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Mutagenic effects of aflatoxin B-1 and G-1 on the Egyptian cotton leaf-worm, *Spodoptera littoralis* (Boisd.)

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Abstract

The aim of this study was to test infertility of the Egyptian cotton leaf-worm, *Spodoptera littoralis*. Three concentrations, 0.8, 1.6 and 2.0 μg/larva of aflatoxin B-1 and G-1 were applied to the final instar of the larval period. Both B-1 and G-1 induced mutagenic effects on spermatogenesis and morphogenesis which consequently reflected in infertility of *Spodoptera littoralis*. The phenomenon of mutagenicity was more obvious in larvae treated with G-1 rather than in those treated with B-1. The two analogues were also capable of inducing malformations in sperms. These abnormalities were transmitted to and inherited by the progeny.

Introduction

Aflatoxins are a group of secondary metabolites which are formed after the logarithmic phase of the fungus. The *Aspergillus flavus* group of fungi is a constituent of the microflora of the air and soil throughout the world. Some strains of *A. flavus* are toxic and cause serious diseases in man and animals. In addition to their biological effects on human beings they are regarded as agents of diseases to several insects (1, 9, 11).

Regarding the mutagenic and other genetically related activities of aflatoxins, some studies have been reported concerning chromosome aberration in seedling roots of *Vicia faba* (8), in a rat Kangaroo cell line (6), in human leukocytes (2), induced dominant lethal mutation in mice (5) and gene mutations in *Drosophila melanogaster* (7).

The Egyptian cotton leaf-worm, *S. littoralis*, as a major polyphagous pest in Egypt, attacks more than 60 different cultivated and wild plants. Cotton plant, which is considered to be one of the essential crops in Egypt, is infected drastically by *S. littoralis*. Thus, the aim of this study was to test the mutagenic effects of aflatoxin B-1 and G-1 on the reproductivity of this pest.

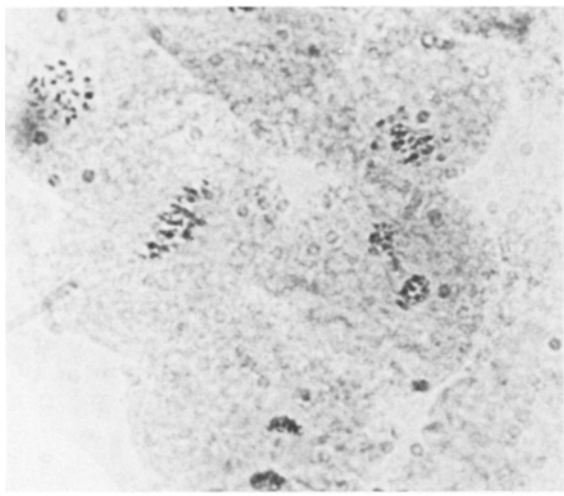
Materials and methods

Isolation and determination of aflatoxins. Aflatoxins were produced by growing a toxic isolate of *A. flavus* on rice medium. Aflatoxins were isolated and purified according to Megalla (10) and determined spectrophotometrically as described by Nabeny & Nesbitt (12).

Larval treatment. Larvae in final instar of Egyptian cotton leaf-worm, which were obtained from the plant protection department, were each injected with three amounts, 0.8, 1.6 and 2.0 μg/larva from aflatoxin B-1 or G-1.

The injection was carried out using propylene glycol solution with a microapplicator. Injection was applied to the first abdominal segment of the ventral side. A total of 30 larvae in triplicate were used for each treatment.

Gonads were obtained after 24 h from the surviving larvae and then imersed in Farmer solution (3:1 ethanol to glacial acetic acid) for 3 days, after while they were preserved in 70% ethanol. They were then examined cytologically using a modified aceto-carmin technique repeatedly used in out laboratory. The gonads were imersed in aceto-carmin solu-



tion for 72 h in small vials at room temperature. Thereafter each gonad was removed and placed in a drop of aceto-carmin on a clean slide. The cover was slightly pressed through a plotting paper avoiding smearing.

Gonads of untreated larvae of the first and second filial generations were exactly treated as previously described in case of parents larvae to be used as reference ones.

Fig. 1. Photomicrograph of primary spermatocytes in gonads of *Spodopetra littoralis* showing cell in anaphase stage with chromosome aberration (bridge).

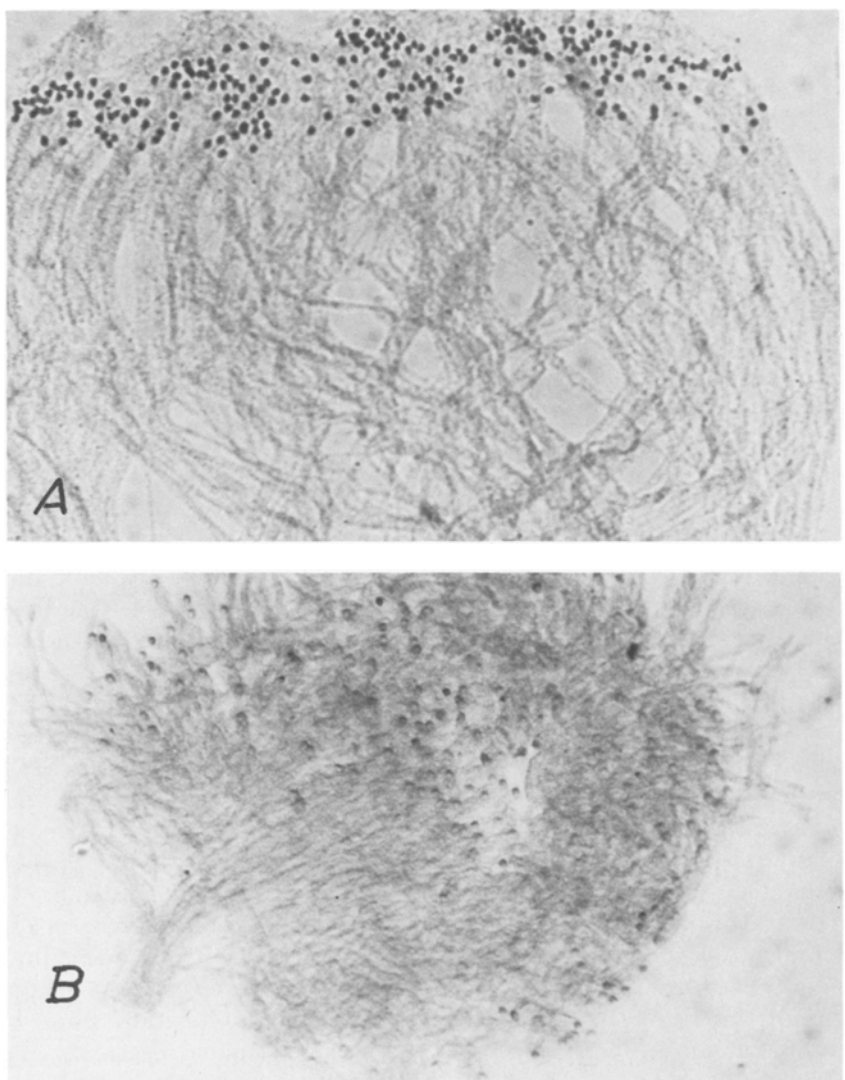


Fig. 2. Photomicrographs of normal and abnormal association of sperms in *Spodopetra littoralis*: (A) oriented group and (B) disturbed group.

Results and discussion

Experimental results revealed that aflatoxin G-1 was more potent in inhibition of the dividing secondary spermatocytes (meiocytes) than aflatoxin B-1. This inhibition was increased as the amount of the aflatoxins increased. Consequently the number of produced sperms was reduced regardless of the amount of aflatoxins used. In addition it was observed that the two analogues were capable of inducing bridges in the anaphase stage of meiotic division (Fig. 1). Such results were also obtained by some other authors (3, 4, 7).

In control treatment, it was observed that sperms

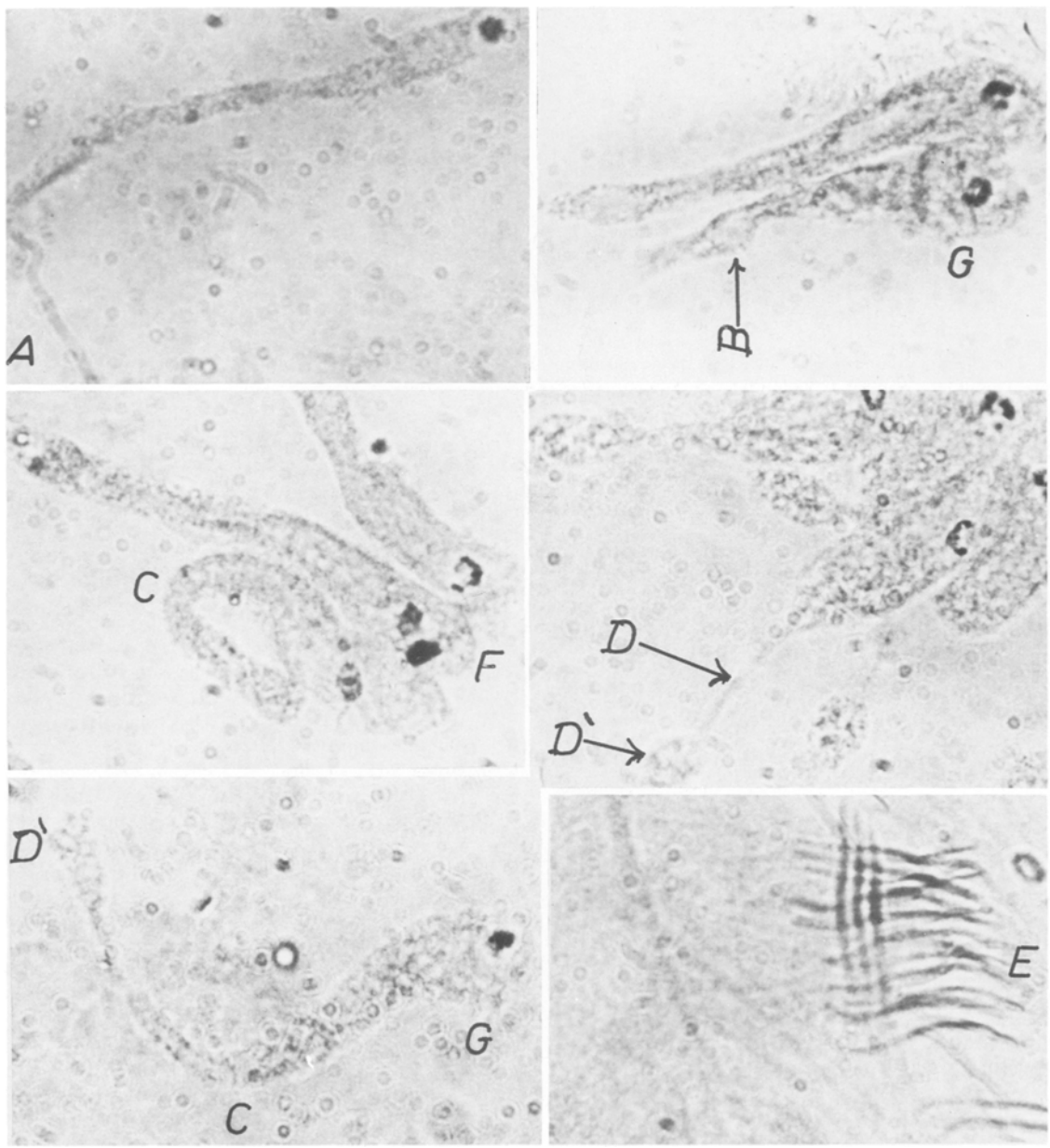


Fig. 3. Photomicrographs of mature sperms in gonads of *Spodopetra littoralis* showing the following abnormalities: (A) normal; (B) missing tail portion; (C) bent tail; (D-D') narrow tail end with a vesicle; (E) narrow head; (F) binucleate head; (G) giant head.

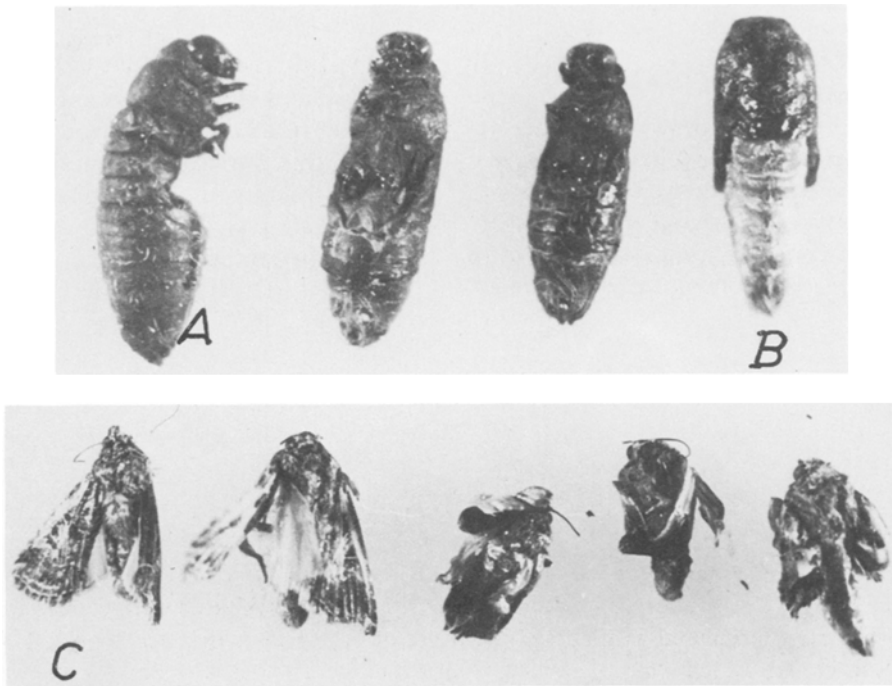


Fig. 4. The effect of aflatoxin B-1 and G-1 on the development of *Spodopetra littoralis*: (A) adultoids, adults retained pupal cuticle; (B) pupal adult intermediate; (C) adultoids, adults with curled and defective wings.

were oriented in groups, where the heads in the anterior side and tails in the posterior side (Fig. 2A).

However, in treated larvae the sperms were disturbed (Fig. 2A). This disturbance was more obvious as the amount of aflatoxins increased.

Concerning the effect of aflatoxins on the viability of sperms, several categories of malformations were observed, missing tail portion, tight tail, narrow tail end with a vesicle; narrow, binucleate, giant heads (Fig. 3).

Moreover, morphological variations in the larvae, pupa and adults in insect generations (F-1 and F-2) were observed (Fig. 4). Thus it can be concluded that the abnormal characters, which were observed in treated larvae (parents), were transmitted to and inherited by their offspring.

From the preceeding results it can be said that low concentrations of aflatoxin analogues cause disturbance in the genial system and therefore lead to blocking the metamorphosis of the insect. This blocking could be due to the derepression, transcription of fresh genetic informations.

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