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Efficacy of the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.), against larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), under laboratory conditions

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Abstract

The efficacy of the entomopathogenic fungus, *Metarhizium anisopliae*, was assessed through applying different conidiospore concentrations of a local isolate against third and fifth larval instars of the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) under laboratory conditions. The lowest tested concentrations (2×10^1, 2×10^2, and 2×10^3 conidiospores/ml) caused low mortality rates on the tenth day post-treatment (2–14% for L_3 and 0–6% for L_5). The highest concentrations (2×10^4 2×10^5, 2×10^6, and 2×10^7 conidiospores/ml) induced (52–90%) mortality rate in L_3 and (50–100%) in L_5 on the seventh day post-treatment. Death of treated larvae started on the fourth day post-treatment with the high concentrations. LC_{50} and LC_{90} values were calculated. They were higher for L_3 than for L_5.

Keywords: *Metarhizium anisopliae*, *Spodoptera littoralis*, Larvae, Efficacy

Background

The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is a polyphagous insect pest attacking a wide range of host plants including vegetables and ornamental plants (Rao et al. 1993). In Egypt, it is considered a serious pest on cotton, corn, vegetables, and medicinal and aromatic plants (Amer et al. 2008). Control of this pest was always practiced by applying chemical insecticides. The continuous use of chemical pesticides against this pest resulted in resistant strains to most applied insecticides (Shad et al. 2012 and Gandhi et al. 2016), environmental pollution, and negative impact on non-target insect species like pollinators, insect parasitoids, and predators. Thus, search became urgent for new alternative and environmentally safe biocide agents such as the entomopathogenic fungi (EPF) (Anand et al. 2009). EPFs were used worldwide for controlling different stages of many insect pests (Liu and Li 2004). They infect insects by penetrating the body, using extracellular cuticle-hydrolyzing enzymes of lipases, proteases, and chitinases (St. Leger et al. 1986). The different isolates of EPFs differ in their efficacy against targeted insect hosts. Petlamul and Prasertsan (2012) related such differences to germination rate, conidia production, radial growth, and enzyme activity.

The present study aimed to test the efficacy of a local isolate of *M. anisopliae* against third and fifth larval instars of the cotton leafworm, *S. littoralis*, reared on a semi-synthetic diet under laboratory conditions.

Materials and methods

S. littoralis larvae

A laboratory strain of *S. littoralis* was reared for many generations on the semi-synthetic diet of Shorey and Hale (1965) at 25 ± 1 °C and 50–60% RH at the Centre of Biological Control, Faculty of Agriculture, Cairo University, Egypt. The prepared diet was poured before solidifying into trays (20 × 30 cm) in a layer of 2 cm in

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thickness. A plastic grid of 80 cubic cells (2 × 2 × 3 cm) was pressed on the diet tray. One larva was confined in each cell on the diet and the structure was covered with a plastic plate perforated with four fine openings over each cell for aeration. The diet amount in each cell is quite enough for the larva to complete development and pupate. The third (L_3) and fifth (L_5) larval instars were used for testing the efficacy of *M. anisopliae* isolate.

Propagation of *M. anisopliae*

A local strain of the fungus originally isolated from a naturally infected mole cricket, *Gryllotalpa gryllotalpa* L. (El-Husseini et al. 2008) was propagated on Czapek's Dox agar medium. The inoculated plates were incubated for 15 days at 25 °C and 50–60% RH. Thereafter, the conidia were harvested from the surface of the cultures by scraping with a sterile solution of 0.01% Tween-80. The concentration of the resulted stock suspension was estimated, using a hemocytometer and stored in the refrigerator till needed.

Efficacy test

Seven concentrations of (2×10^1, 2×10^2, 2×10^3, 2×10^4, 2×10^5, 2×10^6, and 2×10^7 conidiospore/ml) were prepared in a distilled water from the stock spore suspension. For each tested concentration, 4 replicates, each of 25 larvae in the third and fifth instars were treated by direct spray, using a fine perfume atomizer (El-Husseini et al. 2008). Thereafter, the treated larvae were placed in the cells of the diet trays and left to feed on under a perforated cover plate similarly to those in the rearing technique. For the control, the larvae were sprayed with a distilled water containing 0.01% Tween 80 and kept on diet as those of the treatments. The mortality rate was recorded daily for 10 days post-treatment. The dead larvae were kept on moistened filter paper in Petri dishes and checked daily for the Green Muscardine symptoms proving the death by *M. anisopliae*.

Table 1 Mortality % of *Spodoptera littoralis* (L_3 and L_5) treated with different concentrations of *Metarhizium anisopliae* conidiospores

Concentration/ml	Larval instar	Mortality % in days post-treatment									
		1	2	3	4	5	6	7	8	9	10
2×10^1	L_3	0	0	0	0	0	0	2	2	2	2
	L_5	0	0	0	0	0	0	0	0	0	0
2×10^2	L_3	0	0	0	0	4	4	4	4	4	4
	L_5	0	0	0	0	0	0	0	0	0	0
2×10^3	L_3	0	0	0	8	8	10	14	14	14	14
	L_5	0	0	0	2	2	6	6	6	6	6
2×10^4	L_3	0	0	0	16	30	44	52	52	52	52
	L_5	0	0	0	10	22	30	40	50	60	60
2×10^5	L_3	0	0	0	20	42	58	66	66	66	66
	L_5	0	0	0	14	38	44	70	70	70	70
2×10^6	L_3	0	0	0	38	50	70	88	88	88	88
	L_5	0	0	0	30	46	68	78	78	78	78
2×10^7	L_3	0	0	0	44	70	90	90	90	90	90
	L_5	0	0	0	48	92	92	92	100		
Control	L_3	0	0	0	0	0	0	0	0	0	0
	L_5	0	0	0	0	0	0	0	0	0	0

L_3 third larval instar, *L_5* fifth larval instar

Statistical analysis

LC_{50} and LC_{90} as well as LT_{50} and LT_{90} were calculated, using the software "Ldp Line" software (Bakr 2005). Data were processed by analysis of variance (one-way classification ANOVA), followed by a least significant difference, L.S.D. at 5% (CoStat statistical software).

Results and discussion

Beginning from the fourth day post-treatment, death of *S. littoralis* larvae occurred at the concentration (2×10^3 spores/ml) by 8 and 2% mortality for L_3 and L_5, respectively (Table 1). At lower concentrations (2×10^1 and 2×10^2 spores/ml), death of L_3 larvae started on the seventh and fifth day post-treatment. Meanwhile, no mortality

Fig. 1 Cadavers of *Spodoptera littoralis* larvae covered with the dark green mycelium and spores proving death with *Metarhizium anisopliae* (Green Muscardine) in the efficacy test

Table 2 Toxicity of the tested *Metarhizium anisopliae* isolate against L_3 and L_5 larvae of *Spodoptera littoralis* calculated on seventh day post-treatment

Larval instar	LC	Spores/ml	Lower limit	Upper limit	Slope
L_3	LC_{50}	62,514	7836.7	713,797.2	0.5424
	LC_{90}	14,416,410	9,332,985.6	2,038,922,271.2	
L_5	LC_{50}	153,515	30,408.9	573,999.6	0.6296
	LC_{90}	16,656,991	6,420,466.7	243,268,447.1	

appeared among treated L_5 larvae with both lower concentrations till the tenth day post-treatment. One hundred percent mortality in L_3-treated larvae was not reached till the tenth day of the test, but it was recorded for L_5 at the concentration (2×10^7 spores/ml) on the eighth day post-treatment. The present results showed that larval mortality increased by increasing the conidiospores concentrations and that larvae of L_3 were more susceptible to the fungus than L_5 larvae, especially at the lowest tested concentrations. On the contrary, both instars showed a high mortality rate at the highest concentrations (2×10^6 and 2×10^7 spores/ml) ranging between 88 and 90% for L_3 and 78–100% for L_5. The infected dead larvae kept under high humidity in Petri dishes developed the typical symptoms for death by *M. anisopliae* known as "Green Muscardine" (Fig. 1).

The calculated LC_{50} for L_3 was 62,514 (slope 0.5424) and 153,515 spores/ml (slope 0.6296) for L_5. Meanwhile, the LC_{90s} were 14,416,410 and 16656991 spores/ml respectively (Table 2). These results are in line with those of El-Husseini et al. (2008) and Asi et al. (2013).

Conclusion

The resulted high efficacy (up to 100% mortality) of the local isolate of the entomopathogenic fungus *M. anisopliae* against third and fifth larval instars of the cotton leafworm *S. littoralis* encourages its use in the IPM programs concerned with this polyphagous pest. Due to its environmental safety to farm animals, *M. anisolpliae* is recommended for controlling the larvae of *S. littoralis* on the Egyptian clover as forage crop lasting nearly 5–6 months in the field and harboring between 3 and 4 generations of *S. littoralis.* Moreover, this forage crop is shadily contributing to protection of the applied fungus spores from the UV. Besides, it provides suitable humidity for the fungus spore germination more than in other host plants of this pest.

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I agree to all concerned regulations.

Consent for publication
I agree to publish this scientific paper in the EJBPC.

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The author declares that he has no competing interests.

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