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Isolation and pathogenicity of endophytic fungi associated with some maize hybrids against certain Lepidoptera pests

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ABSTRACT

The means of biological control methods in which entomopathogens are being applied to crops has been adopted for several years. However, endophytic entomopathogenic fungi could provide plants with a longer protective effect compared to usual bio-control agents' applications since they are being internally associated with plants and could grant them more sustainable protection. This study was an attempt to isolate the endophytic fungi from Hytech 2031, Pioneer 3444 and SC 132 maize hybrids and investigating their pathogenicity against *Sesamia cretica*, *Pectinophora gossypiella* and *Spodoptera littoralis* in different larval instars. Five fungi were isolated from the maize hybrids, *i.e. Aspergillus flavus* MRDS 301, *Curvularia lunat*a MRDS 302, *Chaetomium madrasense* MRDS 303, *Alternaria alternate* MRDS 304 and *Aspergillus flavus* MRDS 305. *C. lunata* MRDS 302 and *C. madrasense* MRDS 303 treatments showed insecticidal effect (100 %) against *S. cretica* 1st instar larvae. All treatments against *S. cretica* significantly reduced the weight of the resulting full-grown instar larvae. *C. madrasense* caused the highest mortality to *P. gossipiella* 1st instar larvae compared to other treatments. In general, *A. flavus* MRDS 305 was more effective than *A. flavus* MRDS 301 against all the tested pests. Soil infestation was a highly effective method of inoculation to introduce the fungus *C. madrasense* inside maize plants since the fungus was isolated from both roots and leaves.

Keywords: Endophytes, entomopathogens, fungi, maize, Lepidoptera pests

INTRODUCTION

Endophytes are microorganisms able to reside in the plant tissues without causing any detrimental effects to the plant (Carroll, 1988; Petrini, 1991). This kind of intimate association is primarily a mutualistic relationship, in which both partners benefit from each other. The plants accommodate these microorganisms, protect them from adverse environmental conditions and provide them with nutrients. On the other hand, the metabolites of the endophytes can be beneficial for the plant host as they may provide them with some resistant to pathogens and insects or may promote plant growth (Hyde and Soytong, 2008; Rodriguez *et al.* 2009; Moonjely *et al.,* 2016). However, due to biotic or abiotic stresses, some endophytic fungi may switch to a pathogenic mode to the plant host after some time of mutualism, thus some endophytes could be considered as latent pathogens (Sieber 2007; Hyde and Soytong, 2008).

Interestingly, this unique silent association between the plants and the endophytes hides very rich metabolic activities which both sides are accountable for. The secondary metabolites produced by endophytes consist of abundant and diverse types of compounds such as phenols, steroids, flavonoids and alkaloids which retain beneficial biological activities (Strobel, 2003; Murphy *et al.* 2018). In this regard, the mode of action by which the endophytic fungi provide their plants with a level of resistance against pathogens and pests involves various types of bioactive metabolic compounds produced by the fungi (Chutulo and Chalannavar, 2018). Laib *et al.* (2020) found that the extract of the endophytic fungus *Isaria fumosorosea* contains phenols, flavonoids and alkaloids and had an insecticidal effect against *Locusta migratoria* and *Acanthoscelides obtectus*.

Numerous investigations have been focusing on the entomopathogenic activity of the endophytic fungi. *Aspergillus flavus* and *A. terreus* were frequently isolated as endophytes from maize plants and it was suggested that these fungi could be potential entomopathogens against some economic pests (Russo *et al*., 2016). Furthermore, the endophytes, *Cladosporium oxysporum* and *Rigidoporus vinctus* were isolated from different maize varieties and showed entomopathogenic activity against the 2nd instar larvae of *Chilo partelllus* (Renuka and Ramanujam, 2016). Moreover, cotton plants hosting the fungus *Chaetomium globosum* were found to be more resistant to the pierce sucking pest *Aphis gossypii* and the chewing pest *Spodoptera exigua*, in addition to its plant growth-promoting effect (Zhou *et al.,* 2016). A study carried out by Mantzoukas and Grammatikopoulos (2020) showed that sweet sorghum plants that were inoculated with the endophytes *Beauveria bassiana*, *Metarhizium robertsii* and *Isaria fumospora* were less affected by *Sesamia nonagrioides* larvae and the feeding performance and survival of the larvae on the inoculated plants were negatively affected.

Either naturally colonized by or artificially inoculated with entomopathogenic fungi, plants may exhibit a significant level of resistance against economic insect pests (Mantzoukas and Eliopoulos, 2020). Moreover, there has been strong evidence that, in some plant species, these endophytic entomopathogenic fungi (EEF) can be transferred vertically by seeds from the parents to the plants' off-springs due to their systemic colonization of the host without affecting the plant genome (Verma *et al.,* 2008). Therefore, EEF presents a substantial alternative to the application of either fungal or chemical insecticides and genetically modified plants, since they could grant their host plants with systemic and long term protection against pests (Yang, 2015; Bamisile *et al.,* 2018 a & b; Russo *et al.,* 2019; Bubica Bustos *et al.,* 2020).

In Egypt, maize is an economically indispensable crop grown for domestic consumption (Al-Eryan *et al.,* 2019). One of the most important pests that threaten maize crop in Egypt is the stem borer, *Sesamia cretica*, which attack the plant during seedlings stage, however, due to their vulnerability, maize seedlings attacked by this pest could be destroyed (El-Husseini *et al.,* 2018; Al-Eryan *et al.,* 2019). Many endophytic entomopathogenic fungi have potential pathogenic effects against the maize lepidopterous and price sucking insect pests and also promote plant growth and yield (Mahmood *et al.,* 2019 and Russo *et al.,* 2019).

The present study aimed at the isolation of endophytic fungi from maize hybrids and investigating their pathogenicity against some economically important pests: *Sesamia cretica* Lederer (Lepidoptera: Noctuidae) (maize pest), *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) (cotton pest) and *Spodoptera littoralis* (Lepidoptera: Noctuidae) (pest of a wide variety of vegetables and crops) in Egypt.

MATERIAL AND METHODS

Growing maize hybrids:

Seeds of the maize hybrids SC 132, Hytech 2031, and Pioneer 3444 were planted in 30 cm diameter pots containing sandy clay soil under natural conditions in the greenhouse of the Field Crops Research Institute, ARC, Giza. Leaves were excised from plants at the age of 25 days. Leaves were immediately transferred to the lab in plastic bags for carrying out the isolation of endophytes.

Isolation of endophytes from maize hybrids:

The isolation of endophytic fungi was carried out according to Parsa *et al.* (2013) with some modification. Collected maize leaves were cut into segments and sterilized in petri dishes containing 0.5% sodium hypochlorite for two minutes. Leaves segments were then transferred to a sterilized Petri dish containing sterilized distilled water for rinsing. The rinsing was repeated three times and the segments were then placed between layers of filter papers and left to dry.

The leaves segments were cut to get rid of the edges. These segments were further cut up to squared pieces (about 4x4 mm) and then transferred to Petri dishes containing potato dextrose agar medium (PDA) (5 pieces/dish). Four Petri dishes were used as replicates for each maize hybrid. For assuring the efficacy of the sterilization procedure, 100 µL from the water of the final wash step of each hybrid were transferred to Petri dishes containing PDA. Petri dishes were incubated at 25± 2°C for up to 21 days and were checked daily for the emergence of fungi. The emerged fungi were solely transferred to new Petri dishes containing PDA medium and incubated at 25±2°C for 5-7 days. The growing fungal cultures were further used for morphological and molecular characterization.

Characterization of the isolated fungi:

Morphological characterization:

The isolated endophytic fungi were morphologically characterized based on different features such as colony morphology, conidia, conidiophores, ascospores and ascomata (Ames, 1961; Domsch *et al.,* 1980; Wang *et al.,* 2016)

Molecular characterization:

The isolated fungi were grown on PDA Petri dishes for 7 days at 25± 2°C. The mycelia were harvested and grinded in liquid nitrogen using mortars and pestles. The grinded mycelia, ~100 mg, were transferred to 2 mL microtubes and DNA was extracted using DNeasy QIAGEN plant mini kit following the manufacturer instructions. DNA concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific Fisher Scientific, USA) and was then stored at −20°C for further use. Amplification of the partial 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and partial 28S rRNA gene; was carried out using the primer pair ITS1 (TCCGTAGGTGAACCTGCGG)/ ITS4 (TCCTCCGCTTATTGATATGC) (White *et al*., 1990). The amplification was carried out in 25µl reaction volume using 2µl (50-100ng) of genomic DNA, 5 µl 5xGotaq buffer, 1.5µl MgCl2, 0.5µl of 2.5mM dNTPs mixture, 0.5µl of 10µM of each primer, 0.2µl of 5U/µl Taq DNA polymerase (Promega). PCR reaction conditions were: an initial cycle of 95°C of 10 min, 30 cycles of 95°C for 60 sec, 55°C for 60 sec and 72°C for 90 sec, then a final extension on 72°C for 90 sec. Five microliters of the PCR products were visualized by electrophoresis on 1% agarose gel stained with EZView Stain under trans-illuminator. The PCR products were sequenced at Macrogen Inc., (Korea). The consensus sequences were analyzed and evaluated by BLAST search tool and were submitted to the GenBank with unique accession numbers.

Insects Rearing:

Three insect pests were used in this investigation: **a.** The greater sugarcane borer *S. cretica* were collected from the maize fields of ARC, Giza, Egypt. Larvae were reared in the laboratory on an artificial diet as recommended by (El-Metwally *et al.,* 1997) at 25 ± 2°C and 70 ± 5% RH conditions. **b.** A laboratory strain of the cotton pink bollworm, *P. gossypiella*, which was reared according to (Rashad and Ammar, 1985). **c.** The cotton leaf worm *S. littoralis* (Lepidoptera: Noctuidae). The second instar larvae of *S. litorallis* were obtained from Cotton leafworm division, Plant Protection Research Institute (PPRI), ARC,

Giza. Obtained larvae were treated directly. The treatment was conducted according to Khedr and El-Kawas (2013) with slight modification.

Fungal inoculum:
The isolated endophytic fungi were grown on PDA Petri dishes for 7-10 days at 25 ± 2°C. The fungal growth was scratched in sterilized distilled water using a scalpel in order to release and detach the spores in the water. The suspensions concentrations were estimated using a haemocytometer counting slide and were adjusted to 10^7 spore/mL.

Insecticidal activity of isolated fungi:
The isolated endophytic fungi were separately tested against different larval instars of the insect species mentioned above to investigate their potential insecticidal effect.
Prior to the assay, fresh fungal spore suspensions were mixed with an artificial diet free of anti-microbial agents at the rate of 1 ml/3 g diet in sterilized Petri dishes. The artificial diet was mixed with sterilized distilled water (SDW) and set as a control. Screening studies of fungal insecticidal activities was based on insect mortality data (%) which was corrected according to Schneider-Orelli's formula (Püntener, 1981). The bioassay was carried out as follows:
***S. cretica* 1st instar larvae:**
S. critica neonate larvae were transferred to plates containing a diet mixed with fungal spores suspension. Three replicates (10 larvae each) were set for each treatment in addition to the control. All treatments and control were incubated at 25±2°C and 70 ± 5% RH conditions. The treatments were inspected 3 days post-treatment.
The dead larvae were counted and the alive larvae were transferred individually on a recommended fresh diet in a sterilized test tube and inspected daily for 14 days post-treatment. Larval mortalities were recorded.
***S. cretica* 3rd instar larvae:**
Insecticidal potential of the same isolated endophytic fungi against *S. critica* 3rd instar larvae was carried out with the aforementioned described procedure. Three replicates (10 larvae each) were set for each treatment in addition to the control. The individual treated larvae were inspected daily for 28 days until full growth to determine the latent insecticidal effect of treatments. Also, the mycosis percentages were recorded.
***P. gossypiella* 1st instar larvae:**
P. gossypiella 1st instar larvae were treated with the endophytic fungi following the same procedure mentioned above. Three replicates (15 larvae each) were set for each treatment in addition to the control.
***S. littoralis* 2nd instar larvae:**
Leaves of Castor oil plant (*Ricinus comumunis*) were washed, dried and used as food for *S. littoralies* larvae. Leaves were placed in Petri dishes and the spores suspension inoculum of the fungi were placed on the surface of the leaves and left for few minutes to dry. *S. littoralies* 2nd instar larvae were placed on the surface of the leaves. Castor oil leaves treated with sterilized distilled water were used as control. Three replicates (10 larvae each) were set for each treatment. Three days post-treatment, dead larvae were counted and alive ones were transferred to Petri dishes containing untreated Castor oil leaves that renewed every 3 days. Dead larvae were recorded for up to 9 days.

Colonization assay:
This assay was carried out to study the potentiality of the isolated endophytic fungi to colonize maize plants. In this assay, the endophytic fungus *C. madrasense* MRDS 303 was selected form the isolated fungal strains as it was the most effective against the tested insect pests. The maize Inbred line Sk12 seeds were planted in 30 cm diameter pots containing sandy clay soil in the greenhouse of the Field Crops Research Institute, ARC, Giza. Two methods were used in the artificial inoculation of the plants as follows:
1. Foliar treatment: a spore suspension of *C. madrasense* MRDS 303 strain was prepared as mentioned earlier in this study. The foliar of the plants were treated with two techniques *i.e.* spraying and application directed vertically into whorls.
2. Soil infestation: the inoculum of *C. madrasense* MRDS 303 was prepared by growing the fungus in bottles containing sterilized sorghum grain medium (100 g/bottle) to enrich the fungus. The inoculated bottles were incubated at 25±2°C for 10-12 days. The soil in the prepared pots was infested with 3% (w/w) of fungus inocula. The seeds of maize Inbred line Sk12 were planted in the pots one week after soil infestation. The leaves of maize plants from both methods of inoculations mentioned above as well as the control were excised 21 days after inoculation. The leaves were transferred immediately to the laboratory and the isolation of the endophytic fungi was carried out as mentioned earlier in this study.

Statistical analysis:
The experimental design was completely randomized. Obtained data were analyzed using Proc ANOVA in SAS (Anonymous, 2003). Means separation was conducted using Duncan's multiple range test ($P \leq 0.05$) in the same statistical package.

RESULTS

Isolation and identification of endophytic fungi:
The isolated fungi were characterized molecularly based on sequencing of the partial rDNA internal transcribed spacer (ITS). Data generated from the sequencing were deposited in the gene bank database and strains were designated accession numbers and retained in the Mycology Res. and Disease Survey Department fungal collection with unique IDs (**Table 1**). Morphological characterization of the isolated fungi was carried out on a PDA medium and illustrated as follows (**Fig. 1**):

Chaetomium madrasense MRDS 303: The colony surface color was olivaceous to grey-green while the under surface was tinged with yellow and the revers was colorless to yellow. Olivaceous ascomata were ellipsoid, ostiolate and sparse on the surface of the colony. Both terminal and lateral hairs were coiled and finely roughened. Asci emerged from the ascomata and were club-shaped and ascospores were limon shaped. *Aspergillus flavus* MRDS 301/305 The colony growth was rapid, looking floccose and the colour of the colony was light to olivaceous green/dark greyish green while the undersurface was cream to light lime. With microscopic examination, conidiophores erected from hyphae were looking rough and the conidial heads radiated at the top of the conidiophores. Conidia were globose to subglobose and they looked smooth to slightly rough. *Curvularia lunata* MRDS 302 The colony was deep olivaceous to black with illuminating flattened surface. With microscopic examination, septated deep brown conidiophores erected singly or branched, bearing conidia in a sympodial pattern. Conidia looked mostly curved. *Alternaria alternata* MRDS 304 The colony was greyish-green to black. Conidia formed in long chains mostly branched. Conidia smooth or finely rough with a short conical beak.

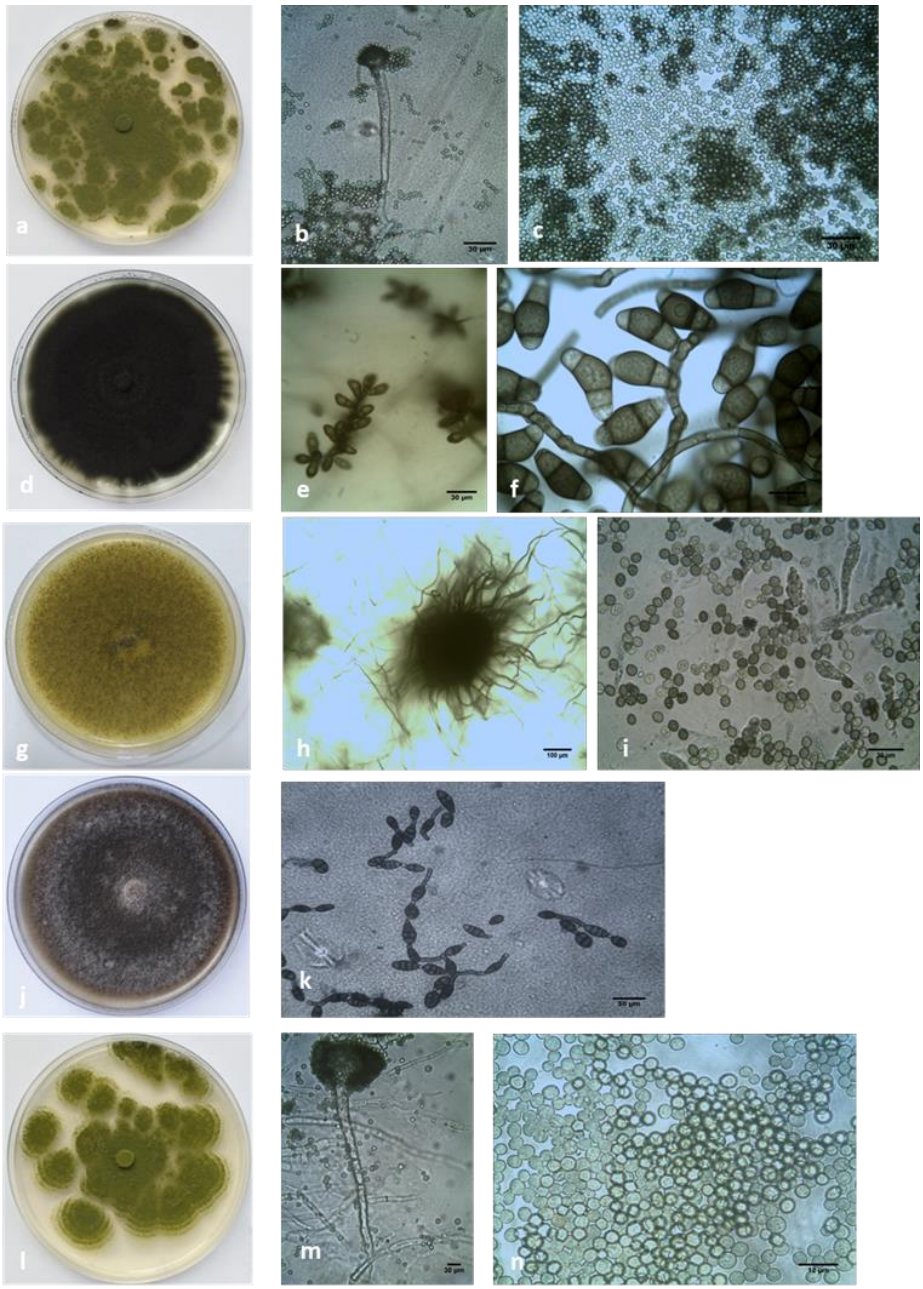

Fig 1. *Aspergillus flavus* MRDS 301: a) colony morphology, b) conidiophores and conidia, c) conidia, bar=30µm. *Curvularia lunata* MRDS 302: d) colony morphology, e) conidiophores bearing conidia, bar=30 µm, f) conidia, bar=10 µm. *Chaetomium madrasense* MRDS 303: g) colony morphology, h) ascomata, bar=100 µm, i) asci and ascosopres, bar=30µm. *Alternaria alternata* MRDS 304: j) colony morphology, k) chains of conidia, bar=50µm. *Aspergillus flavus* MRDS 305: l) colony morphology, m) conidiophores bearing conidia bar=30 µm, n) conidia, bar=12 µm.

Isolation frequency:

Data presented in **Table (1)** show that SC132 maize hybrid was colonized with different species of endophytic fungi while the Hytech and Pioneer hybrids were colonized with single fungal species each. Data revealed that the endophytic fungus, *A. alternata* was more frequent in the SC132 maize hybrid compared to *A. flavus* and *C. madrasense*. Data also showed that *A. flavus* was present in both SC132 and Hytech maize hybrid and *C. lunata* was isolated only from Pioneer maize hybrid.

Table 1. Endophytic fungi isolated from different maize hybrids and their frequency %.

Maize Hybrid	Endophytic Fungi	Code	Accession Number	Frequency %
Hytech 2031	*A. flavus*	MRDS 301	MT509718	6.66
Pioneer 3444	*C. lunata*	MRDS 302	MT509717	6.66
SC 132	*C. madrasense*	MRDS 303	MT452310	6.66
	A. alternate	MRDS 304	MT509716	13.33
	A. flavus	MRDS 305	MT509715	6.66

Insecticidal effect of endophytic fungi:

Data in **Table (2)** show the insecticidal potential and latent effect of isolated endophytic fungi from the three maize hybrids at concentration 10^7 spores /mL against the larval instar (s) of *S. cretica*, *P. gossypiella* and *S. littoralis*. Data revealed that *C. madrasense* MRDS 303, *A. alternata* MRDS 304, and *C. lunata* MRDS 302 showed high significant insecticidal potentials (82.1%, 82.1% and 100%, respectively) against *S. cretica* 1st instar larvae after 3 days of treatment. In addition, *A. flavus* MRDS 305 showed insecticidal activity of 85.7% compared to 67.8% for *A. flavus* MRDS 301. After 14 days of treatment, a highly significant latent pathogenicity effect ranged between 71.5% and 100% for *A. flavus* MRDS 301 and *C. madrasense* MRDS 303, respectively (**Table 2**). The obtained data also showed that all tested isolates did not have insecticidal activity (0.0 %) against *S. cretica* 3rd instar larvae after 3 days of treatment. It was noticed that the mortality percentages of all treatments increased laterally 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. After 28 days of treatment, the accumulated mortality range was 82.7% for *A. flavus* MRDS 305 and 19.3 % for *C. lunata* MRDS 302 against the resulting full-grown larvae (**Table 2**).

Table 2. Entomopathogenicity of endophytic fungi of maize hybrids against the larval instar(s) of *S. cretica, P. gossypiella* and *S. littoralis*.

Endophytic fungi	*S. cretica*					*P. gossypiella*		*S. littoralis*	
	1st instar		3rd instar			1st instar		2nd instar	
	Days post-treatment								
	3	14	3	14	28	3	14	3	9
C. madrasense MRDS 303	82.1 AB	100 A	0.0	2.5 C	71.2 AB	71.1 A	75.2 A	3.3 A	13.3 A
A. alternate MRDS 304	82.1 AB	86.3 B	0.0	7.5 BC	59.6 B	57.8 A	77.8 A	0.0 A	10.0 A
A. flavus MRDS 305	85.7 AB	89.7 B	0.0	15.0 B	82.7 A	66.7 A	77.8 A	3.3 A	10.0 A
A. flavus MRDS 301	67.8 B	71.5 C	0.0	25.0 A	79.8 A	60.0 A	72.2 A	-*	-
C. lunata MRDS 302	100.0 A	100.0 A	0.0	5.0 BC	19.3 C	66.7 A	75.3 A	3.3 A	10.0 A
Control	6.6 C	6.6 D	0.0	3.3 C	13.3 C	0.0 B	10.0 B	0.0 A	0.0 A
P	0.003	0.0001	-	0.001	0.0001	0.69	0.5	0.8	0.98

Means followed by the same letter(s) in the same column are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

*data were not recorded.

Mycosis (2.5%) of treated larvae was recorded for *C. madrasense* MRDS 303 after 7days of treatment. *A. flavus* MRDS 305 also induced mycosis (5% and 2.5 %*)* after 7 days and 20 days of treatment. The same percentages of mycosis were recorded for *A. flavus* MRDS 301 after 7 days and 30 days of treatment (**Fig. 2 and 3**).

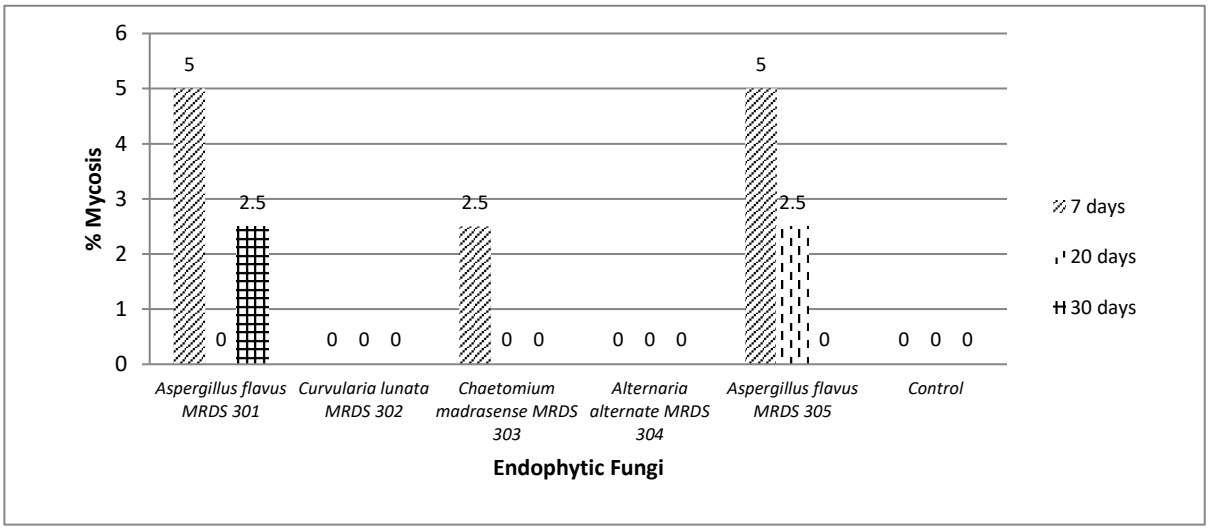

Fig 2. Effect of endophytic fungi in inducing mycosis on *S. cretica* 3rd instar larvae

Moreover, all endophytic fungi isolates caused a highly significant reduction in the weight of resulting full-grown larvae (0.61 to 0.69 g. / 5 larvae) compared to untreated larvae (0.9 g / 5 larvae) (**Fig. 4**). Obtained results revealed that the SC132 EF (C. *madrasense, A. alternata,* and *A. flavus*), Hytech 2031 EF (*A. flavus*) and Pioneer 3444 EF (C. *lunata*) showed insecticidal activity (71.1, 57.8, 66.7 %), (60.7 %) and (66.7 %) and latent effect (75.3, 77.8, 77.8%), (72.2%) and (75.3%) against *P. gossypiella* with non-significant differences after 3 days and 14 days of treatment, respectively (**Table 3**). As shown in **Table (2)**, *C. madrasense, A. alternata, A. flavus* and *C. lunata* induced a very weak insecticidal activity and latent effect (3.3% & 13.3%), (0.0% & 10%), (3.3% &10.0%) and (3.3% &10.0%) against *S. littoralis* larvae with no significant differences after 3 days and 9 days of treatment, respectively.

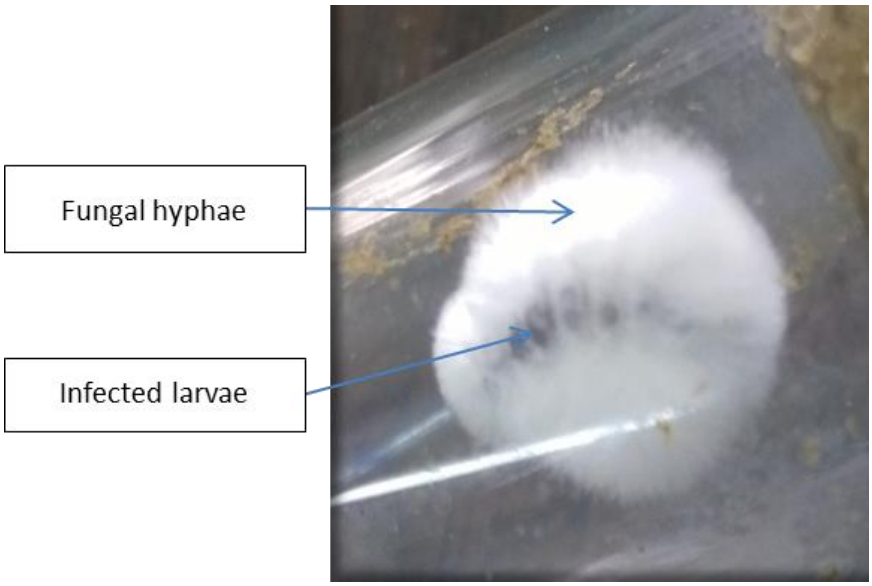

Fig 3. Mycosis of dead *S. cretica* larvae infected with *A. flavus* MRDS 305.

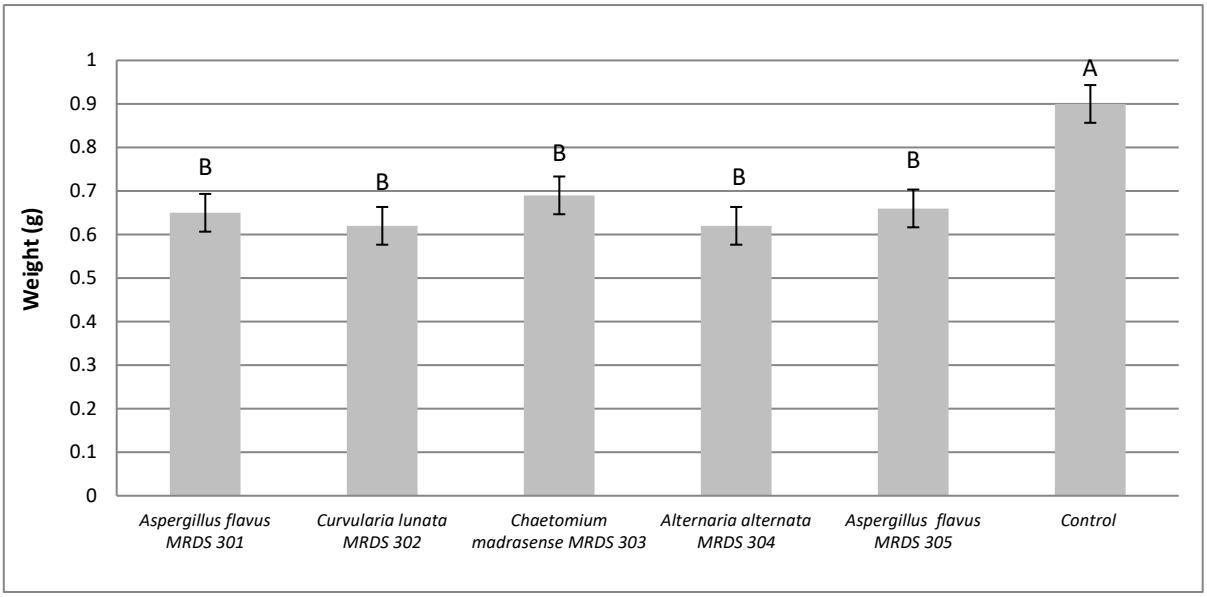

Fig 4. The effect of endophytic fungi on the weight (g) of the full-grown larvae of *S. cretica*

Colonization studies:

Data revealed that *C. madrasense* MRDS 303 was able to endophytically colonize maize inbred line Sk12 by artificial inoculation and that both methods of inoculation, foliar treatment and soil infestation, were effective in delivering the fungus inside the plant tissues. In plants treated with a spore suspension of the fungus, both applications directed into whorl and spraying of the foliar were equally effective in delivering the fungus inside the plant tissues and it was also noticed that no fungi were isolated from plants of the control treatment (**Fig. 5**). Data also indicated that, in plants grown in soil infested with C. *madrasense*, the fungus was able to colonize both roots and shoots tissues (**Fig. 6**). However, in plants of the control treatment, the leaves were free of endophytes contrary to the roots which were naturally infected with *C. madrasenes* as an endophyte (**Fig. 6**).

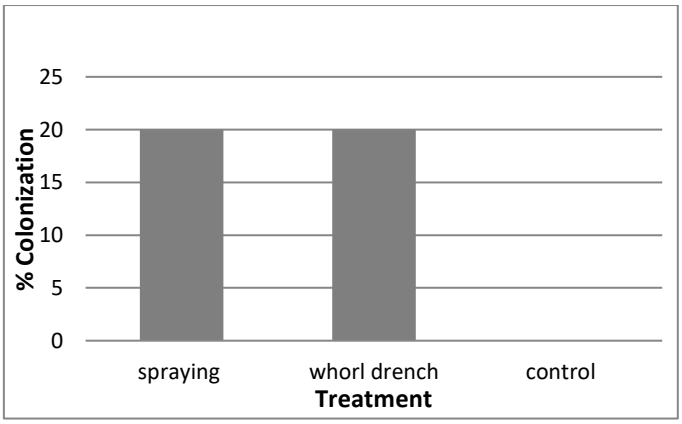

Fig 5. Colonization (%) of maize inbred line Sk12 leaves treated with *C. madrasense* MRDS 303 spore suspension.

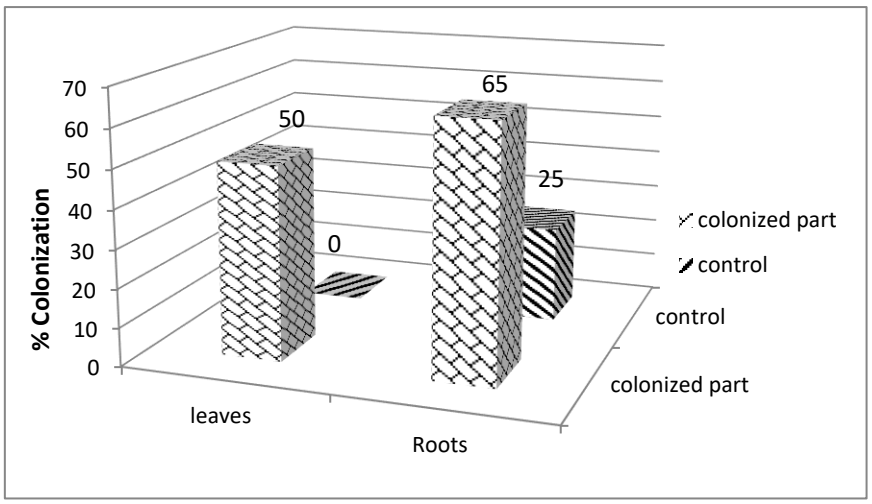

Fig 6. Colonization (%) of roots and leaves of maize inbred line Sk12 grown in soil infested with *C. madrasense* MRDS 303.

DISCUSSION

Towards achieving safe and sustainable agriculture, extensive research has been conducted to employ effective alternatives for chemical pesticides. Some species of fungi that endophytically colonize plant tissues showed entomopathogenic effect against economically important pests (Vega *et al.,* 2008). In this investigation, some endophytic fungi were isolated from different maize hybrids that are commonly grown in Egypt.

In general, the diversity of the isolated endophytes primarily depends on the intensity of work in addition to the isolation procedures (Hyde and Soytong 2008). In our study the capacity of work was quite limited since dealing with maize hybrids is highly restricted, therefore few numbers plants were grown under restricting conditions. Moreover, we adopted a method of isolation that is widely performed in this field. The isolation techniques for endophytes targets very efficient surface sterilization of the plants to completely get rid of all microorganisms present on the plant surface (epiphytes) (Stone *et al.,* 2004). Our results also demonstrated that the frequency of isolation varied between the maize hybrids under investigation which indicates the influence of the plant genotype on the resident endophytes (Hartley and Gange, 2009)

In our investigation, we studied the effect of the endophytic fungi that were isolated from different maize hybrids, against some pests greatly affecting maize and cotton crops in Egypt. Each of the endophytic fungi (EF) isolated from SC132 as well as *C. lunata* MRDS 302 had a high virulence effect against the neonate larvae of *S. cretica*; compared to moderate virulence for the *A. flavus* MRDS 301. These results were compatible with those of Ahmed *et al.* (2018) who found that the numbers of egg and larvae shielded in Hytech 2031 hybrid was higher than that in SC132. Besides, the endophytic fungi *Alternaria* spp. were reported to be entomopathogens for many chewing and pierce sucking pests and their effect was attributed to their secondary metabolites *i.e.*, α-glycosidase inhibitors and tenuazonic acid …etc. (Yang *et al*., 2015; Kaur *et al.,* 2019). Furthermore, the efficiency of *A. flavus* MRDS 305 was higher than that of *A. flavus* MRDS 301 against 1st instar larvae of *S. cretica* after 3 and 14 days of treatment. These differences in the efficiency of *A. flavus* isolates could be attributed to hosting plant properties as indicated by Vidal and Jaber (2015). In this investigation, *C. lunata* MRDS 302 caused 100 % mortality for 1st instar larvae of *S. cretica* after 3 days of treatment. That was in contrast to the study of Renuka and Ramanujam (2016) who found that *C. lunata* was pathogenic to 2nd instar larvae of *Chilo partelllus.* In the present study, all tested isolates didn't induce mortality against 3rdinstar larvae of *S. cretica* after 3 days of treatment. However, their mortality percentage increased gradually with time. Generally, the pathogenicity of endophytic entomopathogens against pests' larvae increases laterally with time and extends to resulting pupae and adults' stages (Kaur *et al*., 2019; Laib *et al.,* 2020).

Furthermore, *C. madrasense, A. flavus* MRDS 305 and *A. flavus* MRDS 301 induced mycosis to *S. cretica* larvae at different ages while *A. alternata* and *C. lunata* did not induce mycosis for the larvae. However, the larval mycosis could be considered as supplementary evidence of the insecticidal effect as well as latent effect of endophytic fungi against pests (Laib *et al.,* 2020). This result suggests that the adverse effect of both *A. alternata* and *C. lunata* could be due to their secondary metabolites. However, Mahmood *et al.* (2019), concluded that the maize endophytic fungi *Beauveria bassiana* have a systemic insecticidal effect against *Sitobion avenae* without mycosis of dead aphid.

Moreover, the weight of the treated *S. cretica* larvae was significantly reduced compared to control. Some reports mentioned that the metabolites of endophytic fungi may have a deterrence effect and could inhibit the digestive enzymes of the pests (Kaur *et al.* 2019). Mantzoukas and Grammatikopoulos (2020) found that the treatment of sweet sorghum endophytes didn't affect the weight of *Sesamia nonagrioides* larvae.

All treatments with the tested endophytes had insecticidal potential against *P. gossypiella* 1stinstar larvae. Zhou *et al.* (2016) found that *Chetomium globosum* protects cotton plants systematically against insect pests with different feeding mode. All treatments of the tested fungi showed a very weak effect against S. *littoralis* 2nd instar larvae. However, dissimilar endophytic fungi effect against an insect may be based on the degree of pest specialism or could be due to the technique of the treatment (Gange *et al*., 2012; Saad *et al.,* 2019).

Several studies showed that the artificial inoculation of plants with entomopathogenic fungi provided them with resistance to pests in addition to the growth-promoting effects of these fungi (Vega, 2018; Russo *et al.,* 2019; Mantzoukas and Eliopoulos, 2020). In the current study, we demonstrated the colonization potentiality of the isolated endophytes inside maize plants using different methods of inoculation. The isolate *C. madrasense* MRDS 303 was used for this purpose since it showed the highest suppressive effect against the selected pests in different larval stages. These experiments were carried out in the maize inbred line Sk12 which is one parent of the SC 132 hybrid. The fungus *C. madrasense* MRDS 303 was retrieved from leaves of the treated plants regardless of the method of inoculation. However, soil infestation was the most efficient method in introducing the fungus inside the plant. These results would suggest a systemic translocation of the fungus inside the host plant.

CONCLUSION

Maize hybrids vary in their associated endophytic fungi in terms of species and frequency. Endophytic fungi could exhibit a substantial insecticidal activity against different pests affecting economic crops. *Chaetomium madrasense* MRDS 303 showed a high insecticidal potential against neonate larvae of both *S. cretica* and *P. gossypiella* therefore it could be considered a promising biological control agent against maize and cotton pests. Entomopathogenicity of isolated endophytic fungi were affected by its plant host variety in addition to the treated pest species and age as well as time post-treatment. *C. madrasense* could be transmitted vertically via maize seeds which suggest a utility in breeding maize hybrids with more resistance against maize stem borers.

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عزل ومرضية الفطريات الداخلية المصاحبة لبعض هجن الذرة الشامية ضد بعض الآفات حرشفية الأجنحه

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الملخص العربي

استخدمت طرق المكافحه البيولوجية للآفات التي تصيب المحاصيل ، والتي يتم فيها المعاملة المباشره للنباتات بالكائن الحيوي ، لسنوات عديده. لكن وجد انه يمكن للفطريات الداخلية المصاحبة للنباتات ان يكون لها تأثير واقي يمتد لفترات طويله بالمقارنه بطرق المكافحه البيولوجية التقليدية حيث أن هذه الفطريات تتواجد داخليا مصاحبة للنبات وتستطيع ان تمنحه الوقايه من الآفات بطريقه مستدامه. وقد كانت هذه الدراسه محاوله لعزل الفطريات الداخلية من بعض هجن الذرة الشاميه وهي هاي تك 2031 و بايونير 3444 و اس سي 132 ، ودراسة كفائتها المرضيه ضد *Sesamia cretica* و *Pectinophora gossypiella* و *Spodoptera littoralis* وذلك في أعمار يرقيه مختلفه. تم عزل خمس فطريات من هجن الذرة الشامية وهي *Aspergillus flavus* MRDS 301 و *Curvularia lunata* MRDS 302 و *Chaetomium madrasense* MRDS 303و *Alternaria alternata MRDS* 304 و *Aspergillus flavus* MRDS 305 . وقد أظهرت المعاملات بالفطريات *C. lunata* MRDS 302 و *C. madrasense* MRDS 303 تأثير مميت ضد العمر اليرقي الأول لـ *S. cretica* . وقد أدت جميع المعاملات الى تخفيض اوزان اليرقات كاملة النمو لـ *S. cretica* .وقد سببت المعامله بالفطر *C. madrasense* الى أعلى نسبة موت للعمر اليرقي الأول لـ *P. gossipiella* بالمقارنة بباقي المعاملات. وقد وجد أن *A. flavus* MRDS 305 هو الاعلى تأثيرا بوجه عام بالمقارنة بالفطر *A. flavus* MRDS 301وذلك ضد كل الآفات المختبرة. ووجد أيضا ان معاملة التربة بالفطر *C. madrasense* هي الطريقة الأكثر كفاءة في حقن الفطر داخل نباتات الذرة الشامية حيث تم عزل الفطر من كل من الأوراق والجذور .

الكلمات المفتاحية: الكائنات الدقيقه الداخلية المصاحبة للنباتات ، ممرضات الحشرات ، فطريات ، الذرة الشامية ، الآفات حرشفية الأجنحه.