RESEARCH ARTICLE

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TOXICITY OF STREPTOMYCES LAVENDULAE (STREPTOMYCETACEAE) CULTURE FILTRATE TO SPODOPTERA LITTORALIS (BOISD.) LARVAE (LEPIDOPTERA: NOCTUIDAE)

ABSTRACT:

The effect of *Streptomyces lavendulae* (Streptomycetaceae) culture filtrate (containing 1x10^4 CFU/ml) on *Spondoptera littoralis* larvae was studied in laboratory experiments. The thin-film technique was employed. Third instar larvae were exposed to different concentrations (226 CFU/cm², 113 CFU/cm², 56.5 CFU/cm² and 28.25 CFU/cm²) for 24h. The LC50 was found to be 151.4 CFU/cm² (95% F. L. = 122.7 CFU/cm² - 201.3 CFU/cm²; Slope = 2.072 ± 0.089), obtained 7days post-treatment. The effects of *S. lavendulae* filtrate on survival, growth, pupation and emergence rates were investigated. Symptoms of exposure included: delay of pupation, formation of intermediate forms (larval-pupal and pupal-adult intermediates) and deformation of wings and mouth parts of the emerged adults. At a concentration of 226 CFU/ cm², the cumulative larval mortality was 82.8%, while the percentage of adult moth emergence was reduced by 89.7%. Deleterious histological effects were observed in the mid-gut of the treated larvae five days post-treatment. Some epithelial cells showed histolysis and cytoplasmic vacuolation. The muscle fibers in the treated individuals were separated from each other. In the meantime, *S. lavendulae* culture filtrate caused a noticeable decrease in the proteins content especially in the cytoplasm of the larval mid-gut cells.

KEY WORDS:


INTRODUCTION:

In the search for new bioactive natural products as a safer source of insecticides, increasing attention is being given to the microorganisms such as bacteria and fungi (Hiort et al., 2004; Quesada-Morga et al., 2006). These can be cultured under fermentation conditions and the resulting insecticidal compounds can be purified, formulated and used effectively against major arthropod pests (Quesada-Morga et al., 2006). Actinomycetes are a large heterogenous group of bacteria tend to form branching filaments, usually Gram-positive, and grow on ordinary laboratory media. The main ecological role of this group is the decomposition of organic matter in and on soil, and production of the most antibiotics. These have found practical application in human and veterinary medicine, agriculture and industry (Madigan et al., 1997). The actinomycete, *Streptomyces* spp. provide an abundant source of bioactive secondary metabolites possessing biological activity against insects with low toxicity level to the non-target organisms (Koga et al., 1987; Thompson et al., 1997) and behave as nematocide (Ishikawa et al., 2003), antibiotic (Wang and Vining, 2003) or antitumor (Arai et al., 1985; Johnson et al., 1997). The cotton leaf worm, *Spondoptera littoralis* is a notorious pest of field crops in Egypt and other near east countries. The polyphagous larval stage of this insect feed on cotton, many ornamentals and vegetable crops, causing severe loss of crop productions (Hosny et al., 1986; Martinez and Van Emeden, 2001).

Although chemical insecticides have been the primary means of controlling the cotton leaf worm for many years, increasing concerns about human safety, effects on the non target organisms, reduction efficacy due to insect resistance have created a dire need for alternative control strategies. Consequently, the purpose of the present study is to evaluate the bioactivity of the *S. lavendulae* crude filtrate against *S. littoralis* larvae.

MATERIALS AND METHODS:

Insect Tested:

*S. littoralis* larvae were reared in the laboratory at 28°C ± 2°C and 65% ± 5% R. H.
The larvae were exclusively reared on castor bean leaves, *Ricinus communis* until they were used in the experiments. Only 3rd instar larvae were used in the present study.

**The Control Agent:**

The *S. lavendulae* culture filtrate containing 1x 10⁴ CFU/ml (where , CFU = colony forming unit) was kindly provided by Dr. S. M. El-Sabagh, assistant Prof. of Microbiology, Botany Dept., Fac. Sci., Menuofia Univ., Egypt.

**Bioassay:**

Third instar larvae were used in this experiment. The Bioassay was performed by applying the tested filtrate as a thin-film on a glass Petri dish (177 cm²). Different amounts of the stock filtrate (containing 1x 10⁴ CFU/ml) were spread onto glass Petri dishes to obtain different concentrations as follows: 226 CFU/cm², 113 CFU/cm², 56.5 CFU/cm² and 28.25 CFU/cm². Control dishes were treated with water. The Petri dishes were left to dry at room temperature. Then 20 healthy 3rd instar larvae were introduced into the middle of each Petri dish. After six hours of treatment, a piece of castor bean leaves was supplied to these larvae. Each treatment was replicated three times. After 24 h post-treatment, these larvae were transferred to clean glass jars, provided with saw dust, until adult emergence. The larvae were exclusively reared on castor bean leaves, and fed with fang-mask mouthparts, adults with un-plasticized wing lobes seen as crippled wings, pupa-adult intermediates, pupae with deformed head region, larval-pupal intermediates, and larvae which had difficulty in shedding their cuticle and died during ecysis.

**RESULTS:**

When *S. lavendulae* culture filtrate was used as a thin-film, it was generally toxic to the 3rd instar larvae of *S. littoralis* (Table 1) even at the lowest concentration. The LC₅₀ value was 151.4 CFU/cm² (95% F. L. = 122.7 CFU/cm² - 201.3 CFU/cm²); Slope = 2.072 ± 0.089 for the tested species 7days post-treatment. The filtrate induced mortality within 24 h post-treatment and mortality became apparent 5 days post-treatment (Fig. 1) in a dose dependent manner. At a concentration of 226 CFU/cm², 82.8% of larvae failed to pupate and died causing reduction in the percentage of pupation and adult emergence (Table 1).

<table>
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<tr>
<th>Conc. CFU/cm²</th>
<th>% Corrected larval mortality</th>
<th>% pupation</th>
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<th>% Adult emergence</th>
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**Data Analysis:**

Mortality data were subjected to Finney’s method (Finney, 1971) to estimate the LC₅₀ and F. L. values. Larval mortality was corrected using Abbott’s formula (Abbott, 1925). The reduction in the adult emergence was calculated according to khazanie (khazanie, 1979).

**Histopathological and Total Protein Determination:**

The effect of *S. lavendulae* culture filtrate on the histology of the mid-gut of the 3rd instar larvae of *S. littoralis* was determined using different concentrations (226 CFU/cm², 113 CFU/cm², 56.5 CFU/cm² and 28.25 CFU/cm²). The mid-guts of the experimented larvae were removed 1, 2, 3, and 5 days post-treatment. At each interval, parts of the larval mid-gut were fixed overnight in either Bouin’s solution or 10 % neutral formalin. After fixation, the specimens were dehydrated in graded series of ethanol, cleared in xylene and embedded in parablast.

Transverse serial sections, 5µm in thickness were prepared and stained using hematoxyline and eosin. The mercury bromophenol blue stain technique (Bonhag, 1955) was used for protein demonstration. The Stained sections were mounted on glass slides in DPX-mounting medium under cover slips. Untreated larvae were included as controls. The photographs were made using Leitz photomicroscope.

**RESULTS:**

When *S. lavendulae* culture filtrate was used as a thin-film, it was generally toxic to the 3rd instar larvae of *S. littoralis* (Table 1) even at the lowest concentration. The LC₅₀ value was 151.4 CFU/cm² (95% F. L. = 122.7 CFU/cm² - 201.3 CFU/cm²); Slope = 2.072 ± 0.089 for the tested species 7days post-treatment. The filtrate induced mortality within 24 h post-treatment and mortality became apparent 5 days post-treatment (Fig. 1) in a dose dependent manner. At a concentration of 226 CFU/cm², 82.8% of larvae failed to pupate and died causing reduction in the percentage of pupation and adult emergence (Table 1).
Figs 2-4. Morphological changes induced by *S. lavendulae* culture filtrate. 2a-d: larval stage of *S. littoralis*. a: healthy 6th instar larva of *S. littoralis*. b-d: abnormal individuals resulted from treated larvae. b: permanent larva (over aged larvae). 2c-d: intermediate forms with larval-pupal characters. Fig. 3a-d: pupal stage of *S. littoralis*. a: healthy pupa of *S. littoralis*. b-c: deformed pupae resulted from the treated larva. b: deformed pupae with soft body. c: pupae with abnormal head and thorax. Fig. 4a-c: adult stage of *S. littoralis*. a: healthy moth. b-c: deformed moth resulted from larvae treated with *S. lavendulae*. b: pupal-adult intermediate. c: deformed moth with crippled wings and a fang-mask mouth parts.

**Histopathology:**

The mid-gut of the control larvae is composed of a simple columnar epithelium including goblet cells and regenerative cells. It rests on a basement membrane and coated from the outside with longitudinal and circular muscle fibers. Some epithelial cells show vesicular secretions at their terminal ends. The food mass is separated from the brush border of the epithelial cells by the peritrophic membrane (Fig. 5).

![Fig. 5. Transverse section of the mid-gut of a control larva of *S. littoralis* stained with H/E.](http://www.egyptseb.org)

When the 3rd larval instar of *S. littoralis* was exposed to thin film of 226 CFU/cm² of *S. lavendulae* culture filtrate, great variations were noted in the columnar cells. The majority of variations are shown in (Fig. 6a-d).
Fig. 6a–d. Transverse section of the mid-gut of 3rd instar larva treated with S. lavendulae culture filtrate at a concentration of 226 CFU/cm² and stained with H/E; showing; a: histolysis of epithelial cells (arrow) and cytoplasmic vacuolation. b: empty epithelial vesicle. c: some cells with pyknotic nuclei (arrow). d: muscle fibers separated from each other leaving a degenerated area in-between. mo = the microorganism S. lavendulae; vs = vesicle; cm = circular muscles; de = degenerated area; L = lumen; lm = longitudinal muscle; p = peritrophic membrane.

The first noticeable histopathological signs appeared at 48h post-treatment, and became more obvious 5 days post-treatment. Few epithelial cells showed apparent histolysis and cytoplasmic vacuolation (Fig. 6a). The apical brush border of the epithelial cells is destroyed. Some of the epithelial cells lost their ability for secretion as indicated by the empty apical vesicles (Fig. 6b). Other cells exhibited ill-defined cell boundaries and lost their nuclei or appeared pyknotic (Fig. 6c). The muscle fibers of treated individuals were separated from each other leaving a degenerated area in-between. Moreover, this layer showed an obvious separation from the epithelial cells (Fig. 6d).

In the epithelial cells of the mid-gut of the control larvae, the protein contents appeared as intensely bluish colouration by the mercury bromophenol blue method. All structures of the cell exhibited positive stainability with varying degrees, reaching its maximum in the nucleus (Fig. 7). Treated 3rd instar larvae exhibited a marked decrease in the proteins of the mid-gut cells (especially in the cytoplasm) in a dose-dependant manner. At a concentration of 226 CFU/cm², there was an obvious decrease in the total mid-gut cell proteins at 5 days post-treatment (Fig. 8).

DISCUSSION:

In the present study, larval growth was inhibited in response to S. lavendulae culture filtrate treatment. Mortality of the 3rd instar larvae of S. littoralis exposed to a thin film of S. lavendulae culture filtrate was dose dependent. Mortality was caused mainly by moult disruption and morphogenetic defects. It is not clearly determined if the mortality was caused by direct contact with the secondary compound(s) released by the tested microorganism or by residues present on the surface of the larvae while walking on the treated Petri dish. However, inhibition of larval growth in the bioassay can arise through the chemical compounds in the culture filtrate released from S. lavendulae and their spores. The same finding was reported by Thompson et al. (1997). They found that, spinosyns A and D which were derived from Saccharopolyspora spinosa had strong insecticidal activity. When the 6th instar S. littoralis larvae were exposed to sawdust treated with the entomopathogen, Serratia marcescens (Eubacteriales: Enterobacteria), this pathogen decreased adult emergence and increased the percentage of malformations (El-Sheikh, 2006). The strong insecticidal activity of S. marcescens was due to a metalloprotease-like insecticide (MPLI) released from this pathogen (Matsumoto et al., 2003). The present
results also coordinate those of Abdel-Aal (2006), who reported that, *B. thuringiensis* kurstaki administered orally to the 4th larval instar of *S. littoralis* decreased the percentages of pupation and adult emergence compared to the control.

The mid-gut epithelial cells were affected by the used filtrate and showed signs of pyknosis manifested as presence of condensed chromatin, with some vacuolation. This process is generally considered as an important mechanism by cell against pathogenic and toxic agents (Dougherty et al., 2006). Similar histopathological findings have been reported by many authors on the toxicity action of other pathogenic toxins, indicating that this action in most cases was due to paralysis in the mid-gut of larvae. For instance, when *Manduca sexta* larvae were injected with exotoxin protein extracted from *Photorhabdus luminescens*, the mid-gut epithelium was disintegrated leaving space between the basal lamina and the mid-gut epithelium, which has pyknotic nuclei (Daborn, et al., 2002). Feeding *S. littoralis* larvae on artificial media mixed with soluble protein extracted from *Metarhizium anisopliae*, caused deterioration and destruction of the mid-gut epithelium (Quesada-Morga et al., 2006). Comparable histological changes have been reported in the mid-gut epithelium of *S. littoralis* larvae fed on a botanical extract of *Artemisia monosperma* (Sakr and Abo-El-Mahasen, 2006). In the 3rd instar larvae of *S. littoralis* exposed to 5-aminopyrazolo-pyrazolone the mid-gut epithelium showed clear signs of pyknosis (Sakr, 2007).

The obvious reduction of the protein content in the mid-gut epithelium of the treated larvae could be due to disturbance in protein metabolism that affected by the histological alterations. The present results find confirmation by Abdel-Aal (2006) and Abo-El-Mahasen (2007). They reported that the total proteins content in the mid-gut cells of *S. littoralis* was decreased after treatment with the exotoxin of *B. thuringiensis*. Sakr and Abo-El-Mahasen (2006) reported that, the vacuolation and cellular lysis in the mid-gut epithelial cells of *S. littoralis* larvae after treatment with *A. monosperma*, affected the ability of these cells to maintain the vital activities and thus decreased the total protein contents in the whole body homogenate of those larvae. However, Sakr (2007) and Asser (2004) observed that the total proteins content was increased in the larval mid-gut of *S. littoralis* and *Parasarcophaga aegyptiaca* after treatment with 5-aminopyrazolo-pyrazolone and abamectin and spinosad, respectively.

CONCLUSION:

The results clearly demonstrated the toxic effects of *S. lavendulae* culture filtrate which caused high mortality percentage, morphogenetic abnormalities, histological abnormalities, destruction of the insect mid-gut, showing clear signs of pyknosis and disturbances in protein metabolism in the mid-gut cells. Agents that induced pyknosis may provide powerful pest control measure. It could be concluded that *S. lavendulae* filtrate has more than one mode of action against the insect. One major route of toxicity found in the present study is the direct inhibition of metamorphosis leading to the intermediate forms. Eventually, *S. lavendulae* filtrate needs further investigations in order to isolate, identify and assay the bioactive compounds for insect control.

REFERENCES:


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