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ORIGINAL ARTICLE

# Bioimpact of application of pesticides with plant growth hormone (gibberellic acid) on target and non-target microorganisms

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**Abstract** The objective of this investigation was to determine the impacts of fungicide, insecticide, plant growth hormone (gibberellic acid) on soil microbiota, and the growth characteristics of *Aspergillus flavus*. In the fungicide or insecticide mixed with plant growth hormone treated soil sample, the total viable number of soil microbiota was found to be higher than that of the soil treated with fungicide or insecticide alone. Moderate effect of insecticide used on the total number of fungi was observed. On the other hand the effect of insecticide on soil bacteria was more than effect of fungicide, and the negative effect of fungicide on soil bacteria was observed particularly at latent periods (15 and 20 days) of application. A great sensitivity to fungicide and insecticide was observed in the case of nitrogen fixing bacteria. At 15 days after fungicide and insecticide application the adverse effect was found. Morphological deformations were clear in *A. flavus* cultivated on medium containing fungicide, the fungus failed to form conidiospores, conidiophores and vesicles. Intermediate and terminal outgrowths like blisters and terminal vesicle originate from hyphae. The addition of plant growth hormone reduced the effect of fungicide on fungus.



## 1. Introduction

Pesticides are applied extensively annually in modern agriculture to increase the production by controlling the harmful effects caused by the target organisms including insects, fungi, bacteria, viruses as well as grasses grown in between the economical crops (Liu and Xiong, 2001). However, less than 5% of these products are estimated to reach the target organisms. The major environmental concern of used pesticides is their capacity to leach down to subsoil and contaminate the ground water (Kookana et al., 1998) or if immobile, they would persist on the top soil where it could accumulate to toxic levels in the soil and become harmful to microorganisms, plants, animals and man. Chemical fertilizers are composed of pure synthetic nutrients facilitating higher plant growth during initial application (Steiner et al., 2007). But when compared to the organic manure it is less effective (Burger and Jackson, 2003).

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The side effects of pesticides on the soil microflora were studied by several authors (Perucci et al., 2000; Dave et al., 2005; Ahmed and Ahmad, 2006; Munees and Mohammad, 2011). Pesticides may affect the microbial population by controlling the survival and reproduction of individual species. On the other hand, several microorganisms were reported to degrade some pesticides (Topp et al., 2000). Population size, enzymatic activity and biodiversity of certain systematic and physiological groups of microorganisms may serve as bioindicators of changes taking place in the soil following herbicide application (Milosevia et al., 1995; Digrak and Kazanici, 2001).

Field doses of many insecticides have inhibitory effects on soil microorganisms (Amirkhanov et al., 1994). Some pesticides stimulate the growth of microorganisms, whereas others have depressive effects or no effects on microorganisms (Ahmed and Ahmad, 2006). Farag et al. (1987) reported the growth and aflatoxin production by *A. parasiticus* in the synthetic medium containing plant hormones, herbicides or insecticides. They observed that the addition of indole-acetic acid to the medium increased aflatoxin production more than gibberellic acid. Bucio-Villalobos et al. (2005) evaluated the effect of plant growth hormones, Gibberellic and Jasmonic acids, *in vitro* to determine their effects on growth, differentiation and mycotoxins synthesis in *A. nidulans* and *A. parasiticus*. Gibbrellic acid has a stimulatory effect on mycotoxin synthesis, the growth factor 2,4-diclorophenoxiaacetic acid has no effect on any of the evaluated parameter in *A. nidulans*, however high concentration of this compound decreased all the parameters in *A. parasiticus* and *A. nidulans*. The use of bacteria for the degradation and detoxification of numerous toxic chemicals such as pesticides is an effective tool to decontaminate the polluted sites. Isolation of indigenous bacteria capable of metabolizing pesticides provides environmentally friendly means of *in situ* detoxification (Mervat, 2009).

Microorganisms are thought to play an important role in the removal and detoxification of these toxicants from the environment. Many bacteria that are able to degrade carbamate pesticides have been isolated from soil around the world (Desaint et al., 2000). Many members of different groups of soil microorganisms (Bacteria, Fungi, Actinomycetes and Algae) isolated from the soil are capable to degrade pesticides (Novaka et al., 1997). The goal of this work was to evaluate the impact of using the insecticide and fungicide with gibberellic acid as a plant growth hormone on soil microbiota at filed rates.

## 2. Materials and methods

### 2.1. Soil samples and pesticides used

Soil samples were taken with the help of sterilized spatula at a depth of 5–15 cm from agricultural fields in menofia governorate egypt cultivated with seedlings of *Vicia faba* treated with plant growth hormone (gibberellic acid), insecticide Cyperkill containing active ingredient Cypermethrin, fungicide Topas containing active ingredient Penconazole, mixture of insecticide with plant growth hormone and mixture of fungicide with plant growth hormone at field application rates. One gm of soil was mixed with 9 ml of sterilized water and mixed thoroughly. 1 ml from the solution was then mixed in 9 ml sterilized water to make $10^{-2}$ dilution of this solution and in the same pattern dilutions up to $10^{-7}$ were prepared to determine the microbial count.

### 2.2. Microbial analysis

After 1, 10, 15 and 20 days of pesticide application at field rates in treated and control soils, the total numbers of microorganisms were counted. The numbers of colony forming units (CFU) in the selective media were determined by means of the serial dilution technique and the spread plate method. Viable counts for fungi were performed using rose bengal-streptomycin agar containing (per liter): 10 g glucose; 5 g peptone;1 g $K_2HPO_4$; 0.5 g $MgSO_4 \times 7H_2O$; 0.033 g rose bengal; 15 g agar. Streptomycin was added after autoclaving at final concentration of 30 mg ml. The plates were incubated at 28 °C and colonies were counted after 5 days. Analyses were performed in three replicates.

Viable counts for bacteria were determined using a Nutrient agar medium containing (per liter of water) Peptone, 5.0 g; Beef extract, 3.0 g; Sodium chloride, 5.0 g; agar, 20.0 g. Viable counts for free nitrogen fixing bacteria were performed using a nitrogen free medium containing (per liter of water) $KH_2PO_4$ 0.2 g; $MgSO_4 \times 7H_2O$, 0.2 g; NaCl, 0.2 g; $CaSO_4$, 0.1 g; $CaCO_3$, 5.0; Mannitol, 10.0 g; agar, 20.0 g. Viable counts for actinomycetes were performed using a starch nitrate medium containing (g/l) of water,: 20.0, starch; 2.0, potassium nitrate; 1.0, dipotassium hydrogen phosphate; 0.5, magnesium sulfate; 0.5, sodium chloride; 3.0 calcium carbonate and 0.01, ferrous sulfate.

### 2.3. Diagnostic criteria of *A. flavus*

Resistant fungus to insecticide and fungicide was isolated and identified according to the current manual of Rapper and Fennel (1965) as *A. flavus*. It was cultivated on Dox medium supplemented with 200 ppm of fungicide, insecticide mixed with 25 ppm of plant growth hormone (Gibberellic acid) was taken for experiment with pH 6, incubated for 7 days at 30 °C, dry mycelium weight was taken as biomass, the hormone was sterilized and added in the medium in 25 ppm concentration after autoclaving. By using software for image analysis (SIS version 2.11, 1996) at the Regional Center for Mycology and Biotechnology at Al-Azhar University Cairo, Egypt.

## 3. Result and discussion

According to guidelines for the approval of pesticides, information about effects of pesticides on soil microorganisms and soil fertility is required, but the relationships of different structures of pesticides on the growth of various groups of soil microorganisms are not easily predicted. Generally some pesticides stimulate the growth of microorganisms, but other pesticides have depressive effects or no effects on microorganisms. The plate count results indicated that insecticide used affects the total number of microorganisms including bacteria, actinomycetes, N-fixing bacteria and fungi. The observed effects were strongly correlated with pesticide type, mixture of pesticide with fertilizer or plant growth hormone and time of exposure. A strong stimulatory effect of fungicide used on the total num-