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Virulence of Some Selected Fungal Isolates against the Cotton Leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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ABSTRACT

The present study was carried out to investigate the virulence of two fungal isolates against cotton leafworm *Spodoptera littoralis*. Six fungal isolates were isolated from a soil sample. Only two isolates were identified as *Cunninghamella sp.* and *Rhizopus sp*. according to the data of preliminary test where they cause higher mortalities. The highest mortality was recorded by isolate *Rhizopus sp*. In addition, the same isolate recorded the lowest LC_{50} values, $2.16x10^7$ conidial concentration/ml. The fungal isolates exhibited an insignificant decrease in the weight of treated larvae. Larval duration was decreased insignificantly at all conidial concentration except the highest conidial concentration that insignificantly increased it. Pupation and adult emergence % were affected. Adult deformities were appeared only by the isolate, *Cunninghamella sp.*. However, the isolate *Rhizopus sp*. exhibited the highest proteolytic and lipolytic activity than isolate *Cunninghamella sp.* while the highest chitinolytic activity was recorded by the isolate *Cunninghamella sp.*

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* in Egypt cause serious damage to many important agricultural crops. Due to the adverse effects of chemical insecticides to human and environment in general, there is a serious need for an insecticidal alternative. Microbial insecticides considered as one of the alternatives that characterized by not leave chemical residues and not insect resistance (Evans, 1999). Entomopathogenic fungi are promising agents for insect control (Lacey & Goettel 1995).

Currently, 66 products related to entomopathogenic fungi have been developed or are being developed (Liu & Li 2004). Various strains of entomopathogenic fungi have been used to control aphids, lepidopteran larva and other pests such as *Lecanicillium* (previous name, *Verticillium*) *sp.* (Jackson *et al*., 1985; Steenberg and Humber, 1999; Jung *et al*., 2006), *Beauveria bassiana* (Quesada *et al*., 2006; Sanchez-Rodriguez *et al*., 2017; Rondot and Reineke, 2018;), and Paecilomyces (Shia and Feng, 2004). Efficacy of entomopathogenic fungi agents against a wide range of insect pests including *Spodoptera* species have been demonstrated (Purwar and Sachan, 2005; Lin *et al.,* 2007; Asi *et al.,* 2013; Gabarty *et al.,* 2014 and Husnain *et al.,* 2014).

The aim of the present study is to evaluate the susceptibility of *S. littoralis* to the two entomopathogenic fungi, *Cunninghamella sp.* and *Rhizopus sp*., and to investigate the enzymatic activity of these isolates in their relation in inducing a mode of action.

MATERIALS AND METHODS

Experimental Insect:

In the laboratory of Entomology, Faculty of Science, Al-Azhar University, Cairo, a culture was established under laboratory controlled conditions (27+2oC, 65+5% R.H., photoperiod 14h L and 10h D). The rearing technique was carried out according to (Hegazi *et al*., 1977).

Fungal Isolation and Identification:

The soil sample was collected from Hadayk El-maady, Cairo, Egypt. Six fungal isolates were isolated using malt extract agar medium. These isolates were preliminary tested against the early late larvae of cotton leafworm *S. littoralis*. Only two fungal isolates exhibited their ability as entomopathogenic.

The two fungal isolates were identified as *Rhizopus sp.* according to Schipper & Stalpers (1984) and *Cunninghamella sp.* according to Baijal and Mehrotra (1980), Cutter (1946), Lunn and Shipton (1983), Mil'ko and Beljakova (1967), Samson (1969), Weitzman (1984), and Weitzman and Crist (1979; 1980).

Insect Treatment:

A series of conidial concentration levels / ml: $1x10^{10}$, $1x10^{8}$ and $1x10^{6}$ were prepared. 50 µ from each concentration was topically applied on the dorsal surface of the thorax of the newly moulted 6th instar larvae. Control congeners were treated with water. Five replicates of treated and control larvae (one larva/replicate) were kept separately in glass vials. The treated larvae were checked daily for recording mortality until adult emergence.

Enzymatic Bioassay:

Extracellular hydrolytic enzymes of the two tested fungal isolates were investigated on solid media. Mycelial disks of each of the two isolates were placed on agar medium containing the relevant enzyme substrate, 0.45% colloidal chitin-agar, 15% milk-agar and 0.1% tributyrin for chitinase, ptorease and lipase test, respectively. Zones (halos) of the degraded substrate that formed around the growing colony were then measured 3 days-post inoculations (3 replicate). Chitinase, protease and lipase activities were evaluated according to Agrawal and Kotasthane (2012), Sarath *et al.* (1989) and Lima *et al.* (1991), respectively.

Calculations:

Mortalities were recorded daily. LC_{50} values were calculated for total mortality by Microsoft Office Excel, 2007 (Finney, 1971). Insect bioassay and extracellular hydrolytic enzymes were expressed as mean±SD and analyzed by the Student's t-distribution (Moroney 1956).

RESULTS

According to the preliminary test, two fungal isolates were determined as entomopathogenic due to their higher induced mortalities to the cotton leafworm larvae. The two isolates were identified microscopically as *Rhizopus sp.* and *Cunninghamella sp.*(Table 1 & Fig. 1).

Table (2) shows the effect of two fungal isolates (*Rhizopus sp.* & *Cunninghamella sp.*) on *S. littoralis* by topical application of 3 conidial concentrations / ml $1x10^{10}$, $1x10^{8}$ and $1x10^{6}$. The highest conidial concentration cause 60% and 40% mortality in the larval stage by isolating *Rhizopus sp.* and *Cunninghamella sp.*, respectively compared to 0% mortality for control insects. In the pupal stage, the isolate *Rhizopus sp* caused 100 % and 25% mortality at $1x10^{10}$ and $1x10^{8}$, respectively. While *Cunninghamella sp.* had no effect on the pupal stage. It induced mortality and deformations of an adult by percentage 66.7 and 20 at conidial concentration/ml $1x10^{10}$ and $1x10^{6}$, respectively. Based on the total mortality, the LC_{50} values were $2.16x10^{7}$ and $4.65x10^{8}$ conidial concentration/ml for isolate *Rhizopus sp*. and *Cunninghamella sp.*, respectively (Fig. 2). The larval weight was

insignificantly decreased irrespective to the fungal isolates. The highest decreased in weight was recorded at the highest conidial concentration $1x10^{10}$ of *Cunninghamella sp.* by 0.418±0.23 mg compared to 0.557±0.09 mg of control insects (Table 3). The highest conidial concentration insignificantly increased the larval duration to 8.5±0.71 and 8.0±1.73 days of *Rhizopus sp.* and *Cunninghamella sp.*, respectively, compared to 7.4±1.34 days of control insects. Pupation % was decreased to 40, 80 and 60% at conidial concentration $1x10^{10}$, $1x10^{8}$, and $1x10^{6}$ of *Rhizopus sp.*. While *Cunninghamella sp.* decreased it only at the two higher conidial concentration to 60 and 80 %. No adult emerged at the highest conidial concentration of *Rhizopus sp.*. However, *Cunninghamella sp.* decreased adult emergence to 33.3 (for more details see Table 3). The recorded deformations were a failure of completely emerged adult from pupa (Fig. 3).

The hydrolytic enzyme activity of lipase, protease and chitinase were studied in agar plate for the two fungal isolates (Fig. 4 & 5). The isolate *Cunninghamella sp.* exhibited extremely significant activity for chitinase 4.10 ± 0.17 mm when compared with isolate *Rhizopus sp*. 2.63 ± 0.15 mm. for protease and lipase the isolate *Rhizopus sp*. exhibited the highest activity. No significant difference was recorded between the two isolates for protease (5.72 ± 0.10 mm, 6.00 ± 0.50 mm for isolate *Cunninghamella sp.* and *Rhizopus sp*., respectively) but a significant difference was recorded for lipase (3.73 ± 0.15 mm and 5.50 ± 0.66 mm for isolate *Cunninghamella sp.* and *Rhizopus sp*., respectively).

Table (1): Preliminary mortality test for 6 fungal isolates against early late larval instar of *spodoptera littoralis* **.**

Fungal isolate	Total Mortality %*
1	80
2	90
3	20
4	10
5	10
6	0
Control	0

*: Total mortality % had been recorded for all stages (larva, pupa & adult) of insect

Table (2): Mortality % of *Spodoptera littoralis* stages after treatment of the early late larval instar topically by spore suspension of two fungal isolates**.**

Fungal isolates	C.C. / ml	Insect stage mortality				LC_{50}
		Larva	Pupa	Adult	Total	
Rhizopus sp.	$1x10^{10}$	60	100	-	100	($2.16x10^{7}$)
	$1x10^{8}$	20	25	0	40	
	$1x10^{6}$	40	0	0	40	
Cunninghamella sp.	$1x10^{10}$	40	0	66.7	80	($4.65x10^{8}$)
	$1x10^{8}$	20	0	0	20	
	$1x10^{6}$	0	0	20	20	
Control		0	0	0	0	-

C.C: Conidial concentration

Table (3): Growth, development and metamorphic changes of *spodoptera littoralis* after treatment of the early late larval instar topically by spore suspension of two fungal isolates**.**

Fungal isolates	C.C. / ml	Larval weight (mean mg±SD)	Larval duration (mean days±days)	pupation %	Adult emergence %	Adult deformities %
Rhizopus sp.	$1x10^{10}$	0.450±0.11ns	8.5±0.71ns	40	-	-
	$1x10^{8}$	0.494±0.03ns	7.3±1.50ns	80	75	0
	$1x10^{6}$	0.557±0.11ns	6.7±1.15ns	60	100	0
Cunninghamella sp.	$1x10^{10}$	0.418±0.23ns	8.0±1.73ns	60	33.3	66.7
	$1x10^{8}$	0.483±0.06ns	7.0±1.15ns	80	100	0
	$1x10^{6}$	0.498±0.10ns	6.8±1.30ns	100	80	20
Control		0.557±0.09	7.4±1.34	100	100	0

C.C: Conidial concentration. ns: nonsignificant data: p>0.05

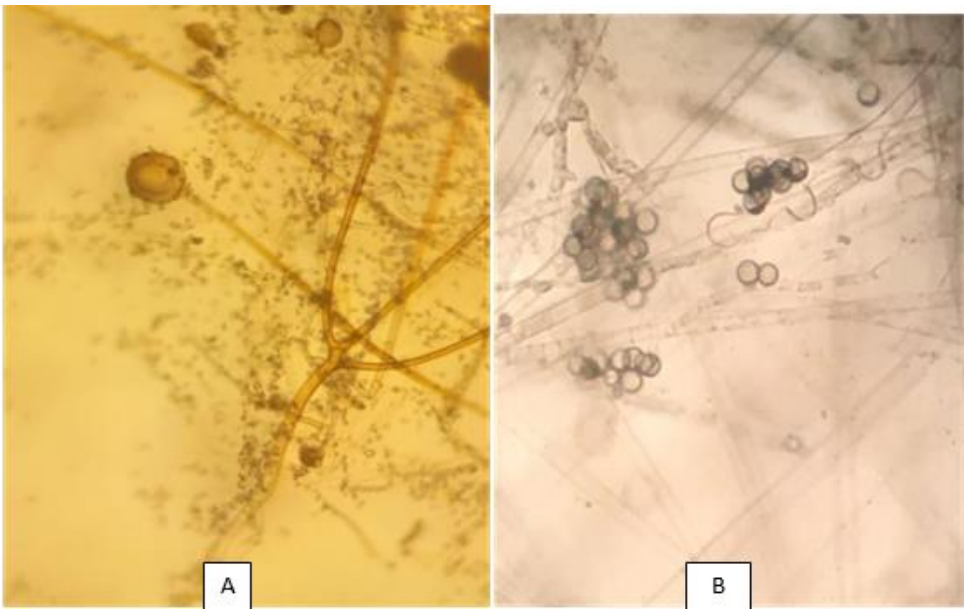

Fig. (1): Photos of preliminary identification of fungal isolates. Where A: *Rhizopus sp*. and B: *Cunninghamella sp.*.

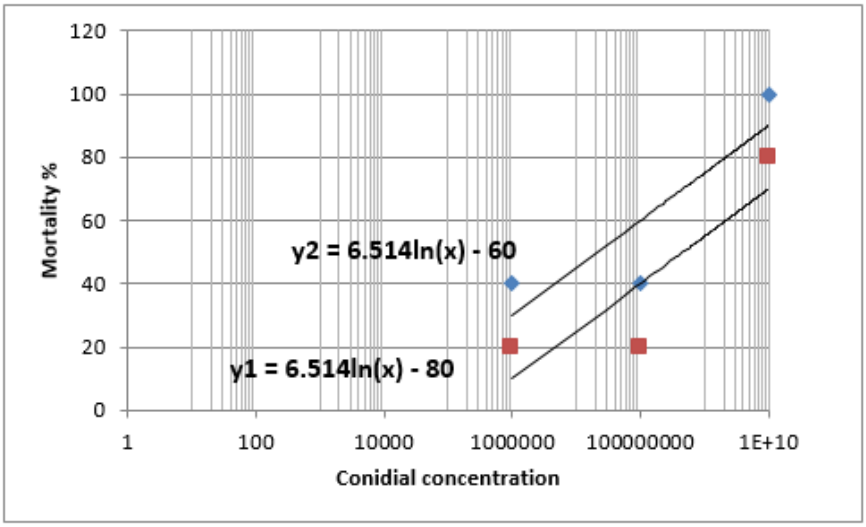

Fig. (2): Regression line of total mortality in *Spodoptera littoralis*. Where: y1: *Rhizopus sp.*, y2: *Cunninghamella sp.*.

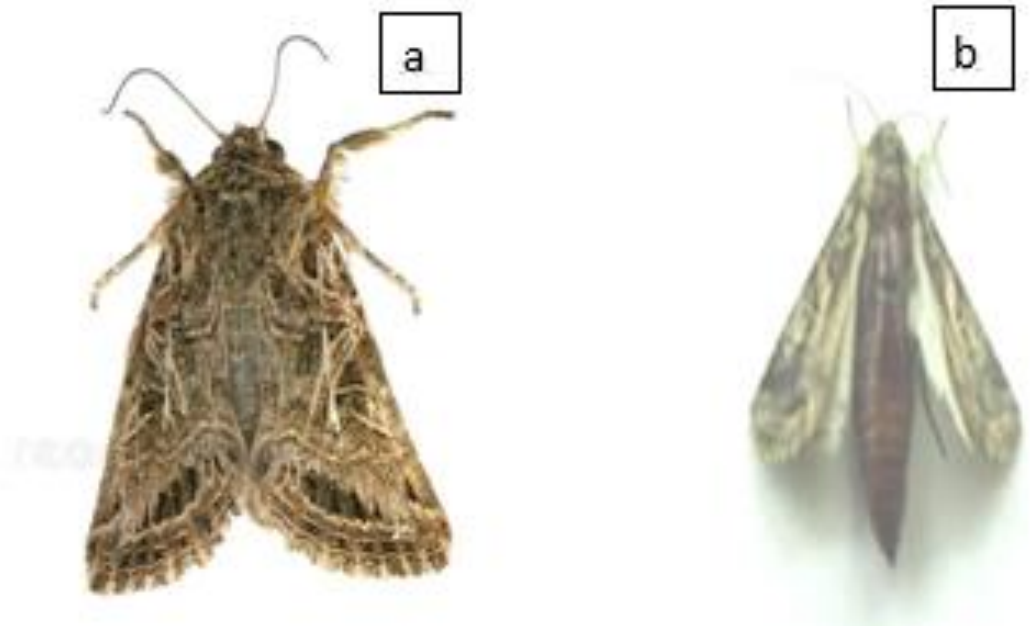

Fig. (3): Deformed *Spodoptera littoralis* adult after treatment the early last instar larvae by spore suspension of two fungal isolates. Where a): normal adult and b): adult failed to completely emerge from the pupa.

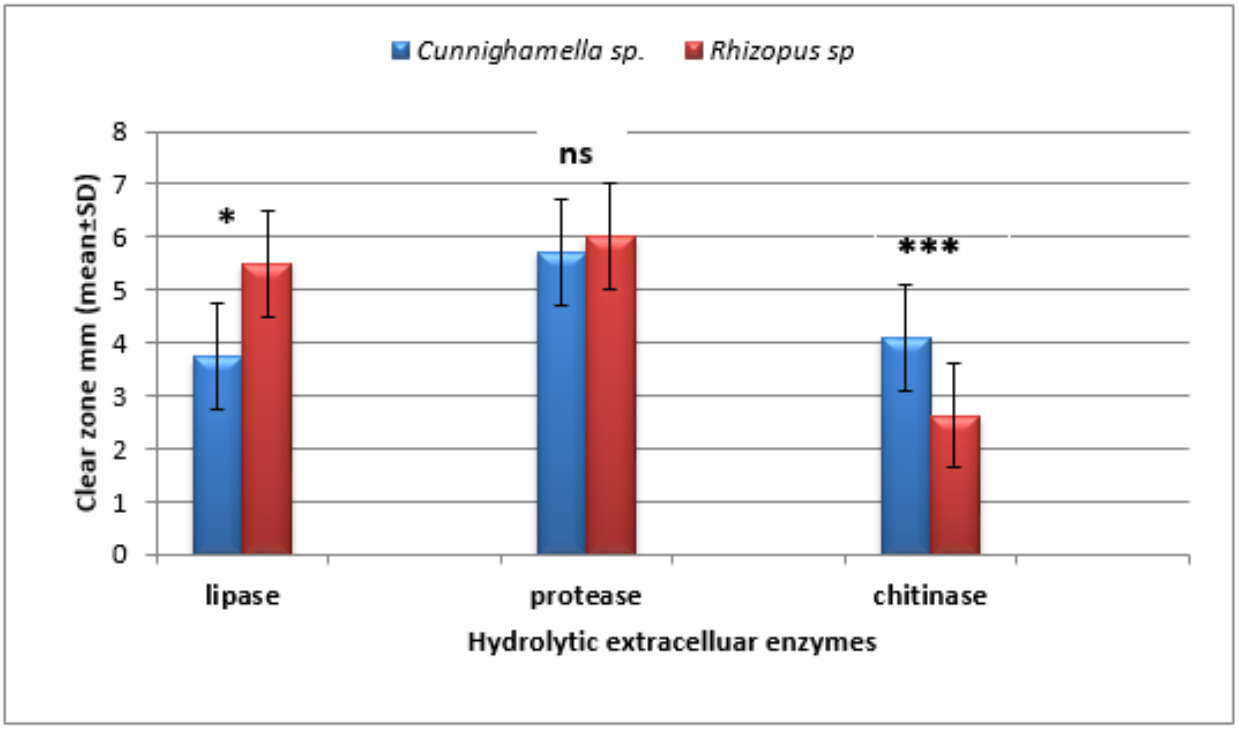

Fig. (4): Hydrolytic activities of extracellular lipase, protease and chitinase enzymes for two fungal isolates. Where: ns: nonsignificant $p>0.05$, *: significant $p<0.05$, ***: extremely significant $p<0.001$

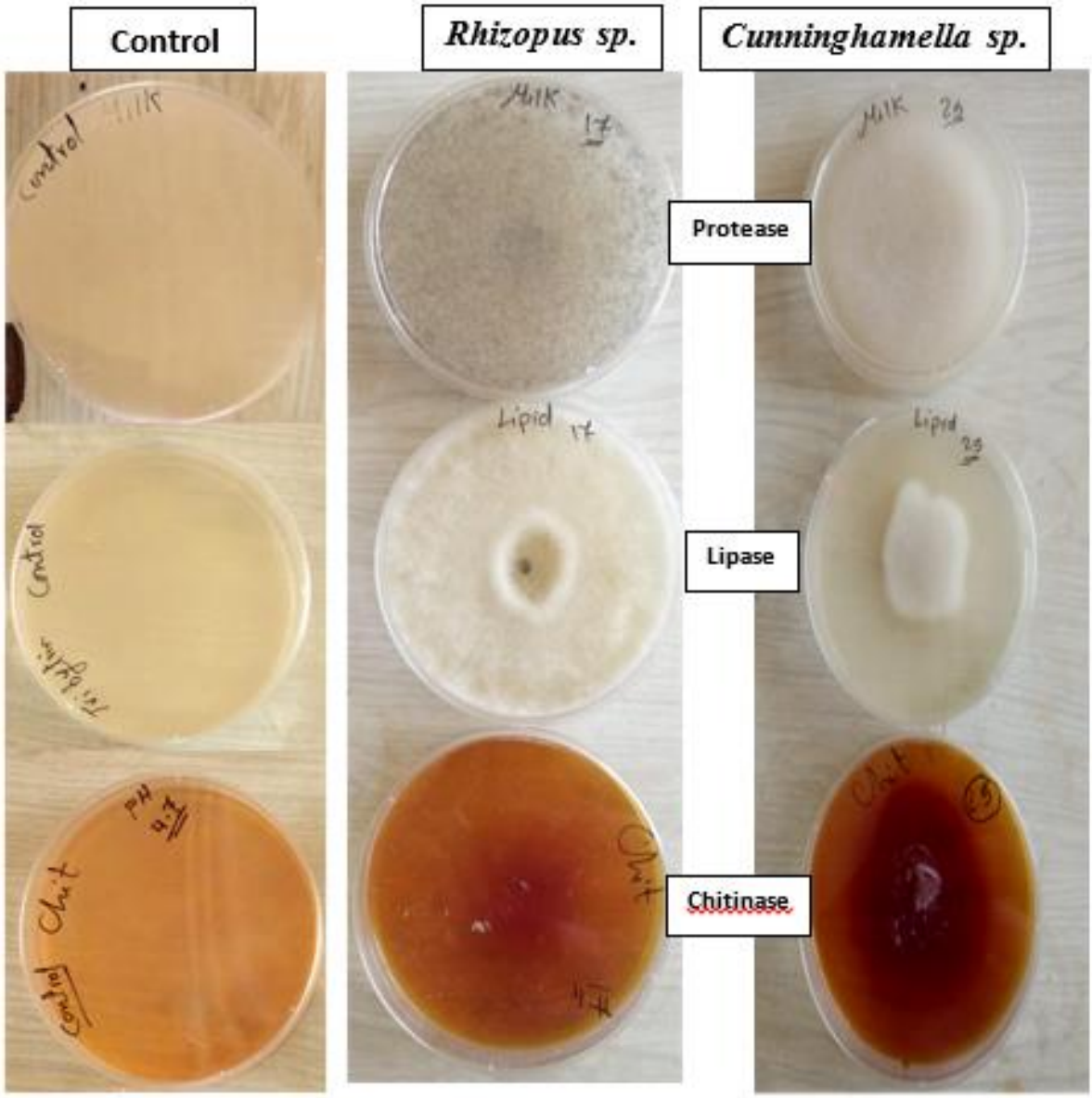

Fig. (5): Hydrolytic enzyme activity in agar medium for the two fungal isolates.

DISCUSSION

There is increasing interest in the use of entomopathogenic fungi for the biocontrol of insect pests (Hajek & St. Leger, 1994 and Evans, 1999).

In the current study, two fungal isolates were identified as *Cunninghamella sp.* and *Rhizopus sp.* and tested against *S. littorallis.* The obtained data revealed that the ability of fungal isolates in inducing mortality. However, the lowest LC_{50} values was recorded by isolate *Rhizopus sp.*, $2.16x10^7$ conidial concentration / ml

This finding was recorded in the same insect with other entomopathogenic fungi. *Trichoderma harzianum* T24 showed 80% larval mortality only when applied at its highest conidial concentration (1×10^8 conidial spores ml^{-1}), while *Aspergillus flavus* showed 100% pupal mortality only at all of its conidial concentrations (Ahmed and El-

Katatny, 2007). The LC_{50} of *Beauveria bassiana* and *Metarhizium anisopliae* against 3rd instar larvae were $7.84x10^{11}$ spores ml^{-1} and $2.89x10^{8}$ spores ml^{-1} after 7 days of treatment (Shairra and Noah, 2014).

Efficacy of entomopathogenic fungi products (i.e. Bio- Power (*Beauveria bassiana*), Bio-Catch (*Lecanicillium lecanii*) and Priority (*Paecilomyces fumosoroseus*) was studied by El-Hawary and Abd El-Salam (2009) against *S. littoralis* larvae. They found that Bio- Power was the most effective product followed by Bio-Catch and Priority against *S. littoralis* 3rd instar larvae.

The two fungal isolates have an effect on pupation and adult emergence %. These data are in accordance with El-Hawary and Abd El-Salam (2009) who recorded that different formulations of fungi products had delayed effects on the percentage of pupae and adults emergence % for the same insect.

The highest conidial concentration of the two fungal isolates insignificantly increased larval duration. Such extended development of infected larvae has been reported in several investigations (Mitchell & Cali, 1994; Henn & Solter, 2000; Hussain *et al*., 2009).

The growth of the larvae was reduced insignificantly irrespective to the fungal isolate. Other insects treated with entomopathogenic fungi exhibited reduction of growth as the eastern spruce budworm larvae, *Choristoneura fumiferana*, with a medium lethal spore dose (2×10^4 spore/mL) of the microsporidium *Nosema fumiferanaei* (Thomson) (Bauer & Nordin, 1988) and *Ocinara varians* larvae with *Beauveria bassiana, Metarhizium anisopliae* and *Isaria fumosorosea* (Hussain *et al.*, 2009).

Several studies suggested that the immune responses in some insects may be under hormonal control and that the parasite can alter the hormonal system, and subsequently, modulate the host immune response (Nappi & Stoffalono, 1972; Nappi, 1975). So the disturbance of hormone may reflect on growth and development of insects.

Mode of action of *Cunninghamella sp.* and *Rhizopus sp.* as biocontrol was studied. The study revealed that the isolates have hydrolytic enzyme activity as chitinase, protease and lipase. Catalyzing activity using specific enzymes is considered one of the main mechanisms of fungal infection to insect host and inducing mortality. Evidence for this has been provided by Shakeri and Foster (2007) who reported production of protease and chitinase during the growth phase of *Trichoderma*. These proteases are believed to assist the penetration of fungal hyphae into the host tissue by degrading the protein linkages in the insect cuticle and/or the utilization of the host proteins for fungal nutrition (Pozo *et al.*, 2004).

The strategy of two fungal isolates in inducing mortality seems to be they produced chitinase, protease and lipase enzymes that are capable of histolyzing insect cuticle (that contain chitin, protein and lipid) and induced mortality by desiccation through the

imperfect cuticle. Failure of adult emergence from pupae by isolate *Cunninghamella sp.* may result from a decrease in chitin because the present work proved that isolate had high chitinase activity. Thus, chitinases are produced by the entomopathogenic fungi to degrade this protective structure (Herrera-Estrella and Chet, 1999).

The use of pathogen may offer an environmentally sound method for the management of insects and fungi are the most promising candidates.

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ARABIC SUMMARY

قدرة تأثير بعض العزلات الفطرية ضد دودة ورق القطن *سبودوبترا ليتوراليس* (حرشفيات الأجنحة: الليليات)

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أجريت هذة الدراسة لفحص قدرة اثنان من العزلات الفطرية ضد دودة ورق القطن *سبودوبترا ليتوراليس*. تم عزل ست عزلات فطرية من عينة التربة. اثنان فقط تم تعريفهم بنوع *كانينجاميلا* ونوع *الريزوبس* بناءاً على نتائج التربة المبدئية والتي أظهرت قدرت هذه العزلات في إحداث أعلى نسبة وفيات. تم تسجيل أعلى نسبة وفيات بواسطة العزلة الفطرية *الريزوبس*. بالإضافة إلى ذلك سجلت نفس العزلة أدنى قيمة للتركيز المميت ل 50% وهو 2.16×10^7 تركيز جرثومي/مل. أظهرت العزلات الفطرية إنخفاضا غير معنوي في أوزان اليرقات المعاملة. انخفضت فترة نمو اليرقات غير معنوياً عند كل التركيزات ما عدا أعلى تركيز والذي أحدث زيادة غير معنوية في حياة اليرقات. أيضا تأثرت نسبة التعذر ونسبة خروج الحشرة الكاملة من العذارى. ظهرت تشوهات الحشرات الكاملة بواسطة العزلة الفطرية *كانينجاميلا*. علاوة على ذلك، العزلة الفطرية *الريزوبس* أظهرت نشاط عالي لإنزيمي الليباز والبروتياز بينما أعلى نشاط لإنزيم الكيتيناز تم تسجيله بالعزلة الفطرية *كانينجاميلا*.