

and Maheshwari, 2000; Ismail, *et al.*, 2003 and Thind, *et al.*, 2004). Furthermore, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum*, is an efficient bio-agent that protects cucurbits plants against powdery mildew disease (Bettiol, *et al.*, 19972; Urquhart *et al.*, 1994; Koumaki, *et al.*, 2001; Apablaza, *et al.*, 2002; Levy, *et al.*, 2004; Schmitt, 2006 and Zhang, *et al.*, 2008).

Application of different concentrations of four chemical compounds, *i.e.*, (KNO<sub>3</sub>, KH<sub>2</sub>PO<sub>4</sub>, K<sub>2</sub>HPO<sub>4</sub> and salicylic acid) on the induction of resistance against the pathogen was studied *in vivo*. They were sprayed, each alone, on 40-day-old squash plants, 2 days before and/or after inoculation with the pathogen. Commonly used for induction of resistance against powdery mildew disease (Casulli, *et al.*, 2002; Dik *et al.*, 2003 and Abd-El-Kareem, *et.al.*, 2004 ).

The main objectives of this research are studying the efficacy of using the different concentrations of four chemical compounds for inducing resistance as well as antagonistic isolates against powdery mildew of squash.

## **MATERIALS AND METHODS**

### **Isolation and identification of the causal pathogen:**

Leaves of squash plants showing typical symptoms of powdery mildew were collected from different localities of Beheira Governorate in 2007 growing season. Plants (5 weeks old) of squash, cv. Eskandarani, and infected with *S. fuliginea* were obtained. The conidia were liberated, by scrapping the surface of the infected leaves with sterilized needle, and suspended in a Petri dish containing 20 ml of sterilized distilled water, then filtered through cheesecloth to remove most of the mycelial fragments (Elad *et al.*, 1989 and Reuveni *et al.*, 1995). The concentration of the spore suspension was determined by aid of a Haemocytometer slide and adjusted to  $3 \times 10^5$  conidia/ml.

### **Fungal and bacterial bioagents:**

Three bacterial isolates, *i.e.* *Bacillus subtilis* (2 isolates) and *Pseudomonas fluorescens* (one isolate) and fungi, *i.e.* *Trichoderma harzianum* (3 isolates), were obtained from the stock cultures collection of the Bacterial and Biological Control Dept., Plant Pathology Research Institute, Agriculture Research Center Giza, were used as biocontrol.

### **Pathogenicity tests:**

Different isolates of *S. fuliginea* collected from various localities, from different localities of Beheira Governorate, were used in this study. Spore suspensions of these isolates were prepared, as mentioned before, ( $3 \times 10^5$  conidia/ml). Three weeks old squash seedlings cv. Eskandarani grown in pots (25 cm in diam.) were sprayed with the spore suspensions using an automizer, and incubated under greenhouse conditions (25±5°C and 75-90% R.H.) for 10 and 20 days, then the aggressiveness of the tested isolates was determined by calculating the disease severity.

**Biological control:**

**In vivo evaluation of antagonistic microorganisms:**

The highly antagonistic isolates of *B. subtilis*, *P. fluorescens* and *T. harzianum* were used against *S. fuliginea* on squash plants. Three seeds of squash cv. Eskandarani, were sown in each pot (25 cm in diam.) filled with sandy-clay soil (1:1 w/w). Squash plants (40-days-old) were sprayed with each of the bacterial suspensions alone at the concentration of  $10^8$ cfu/ml. The growing plants were sprayed with the tested bioagents, and then sprayed after three days with *S. fuliginea* spore suspension ( $3 \times 10^5$  conidia/ml).

The fungal isolates were used at concentration of  $5 \times 10^8$  cfu/ml prepared from 10-days-old cultures grown on PDA. The bioagents were sprayed, each alone, on squash plants by using a hand atomizer. Bioagents were applied at the same time of inoculation and/or three days before inoculation with the pathogen. The foliar fungicides (Topase-100; Afugan and Amistar 250 SC) were used for comparison purpose with the biocontrol agents in controlling the disease incidence. The experiment was repeated twice under greenhouse condition in 2007 and 2008. Pots (25-cm-diameter) were filled with autoclaved clay soil. Three replicates were used. Each replicate consisted of four pots and each pot consisted of 2 plants. Disease assessment was recorded at 10 and 20 days after inoculation.

**Tests of induced resistance:**

The effect of different concentrations of four chemical compounds, *i.e.*, potassium nitrate ( $\text{KNO}_3$  25, 50 and 100 mM), potassium phosphate monobasic ( $\text{KH}_2\text{PO}_4$  25, 50 and 100 mM), potassium phosphate dibasic ( $\text{K}_2\text{HPO}_4$  25, 50 and 100 mM) and (salicylic acid 3, 5 and 10 mM) on the induction of resistance against the pathogen was studied *in vivo*. They were sprayed, each alone, on 40-day-old squash plants, 2 days before and/or after inoculation with the pathogen. The experiment was repeated twice under greenhouse conditions, with 3 replicates. Each replicate consisted of four pots and each pot consisted of 3 plants. Disease severity of each treatment was recorded after 10 and 20 days from inoculation date.

**Greenhouse experiments:**

The effects of above chemical inducers were evaluated on incidence of squash powdery mildew diseases under artificial inoculation conditions with the causal fungi under greenhouse conditions. Squash seeds cv. Eskandarani, were sown in plastic pots (25-cm-diam.) containing loamy soil at rate 3 seeds/pot. Ten pots were used for each treatment. Traditional agricultural practices, *i.e.* irrigation and fertilization, were carried out as needed.  $\text{KNO}_3$  25, 50 and 100 mM,  $\text{KH}_2\text{PO}_4$  25, 50 and 100 mM  $\text{K}_2\text{HPO}_4$  25, 50 and 100 mM and salicylic acid 3, 5 and 10 mM were applied for testing their efficacy against powdery mildew disease severity. The prepared concentrations were sprayed on the grown plants at the first true leaf growth stage 2 days before and/or after inoculation with the causal fungi. Plant inoculation was carried out at the second true leaf growth stage by spraying of squash leaves with spore suspension ( $3 \times 10^5$  conidia/ml) of *S. fuliginea* the causal of powdery mildew. Plants sprayed with tap water served as check. Assessment of disease severity according to (Descalzo *et al.*, 1990) was