological description were conducted.

The endophytic isolate *A. nidulans* was further identified based on their sequence of ITS regions. The purified amplicons (650 bp) of *A. nidulans* was deposited on Genbank with accession MK367603. From the alignment profile and phylogenetic analysis of rDNA sequences, five phylogenetic clades for *A. nidulans* isolates were evolved from the non-redundant BLAST research and phylogenetic analysis of ITS sequence of *A. nidulans* (Figure 1). The retrieved sequence of *A. nidulans* displayed a 96.6% similarity (E value zero) with various isolates of *A. nidulans* namely; *A. nidulans* MK026965, MH861048, MH858232, MK991576, and KY074657.

Larvicidal activity of A. nidulans extracts on S. littoralis 2nd larval instar

The biological activity of ethylacetate and dichloromethane extracts of *A. nidulans* against 2nd instar larvae of *S. littoralis* was assessed. After incubation for 20 days, the cultures were harvested, filtered, and the extracellular metabolites was extracted with ethylacetate and dichloromethane, and their larvicidal activity were evaluated. From the results (Table 2), the tested extracts of *A. nidulans* displayed an obvious larvicidal activity in addition to retardation the larval growth and elongation in period of *S. littoralis* life cycle stages. The larval mortality may be attributed to direct insecticidal action due to feeding inhibition or gustatory repellency or impairment in the food assimilation after feeding on leaves treated with the fungal extracts. Additionally, some larvae failed to reach more next advanced instar. The mortality rate was proportionally increased with the fungal extract concentration. The ethylacetate and dichloromethane extracts of *A. nidulans* caused accumulative mortality 40.7 and 55.6%, respectively, after 18 days of post treatments. Similarly, the ethylacetate extract of *Alternaria alternata* induced significant inhibitory effect on survival and reproductive potential of *Spodoptera litura* (Namasivayam et al., 2014, Kaur et al., 2016). The mortality percentages of the tested extracts were recorded daily for 18 days of treatment. The LC_{50} values were

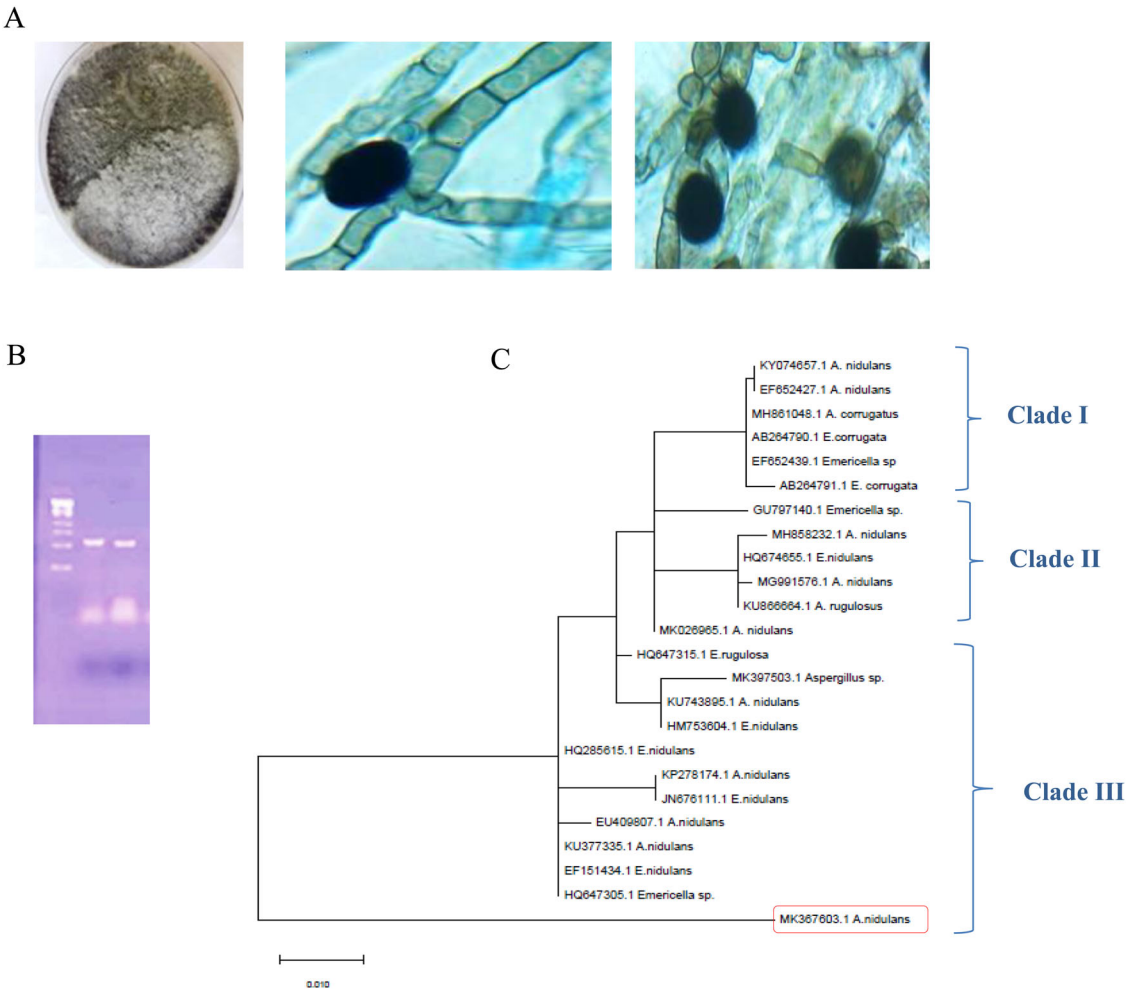

Figure 1. Morphological and molecular identification of *Aspergillus nidulans*. A, plate culture and microscopical view of *A. nidulans*. B, PCR amplicons of ITS sequence, C, phylogenetic tree of *A. nidulans* using Maximum Likelihood method (Tamura et al. 2011).

determined from the plotted regression lines for tested fungal extracts against the 2nd instar larvae of *S. littoralis*. From the LC_{50} values, 2nd instar larvae of *S. littoralis* were more susceptible to all treatments with the time increase. The values of LC_{50} were 22.6 and 34.4 µg/mL for dichloromethane and ethylacetate extract, respectively after 18 days of treatment (Figure 2).

Table 2. Mortality of S. *littoralis* by *Aspergillus nidulans* extracts after 48 h. treatment of 2nd instars larvae.

		Cumulative mortality (%)				
Treatment	Conc. (µg/ml)	1 day	3 days	7 days	12 days	18 days
Water control	–	0.0	0.0	0.0	0.0	0.0
EtOAc control	–	0.00	3.70	3.70	3.70	3.70
DCM control	–	0.0	7.41	7.41	7.41	7.41
EtOAc Extract	**12.5**	0.00	14.81	29.63	29.63	29.63
	25	0.00	29.63	29.63	29.63	40.74
	50	11.11	40.74	48.15	59.26	59.26
DCM extract	**12.5**	0.00	22.22	33.33	33.33	37.04
	25	11.11	37.04	44.44	51.85	55.56
	50	25.93	55.56	59.26	59.26	62.96

Effect of the fungal extracts on the development of immature stags of S. littoralis

From the obtained results (Table 3), *A. nidulans* ethylacetate extract significantly prolong the total larval duration of *S. littoralis* from 8 days (control) to 18 days upon treatment with the extract. As well as, *A. nidulans* ethylacetate and dichloromethane extracts affected total larval period and total development period of immature stages but with non-significant effect on pupal stage period. Noticeably, elongation of the immature stages was non-significantly affected by the tested extract concentration. The larvae deformation percentage was 10.0%, while that for the positive and negative controls was 1.67% and zero for 2nd instar larvae, respectively. Recent studies have demonstrated the detrimental effects of *Alternaria alternata* toxin on the reproduction of rose aphid, *Mcrosiphum rosivorum* Zhang (Sharma et al., 1988, Yang et al., 2012, El-Sayed et al., 2020). Consistently Penicinoline, an alkaloid isolated from *Penicillium* sp showed a

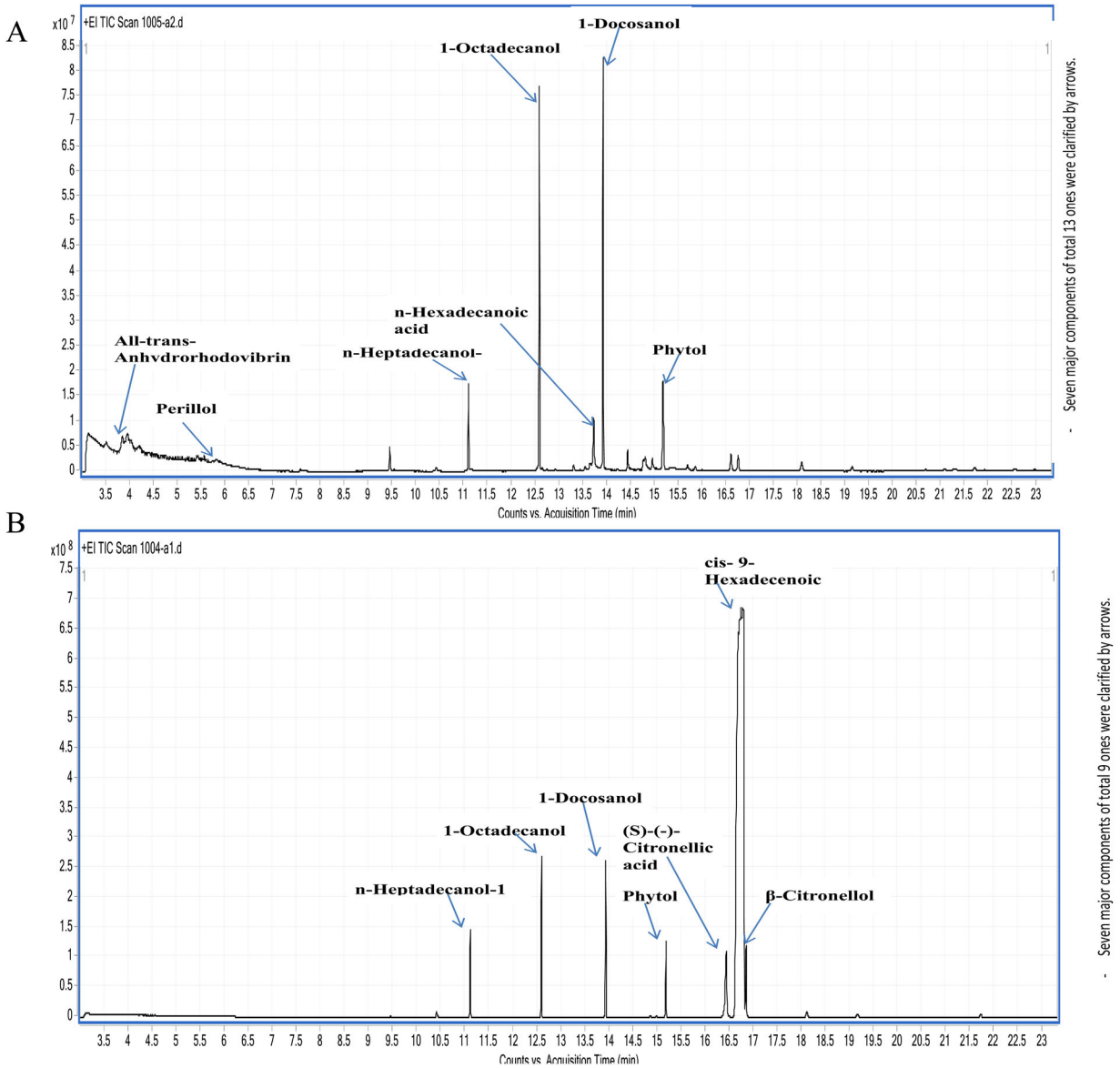

Figure 2. GC/MS-MS chromatogram of *A. nidulans* EtOAc extract (A) and DCM extract (B).

strong insecticidal activity against the sucking pest, Aphis *gossypii Glover* (Theantana et al., 2012, Shao et al., 2010).

Chemical characterization of bioactive compounds of A. nidulans

The chemical identities of ethylacetate and dichloromethane extracts of *A. nidulans* were used for GC-MS analysis. GC-MS analysis indicated that the fungus *A. nidulans* produced bioactive compounds. From the GC-MS/MS profiling (Table 4), sixteen compounds in *A. nidulans* ethylacetate extract and nine compounds in *A. nidulans* dichloromethane extract were extracted. The identification was based on peak area, retention time, molecular weight and formula, according to the database of National Institute standard and Technology (NIST) and WILEY libraries of the mass spectrum. The identification of these metabolites revealed that endophytic *A. nidulans* has the capacity to produce bioactive compounds. GC-MS analysis of crude ethylacetate extract of *A. nidulans* revealed a major seven compounds as 1-docosanol, 1-octadecanol, all-trans- anhydrorhodovibrin, perillol, phytol, n-heptadecanol-1 and n-hexadecanoic acid. While, for crude dichloromethane extract of *A. nidulans*, the major five components were cis-9-hexadecenoic acid (palmitoleic acid) which recorded the highest peak area value 83.24%, followed by 1-octadecanol, 1-docosanol, (*S*)-(-)-citronellic acid and *β*-citronellol with peak areas 3.57, 3.36, 3.12 and 1.66%, respectively (Table 4). In the ethyl acetate extract of fungi *A. nidulans*, the main compounds 1-docosanol ($C_{22}H_{46}O$) with peak area 21.87 and retention time was 13.93; 1-octadecanol ($C_{18}H_{38}O$) with retention time 12.59 and peak area was 21.16, all-trans- anhydrorhodovibrin which molecular formula is ($C_{41}H_{58}O$) along with retention time and peak area is 3.8 and 15.48, respectively. Perillol ($C_{10}H_{16}O$) which retention time was 5.8 and had 8.82 peak areas. n-heptadecanol-1 ($C_{17}H_{36}O$) had 11.11 retention time and peak area was 5.28. n-hexadecanoic acid ($C_{16}H_{32}O_2$) had 13.8 retention time and peak area was 4.23. Minor constituents of *A. nidulans* ethyl acetate extract were 1-hexadecanol ($C_{16}H_{34}O$). Similarly, hexadecanoic acid was mainly found in endophytic {Formatting Citation}. Similar results were reported *Sarocladium strictum* against *S. littoralis* (El-Sayed et al. 2020) The presence of phytochemicals such as primary fatty alcohol, saturated fatty acid monoterpenes and tetraterpenoid with high concentrations in ethylacetate extract; unsaturated fatty acid, monoterpenes and fatty alcohols might be the reason for insecticidal activity of these extracts. Our findings suggest that endophytic fungi are rich source for important bioactive compounds in agriculture and medicine.

Table 3. Effect of *A. nidulans* extracts on larval and pupal development periods.

	EtOAc			DCM		
Conc (µg/ml)	TLP	TPP	TDP	T LP	TPP	TDP
12.5	15.2^{b}	7.75^{a}	22.9^{a}	16.6^{a}	8.33^{a}	24.9^{a}
25	16.9^{a}	7.5^{a}	24.4^{a}	16.5^{a}	8.00^{a}	24.5^{a}
50	16.6^{a}	7.5^{a}	24.1^{a}	16.2^{a}	8.10^{a}	24.3^{a}
Control	8.4^{c}	7.5^{a}	21.9^{a}	13.4^{b}	8.00^{a}	21.4^{b}
LSD	0.57	0.58	3.71	0.51	0.36	0.58
***P* value**	*0.00	$^{n.s}$0.58	$^{n.s}$0.25	*0.000	$^{n.s}$0.26	*0.00

The mean values followed by letters
a,b,cwith in the same column are significantly
different (ONE Way ANOVA, Tukey's test, $p \leq 0.05$).
LSD: the least significant difference.
n.s: non-significant,
*means highly significant.
TLP, Total larval period. **TPP**, Total pupal period. **TDP**, Total development period

Table 4. GC-MS/MS metabolic profiling of *A. nidulans* EtOAc and DCM crude extracts.

	No.	Compound name	RT	Area (%)	Phytochemical category
EtOAc Extracts	1	Trans-anhydrorhodovibrin	3.8	15.48	Tetraterpenoid
	2	Perillol	5.8	8.82	Monoterpenes
	3	1-Tridecene	9.45	1.78	Hydrocarbon, acyclic olefin
	4	Methyl- *β* -ionone	10.4	0.65	Monoterpene
	5	n-Heptadecan-1-ol	11.11	5.28	Long-chain primary fatty alcohol
	6	1-Octadecanol	12.59	21.16	Long-chain primary fatty alcohol
	8	n-Hexadecanoic acid	13.8	4.23	Straight-chain saturated fatty acid
	9	1-Docosanol	13.93	21.87	Long-chain primary fatty alcohol
	10	1-Hexadecanol; cetyl alcohol	14.44	1.98	Long chain primary fatty alcohol
	11	Sebacic acid; dibutyl ester	14.87	2.58	Dicarboxylic Acids
	12	Cis-13-octadecenoic acid	14.91	2.07	Monounsaturated, Oleic Acids
	13	Phytol	15.19	8.71	Acyclic diterpene
	14	Arg-Ser-Lys	16.64	1.78	Short peptide
	15	Cis- 9-hexadecenoic acid	16.67	1.47	Unsaturated fatty acid
	16	γ-L-glutamyl-L-glutamic acid	18.16	1.52	Dipeptide
DCM Extracts	**1**	Methyl- *β* -ionone	10.4	0.94	Monoterpene
	2	n-Heptadecan-1-ol	11.11	1.67	Long-chain primary fatty alcohol
	3	1-Octadecanol	12.59	3.57	Long-chain fatty alcohol
	4	1-Docosanol	13.93	3.36	Long-chain primary fatty alcohol
	5	Phytol	15.19	1.61	Acyclic diterpene
	6	(S)-(-)-Citronellic acid	16.3	3.12	Acyclic caprylates monoterpenoid
	7	Cis-9-hexadecenoic acid	16.67	83.24	Unsaturated fatty acid
	8	*β*-Citronellol	16.79	1.66	Monoterpenoid
	9	γ-L-glutamyl-L-glutamic acid	18.16	0.84	Dipeptide

In conclusion, endophytic fungi from *Lantana camara* were isolated and their biological activity towards *S. littoralis* were assessed. The ethylacetate and dichloromethane extracts of *A. nidulans* displayed significant deleterious effects to *S. littoralis* as revealed from the tested biochemical parameters. A strong deformation in adults of *S. littoralis* by about 25.1% was observed on the individuals descending from larvae, comparing to positive and negative controls. GC-MS analysis of *A. nidulans* ethylacetate revealed major compounds namely 1-docosanol, and 1-octadecanol, while, for dichloromethane extract components were cis-9-hexadecenoic acid and 1-octadecanol.

To the best of our knowledge, this is the first report describing the potentiality of *A. nidulans* for controlling the cotton leave worm *S. littoralis*, that could open a new avenue for biocontrol strategies of various diseases caused by this pest. Further studies are ongoing to implement this fungus and its bioactive compounds with different formulating agents against *S. littoralis* infecting different plants, and evaluating their effect regarding to the transcriptomic and proteomic responses of the host plant.

Disclosure statement

There is no any conflict of interest.

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References

Ali GS, El-Sayed ASA, Patel JS, Green KB, Ali M, Brennan M, Norman D. 2016. Ex Vivo application of secreted metabolites produced by soil-inhabiting *Bacillus* spp efficiently controls foliar diseases caused by *Alternaria* spp. Appl Environ Microbiol. 82(2): 478–490.

Demain AL, Hayes MA, Thomas R, Chrystal EJT, Nicholson N. 2000. Microbial natural products: A past with a future. In: Biodiversity. The Royal Society of Chemistry, Cambridge, p. 3–16.

Desire MH, Bernard F, Forsah MR, Assang CT, Denis ON. 2014. Enzymes and qualitative phytochemical screening of endophytic fungi isolated from *Lantana camara* Linn leaves. J Appl Biol Biotechnol. 2:1–6.

Dua VK, Pandey AC, Dash AP. 2010. Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. Indian J Med Res. 131:434–440.

Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinf. 5:113.

Edriss AE, Satti AA, Alabjar Z. 2012. Preliminary studies on phytochemicals and larvicidal effects of *Acacia nilotica* L. extracts against *Anopheles arabiensis* Patton. Sci Res Essays. 7:4253–4258.

El-Sayed ASA, Yassin MA, Ali GS. 2015. Transcriptional and proteomic profiling of *Aspergillus flavipes* in response to sulfur starvation. Plos One. 10(12): e0144304.

El-Sayed AS, Shindia AA, Zaher Z. 2012. L-Amino acid oxidase from filamentous fungi: screening and optimization. Ann Microbiol. 62(2):773–784.

El-Sayed AS, Khalaf SA, Aziz HA. 2013. Characterization of homocysteine γ-lyase from submerged and solid cultures of Aspergillus fumigatus ASH (JX006238). J Microbiol Biotechnol. 23(4):499–510.

El-Sayed ASA, Safan S, Mohamed NZ, Shaban L, Ali GS, Sitohy MZ. 2018a. Induction of Taxol biosynthesis by *Aspergillus terreus*, endophyte of *Podocarpus gracilior* Pilger, upon intimate interaction with the plant endogenous microbes. Process Biochem. 71:31–40.

El-Sayed ASA, Moustafa AH, Hussein HA, El-Sheikh AA, El-Shafey SN, Fathy NAM, Enan GA. 2020 Potential insecticidal activity of *Sarocladium strictum*, an endophyte of *Cynanchum acutum*, against *Spodoptera littoralis*, a polyphagous insect pest. Biocatal Agric Biotechnol. 24:101524.

El-Sayed ASA, Abdel-Ghany SE, Ali GS. 2017a. Genome editing approaches: manipulating of lovastatin and taxol synthesis of filamentous fungi by CRISPR/Cas9 system. Appl Microbiol Biotechnol. 101:3953–3976.

El-Sayed ASA, Ruff LE, Ghany SEA, Ali SEA, Esener S. 2017b. Molecular and spectroscopic characterization of aspergillus flavipes and pseudomonas putida L-methionine γ-lyase in vitro. Appl Biochem Biotechnol. 181: 1513–1532.

El-Sayed ASA, Ali GA. 2020. Aspergillus flavipes is a novel efficient biocontrol agent of Phytophthora parasitica. Biol Control. 140:104072.

El-Sayed ASA, Akbar A, Iqrar I, Ali R, Norman D, Brennan M, Ali GS. 2018b. A glucanolytic *Pseudomonas* sp. associated with *Smilax bona-nox* L. displays strong activity against *Phytophthora parasitica*. Microbiol Res. 207:140–152.

El-Sayed MT, El-Sayed AS. 2020. Biosynthesis and comparative bactericidal activity of silver nanoparticles synthesized by *Aspergillus flavus* and *Penicillium crustosum* against the multidrug-resistant bacteria. J Microbiol Biotechnol. 30(2):226–236.

El-Sayed ASA, Shindia AA, Zeid AAA, Yassin AM, Sitohy MZ, Sitohy B. 2019a. *Aspergillus nidulans* thermostable arginine deiminase-Dextran conjugates with enhanced molecular stability, proteolytic resistance, pharmacokinetic properties and anticancer activity. Enzyme Microb Technol. 131(12):109432.

El-Sayed ASA, George NM, Bolbol AA, Mohamed MS. 2019b. Purification and biochemical characterization of *Aspergillus terreus* ornithine decarboxylase: Curcumin is a potent enzyme inhibitor. Molecule. 24:1–16.

El-Sayed ASA, Mohamed NZ, Safan S, Yassin MA, Shaban L, Shindia AA, Shad Ali G, Sitohy MZ. 2019c. Restoring the Taxol biosynthetic machinery of Aspergillus terreus by Podocarpus gracilior Pilger microbiome, with retrieving the ribosome biogenesis proteins of WD40 superfamily. Sci Rep. 9(1):11534.

Eldefrawi ME, Toppozada A, Zeid M. 1964. Toxicological studies on the Egyptian cotton leafworm, Prodenia litura. I. Susceptibility of different larval instars of Prodenia to Insecticides1. J Econ Entomol. 57(4): 591–593.

Faeth SH. 2002. Are endophytic fungi defensive plant mutualists? Oikos. 98(1):25–36.

Finney DJ. 1952. In: Finney DJ, editor. Probit Analysis. 2nd ed. Cambridge University Press, New York: J. American Pharmac. Assoc. John Wiley & Sons, Ltd, 41(11).

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A. 2015. The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev. 79(3): 293–320.

Kaur T, Kaur J, Kaur A, Kaur S. 2016. Larvicidal and growth inhibitory effects of endophytic Aspergillus Niger on a polyphagous pest, Spodoptera litura. Phytoparasitica 44:465–476.

Kebeish R, El-Sayed ASA. 2012. Morphological and molecular characterization of L-methioninase producing *Aspergillus* species. Afr J Biotechnol. 11:15280–15290.

Maxmen A. 2013. Crop pests: Under attack. Nature. 501(7468):S15–S7.

Namasivayam SKR, Sekar S, Bharani RSA. 2014. Pesticidal activity of endophytic fungal metabolites against major groundnut defoliator *Spodoptera litura* (Fab.). J Biopestic. 7:116.

Rajashekar Y, Ravindra KV, Bakthavatsalam N. 2014. Leaves of *Lantana camara* Linn. (Verbenaceae) as a potential insecticide for the management of three species of stored grain insect pests. J Food Sci Technol. 51(11):3494–3499.

Selvanathan S, Indrakumar I, Johnpaul M. 2011. Biodeversity of the endophytic fungi from *Calotropis giganatea*. Recent Res Sci Technol. 3:94–100.

Shao C-L, Wang C-Y, Gu Y-C, Wei M-Y, Pan JH, Deng DS, She ZG, Lin YC. 2010. Penicinoline, a new pyrrolyl 4-quinolinone alkaloid with an unprecedented ring system from an endophytic fungus Penicillium sp. Bioorg Med Chem Lett. 20: 3284–3286.

Sharma OP, Makkar HPS, Dawra RK. 1988. A review of the noxious plant *Lantana camara*. Toxicon. 26(11): 975–987.

Strobel G, Daisy B. 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev. 67(4):491–502.

Sun Y-P. 1950. Toxicity index-an improved method of comparing the relative toxicity of Insecticides1'. J Econ Entomol Narnia. 43(1):45–53.

Sunitha VH, Nirmala Devi D, Srinivas C. 2013. Extracellular enzymatic activity of endophytic fungal strains isolated from medicinal plants. World J Agric Sci. 9:1–9.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 28(10):2731–2739.

Theantana T, Kanjanapothi D, Lumyong S. 2012. In vitro inhibition of lipid peroxidation and the antioxidant system of endophytic fungi from Thai medicinal plants. Chiang Mai J Sci. 39:429–444.