Egypt. J. Plant Prot. Res. Inst. (2021), 4 (1): 150 –156

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Insecticidal , biochemical efficacy and digestive enzymes of isolated endophytic fungi on *Agrotis ipsilon* larvae (Lepidoptera: Noctuidae)

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ARTICLE INFO

Article History

Received: 6 /1 /2021

Accepted: 27 / 2 /2021

Keywords

Agrotis ipsilon, Aspergillus flavus, insecticidal , biochemical and digestive enzymes.

Abstract:

The black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is a worldwide pest of different economic crops. The aim of the present work is to study the effect of five isolated endophytic fungi *Aspergillus flavus* MRDS 301, ***Curvularia*** *lunata* MRDS 302, *Chaetomium madrasense* MRDS 303, *Alternaria alternate* MRDS 304 and *Aspergillus flavus* MRDS 305 against the 3rd larval instar of *A. ipsilon* , to investigate the cumulative percentage mortality of the larvae was treated with five isolated endophytic fungi can be arranged according to the most effective compounds 50, 58.88, 45.55, 42.22 and 40%, respectively. The results showed that biochemical effect on the metabolites (Total carbohydrates, total protein and free amino acids) and digestive enzymes activity (Amylase , invertase, proteases and lipase), the total carbohydrate content in *A. ipsilon* treated larvae was decreased for the larvae fed for 3day on treated leaves with all isolated endophytic fungi at concentration10^7 spores /mL sterilized water. While *A. flavus* MRDS 301 and C. *lunata* MRDS 302 caused decreases in proteins and free amino acid percentage compared to control. A significant reduction in α-Amylase, invertase, proteases and lipase activated were observed in most treated relative to control. In other hand, larvae treated with *C. madrasense* MRDS 303 *and A. alternate* MRDS 304 recorded an increase in invertase, proteases and lipase activities compared to control.

Introduction

The black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is one of the most severe insect pests in Egypt, this noctuid is polyphagous and attacks a large number of field and vegetable crops (Abo El-Ghar *et al.,* 1996). The growers usually use the conventional insecticides, especially organophosphates, in controlling this pest. However, the use of pesticides has resulted in environmental contamination (Frank *et al.,* 1990) negative effects on non-target organisms (Franz, 1974). One very exciting area in mycology involves the study of fungal endophytes. The term ‘‘endophyte” was coined by De Bary (1884) and is used to define fungi or bacteria that occur inside asymptomatic plant tissues. Fungal endophytes are ubiquitous and are dominated by Ascomycota (Arnold and Lutzoni, 2007). Different genera of fungal

entomopathogens have been reported as naturally occurring fungal endophytes, and it has been shown that it is possible to inoculate plants with fungal entomopathogens, making them endophytic. Their mode of action against insects appears to be due to antibiosis or feeding deterrence. Research aimed at understanding the fungal ecology of entomopathogenic fungi, and their role as fungal endophytes, could lead to a new paradigm on how to successfully use these organisms in biological control programs (Vega, 2008).

The present work aimed to investigate insecticidal and biochemical efficacy of five isolated endophytic fungi (*Aspergillus flavus* MRDS 301, *Curvularia lunata* MRDS 302, *Chaetomium madrasense* MRDS 303, *Alternaria alternate* MRDS 304 and *Aspergillus flavus* MRDS 305) against the 3rd larval instar of *A. ipsilon.*

Materials and methods

1. Isolation of endophytes:

The isolations of endophytic fungi from maize hybrids were carried out according to Parsa *et al.* (2013) with some modification by El-Lebody *et al.* (2021).

2. Insect culture:

Susceptible strain of the black cutworms, *A. ipsilon* was reared on castor leaves away from any insecticidal contamination in the laboratory of Pest Physiology Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt under controlled laboratory conditions at 25 ± 2°C and 65 ± 5 % RH.

3. Insecticidal activity:

The isolated endophytic fungi were tested individually against *A. ipsilon* 3rd instar larvae to investigate its Insecticidal activity. The castor leaves (*Ricinus comumunis*) were washed, dried and used as food for *A. ipsilon* larvae. The isolated endophytic fungi at concentration 10^7 spores /mL were placed on the surface of the leaves and left for a few minutes to dry. Each *A. ipsilon* 3rd instar larvae were transferred singly in small vials (3.5diam, 8cm high) with treated leaves. Castor leaves treated with sterilized_distilled water were used as control. Three replicates (10 larvae each) were used for each treatment. Three days post-treatment, dead larvae were counted, and alive ones were transferred to small vials containing untreated castor leaves and renewed as needed. Dead larvae were recorded at 3, 6, 9 and 12 interval days.

4. Biochemical studies:

For the biochemical studies 3rd instar larvae were treated as previously described (30 larvae / three replicates) with the isolated endophytic fungi at concentration 10^7 spores /mL for 3 days. Ten live larvae per treatment and control were randomly selected for the biochemical analysis.

5. Preparation of insects for biochemical analysis:

The insects were prepared as described by Amin (1998). They were homogenized in distilled water (50 mg of larvae /5ml sterilized_distilled water). Homogenates were centrifuged at 8000 rpm for 15 min at 2°C in a refrigerated centrifuge. The deposits were discarded and the supernatants, can be stored at least one week without appreciable loss of activity when stored at 5°C.

6. Biochemical analysis:

Total carbohydrate was estimated according to Dubois *et al.* (1956). Total protein content was determined according to the method of Bradford (1976). Total amino acids were assayed according to the method described by Lee and Takabashi (1966). Total carbohydrate was extracted and prepared for assay according Cromppton and Birt (1967). Amylase and invertase activities were assayed according to the method described by Ishaaya and Swiriski (1976). Starch and

sucrose was used as substrates for amylase and invertase, respectively. Proteolytic activity was measured as described by Tatchell *et al.* (1972), Lipase activity was determined by a slight modification of the procedure of Tahoun and Abdel-Ghaffar (1986).

7. Statistical analysis:

The data were statistically analysed by means of analysis of variance (ANOVA) (Tukey's test) at $P<0.05$ using IBM® "SPSS" STATISTICS Ver.26 software, most of the results were expressed in percentage, although actual numbers were used for statistical tests. Results were recorded as mean ± standard deviation (SD).

Results and discussion

1. Insecticidal activity:

The accumulated mortality and latent effect of isolated endophytic fungi from the maize hybrids at concentration 10^7 spores /mL against the 3rd larval instar of *A. ipsilon* were presented in Table (1) revealed that *A. flavus* MRDS 301 recorded high mortality percentage (14.44%) and *C. lunata* MRDS 302 recorded (10%) where *C. madrasense* MRDS 303 and *A. alternata* MRDS 304 recorded (5.55%) and A. *flavus* MRDS 305 was lowest insecticidal potentials (2.22%) after 3 days of treatment. After 6 days of treatment, *C. lunata* MRDS 302, *A. flavus* MRDS 301 , *A. alternata* MRDS 304 *C. madrasense* MRDS 303and *A. flavus* MRDS 305 were (27.77, 24.44, 22.22, 17.77 and 15.55%),respectively. After 9 days were (31.11, 27.77, 2.22, 18.88 and 15.55%), respectively. The obtained data also showed that after 12 days of treatment, insecticidal activity arranged according the highest mortality percentage for *C. lunata* MRDS 302 , *A. flavus* MRDS 301 , *C. madrasense* MRDS 303, *A. alternata* MRDS 304, and *A. flavus* MRDS 305 were (58.88,50, 45.55 ,42.22 and 45.55%), respectively. It was noticed that the mortality percentages of all treatments increased lateral with time. The obtain result agree with El-Shershaby (2010) as slight toxicity by testing capparis extracts on the black cutworm, *A. ipsilon* and El-Lebody *et al*. (2021) noticed that the mortality percentages of all treatments increased lateral with time. After 14 days of treatment, latent pathogenicity ranged between 2.5% to 25% for *C. madrasense* MRDS 303 and *A. flavus* MRDS 301, respectively.

Table (1): Insecticidal effects of endophytic fungi of maize hybrids against the 3rd larval instar of *Agrotis ipsilon*.

Endophytic fungi	Days			
	3	6	9	12
***Chaetomium madrasense* MRDS 303**	**5.55%**	**17.77%**	**18.88%**	**45.55%**
***Alternaria alternate* MRDS 304**	**5.55%**	**22.22%**	**22.22%**	**42.22%**
***Aspergillus flavus* MRDS 305**	**2.22%**	**15.55%**	**15.55%**	**40%**
***Aspergillus flavus* MRDS 301**	**14.44%**	**24.44%**	**27.77%**	**50%**
***Curvularia lunata* MRDS 302**	**10%**	**27.77%**	**31.11%**	**58.88%**
Control	**2.22%**	**3.33%**	**4.44%**	**5.55%**

2. Biochemical analysis:

Carbohydrates are of vital importance since they can be utilized by the insects' body for production of energy or conversion of lipids or proteins (Fahmy, 2017). That explains the total carbohydrate content in *A. ipsilon* larvae was decreased for the larvae fed on treating leaves with the isolated endophytic fungi at concentration10^7 spores /ml for 3 days (Table 2). Under stress conditions, more sugars might be metabolized to meet out the energy expenses. This

could be the reason for the carbohydrate level depletion in the treated insects (Fahmy , 2008)., that explain the increased in proteins and free amino acid percentage in the *A. ipsilon* larvae treated with *C. madrasense* MRDS 303, *A. alternata* MRDS 304 and A. *flavus* MRDS 305 compared to controls.

Table (2: Effect of Endophytic fungi on the Metabolites of *Agrotis ipsilon* 3rd larval instar.

Endophytic fungi	Total proteins (mg/g.b.wt)		Total carbohydrates (mg/g.b.wt)		Free amino acids(ug alanine/g.b.wt)	
	Mean ± SD	% Change to control	Mean ± SD	% Change to control	Mean ± SD	% Change to control
***Chaetomium madrasense* MRDS 303**	22.13± 1.52 a	47.53	5.24±0.26 b	-8.23	61.9±1.73 a	16.01
***Alternaria alternate* MRDS 304**	21± 1.80 a	40	5.08±0.13 b	-11.03	63±2.29 a	18.07
***Aspergillus flavus* MRDS 305**	16.23± 0.58 b	8.2	4.85±0.19 b	-15.06	55.73±1.48 b	4.44
***Aspergillus flavus* MRDS 301**	12.76± 0.81 c	-14.93	3.34±0.16 c	-41.51	40.96±1.74 c	-23.24
***Curvularia lunata* MRDS 302**	11.73±0.49 c	-21.8	5.65±0.17 a	-1.05	43.16±2.02 c	-19.12
Control	15± 1.05 b	0	5.71±0.33 a	0	53.36±4.56 b	0
LSD	2.05		0.39		4.5	
F	41.22		45.65		40.15	
P	.0001***		.0001***		.0001***	

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Metabolism of carbohydrates is controlled mainly by carbohydrate hydrolyzing enzymes. In Table (3) there is a significant reduction in digestive enzyme amylase and Invertase in most treatment of larvae feeding on endophytic fungi relative to control. Amylases hydrolyze starch into the monosacccharides, glucose, and fructose and secreted by larva salivary glands and midgut (Ribeiro *et al.*, 2000). Invertases cleave sucrose into the mono saccharides, glucose, and fructose and are secreted and worked in the gut of the larvae (Heil *et al.*,

2005).The final product of carbohydrate metabolism is glucose, the increase of these enzymes during the larval stage suggested that these enzymes degrade carbohydrates to glucose for chitin build-up (Wyatt, 1967). In this study isolated endophytic fungi decreased the activity of digestive enzymes. So, degradation of carbohydrates also decreases. This leads to disturbance in chitin building and the failure of the molting process. In recently, attention has been focused on the idea of using digestive enzyme inhibitors that affect the growth and development of pest species. Inhibitors of insect amylases and proteases obtained have been demonstrated to be important in the control of insect pests (Franco *et al.*,2000 and Mehrabadi *et al.*, 2012).

Table (3):Effect of Endophytic fungi on the digestive enzymes of *Agrotis ipsilon* 3rd larval instar.

Endophytic fungi	Invertase(ug glucose/min/g.b.wt)		Amylase (ug glucose/min/g.b.wt)		Proteases(ug D,L alanine/min/g.b.wt)		Lipase (mU/g.b.wt)	
	Mean ± SD	% Change to control	Mean ± SD	% Change to control	Mean ± SD	% Change to control	Mean± SD	% Change to control
***Chaetomium madrasense* MRDS 303**	272.33±7.76 a	8.21	119.33±6.65 c	-37.85	56.5±3.04a	37.37	108.33± 4.50bc	4.84
***Alternaria alternate* MRDS 304**	257.33±10.59 b	2.25	109. 33±8.38 c	-43.06	50.63±2.55 b	23.09	126.66±5.68 a	22.58
***Aspergillus flavus* MRDS 305**	202±5.29 d	-19.73	150±9.64 b	-21.87	31.9±1.85 d	-22.44	74± 5.29 e	-28.38
***Aspergillus flavus* MRDS 301**	144.66±8.96 e	-42.52	108.33±5.68 c	-43.58	22.96±3.32 e	-44.18	93.66± 4.04d	-9.35
***Curvularia lunata* MRDS 302**	238.33±3.78 c	-5.30	146±7 b	-23.96	*36.3*±2 .28 cd	-11.74	114.66± 6.50b	10.96
Control	251.66±10.40 bc	0	192±13 a	0	*41.13*±4.56 c	0	103.33± 3.78 c	0
LSD	14.59		15.54		5.47		9.01	
F	99.11		40.65		48.02		38.69	
P	.0001***		.0001***		.0001***		.0001***	

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