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Efficacy of essential oils in the control of cumin root rot disease caused by Fusarium spp.

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ABSTRACT

The aim of this study was to evaluate the effectiveness of some natural safe essential oils in control of cumin root rot disease to reduce the pollution of environment as a result of wide distribution of synthetic chemicals which are employed as fungicides.

Pathogenicity test of eight *Fusarium* isolates belonging to six species (*F. oxysporum*, *F. solani*, *F. moniliforme*, *F. dimerum*, *F. equiseti* and *F. lateritium*) isolated from root-rot symptomized cumin plants has proved their ability to infect the same crop involving in emerging symptoms of root rot disease in various degrees.

In vitro, essential oils extracted from cumin, basil and geranium showed the highest antagonistic effect against the candidate pathogens and produced significant inhibition zones against them.

Under greenhouse conditions, the treatment of cumin seeds with 3 essential oils resulted in the reduction of mean disease rating of root rot caused by all *Fusarium* spp. Both *Fusarium oxysorum* 112 and *F. moniliforme* 235 which gave the highest mean disease rating, were significantly inhibited by geranium and basil oils. All growth parameters (e.g. plant height, shoot fresh weight, root fresh weight and number of branches) which were altered as a result of infection with different *Fusarium* spp., were recovered when the essential oils were applied.

Results of field experiment during two successive seasons have confirmed the results obtained from laboratory and greenhouse treatments, indicating that the three selected oils have a promising effect in the control of root rot disease of cumin as biological alternatives to chemical pesticides either singly or as a part of integrated control to conserve and protect the natural environment from chemical pollutants.

1. Introduction

Medicinal and aromatic plants have a great economical importance in Egypt as they are of export priority in the first place. They also represent an essential part in the pharmaceutical and cosmetic products.

Recently, an increasing interest in the cultivation and production of medicinal and aromatic plants has been noticed (Mahmoud, 1996), but the farmers are facing a significant low productivity and low content of active ingredients in these plants because of fungal diseases and also the synthetic chemicals which are employed as fungicides in crop protection (Fiori et al., 2000). These compounds have cautioned due to their carcinogenicity, teratogenicity and other residual toxicities (Arya, 1988; Lingk, 1991; Hashem, 2004). Also many synthetic fungicides were reported to cause adverse effect on treated soil ecosystems because of their non biodegradable nature (Thomas and Willis, 1998). Therefore, biological control seems to be a good alternative for chemicals (Mares et al., 2004). Aromatic plants are used in folk medicine as antimicrobial agent and their essential oils have been known to have antibacterial and antifungal proprieties (Pinto et al., 2007; Hussain et al., 2008). The antimicrobial activity of plant oils and extracts has formed the basis of many applications (Falerio et al., 2003; Sohn et al., 2004; Boyraz and Özcan, 2005; Ghorbany and Salary, 2005; Dambolena et al., 2010). The genus Fusarium is widely found in plant debris and crop plants worldwide (Marasas et al., 1984). Several species from this genus are economically relevant because, apart from their ability to infect and cause tissue destruction on important crops such as corn, wheat and other small grains on the field, they produce mycotoxins on the crops in the field and in storage grains (Dambolena et al., 2010).

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Consequently, the aim of this study was to evaluate the efficacy of natural essential oils in control of cumin root disease caused by different *Fusarium* spp., as a safe biological alternative to reduce the pollution of environment resulted from wide distribution of synthetic chemicals.

2. Materials and methods

2.1. Isolation and identification of fungal pathogens

Cumin (*Cuminum cyminum* L) plants showing symptoms of wilt or root-rot were collected from different farms in Assiut and were transferred in ployethylene bags to the laboratory. The fungal pathogens were isolated from root tissues on PDA medium (potato dextrose agar) according to the growth requirements of organisms (Gamliel et al., 1996) at 25 ± 1 °C for 7 days. The developed *Fusarium* species were purified on the same medium and identified according to the morphological characteristics of their mycelia and spores as described by Booth (1977) and Nelson et al. (1983).

2.2. Pathogenicity test

The dominant *Fusarium* isolates including *Fusarium oxysporum* 110, *F. oxysporum* 112, *F. solani* 225, *F. solani* 234, *F. moniliforme* 235, *F. dimerum* 289, *F. equiseti* 315 and *F. lateritium* 324 which were isolated from cumin were selected to carry out their pathogenicty on the same plant. Inoculum of each isolate was prepared by growing the isolate in 500 ml glass bottles containing 150 g sterilized barley grains at 25 °C for 21 days. Then the inoculum was mixed thoroughly with sterilized clay soil at the rate of 3% and then put in sterilized pots (25 cm in diameter). Pots containing soil infected with a proper inoculum (5 kg/pot) were watered for three days before cultivation. Ten surface disinfested cumin seeds were sown in each pot. Sterilization of seeds was done by dipping in 0.1% mercuric chloride for 2 min. In control, pots were filled with the same soil and sterilized barley but without organisms. Three pots were used for each treatment which were irrigated when needed.

Percentage of germination was determined after 25 days, then plants were harvested after 4 and 8 weeks from inoculation. Plant height, plant weight, root weight and branching number were determined as growth parameters as demonstrated by Gamliel et al. (1996).

The mean disease rating (MDR) was detected and compared by the following disease indices as described by Pal et al. (2001) with minor modification. The different degrees of root discoloration were classified to 5 degrees scale as follows:

0 = healthy (no discoloration).
1 = 1–25% discoloration of roots.
2 = 26–50% discoloration of roots.
3 = 51–75% discoloration of roots.
4 = 75–100% discoloration of roots.

$$\text{Mean disease rating (MDR)} = \sum \frac{(A0 + B1 + C2 + D3 + E4)}{(A + B + C + D + E)}$$

where *A*, *B*, *C*, *D*, and *E*, are the number of plants with the disease rating of 0, 1, 2, 3, and 4, respectively.

2.3. In vitro studies

The antifungal activities of nine different essential oils: Rose geranium (*Pelargonium graveolens* L. Her), Marjoram (*Majorana hortensis* L), Sweet basil (*Ocimum basilicum var basilicum* L.), Bush basil (*Ocimum basilicum var. minimum*), Caraway (*Carum carvi* L.), Cumin (*Cuminum cyminum* L.), Fennel (*Foeniculum vulgare* Miller), Parsley (*Petroselinum crispum* L.) and Anise (*Pimpinella anisum* L.) were tested (data not included) and the most three effective oils (Cumin, Rose geranium and Sweet basil) were used in this study and tested on the most aggressive *Fusarium* species using the agar diffusion method (Mangena and Muyima, 1999 and Sahin et al., 2003). All tested oils were supplied by Beni-suef Cooperative Society for oil production, Beni-suef governorate, Egypt.

2.4. In vivo studies

Under greenhouse conditions, 48 earthen pots, 24 for control (3 for each fungus) and 24 for each treatment (3 pots for each fungus) were filled with the clay soil at rate 5 kg/pot, and inoculated with the tested *Fusarium* species then kept for 3 days before planting to establish the infection (as described in pathogenicity test). Ten grams of cumin seeds were soaked for 5 min at minimum fungitoxic dose of the oils (4% concentration). In control, seeds were surface disinfested in 0.1% mercuric chloride for 2 min. and washed three times with sterile distilled water (Land et al., 2001). Ten seeds per pot were sown and watered when needed with equal amounts of water.

Growth parameters (plant height, plant weight, root weight and number of branches) were recorded and analyzed after 4 and 8 weeks from date of planting according to Pandey and Dubey (1994). At the end of the experiment, plants were uprooted and subjected to the mean disease rating scale to determine the severity of the root disease according to Pal et al. (2001). The percentage of disease reduction (%DR) was measured according the following equation:

$$\%DR = (\text{MDR of control} - \text{MDR of treatment}/\text{MDR of control})\,100$$

2.5. Field experiment

The experiment was conducted at the farm of Agricultural Research Station, Assiut governorate, Assiut Egypt. Plots were selected and divided on the basis of complete randomized blocks with three replications, the plot of ground was divided into three sections, each of a single row 50 cm wide and was separated from neighboring plots by a 1.5 m guard area.

Cumin seeds were treated with three effective essential oils (cumin, basil and geranium). Two types of treatments (seed and soil) were applied as the following:

2.5.1. Seed treatment

The oil (4% concentration) was placed in conical flask and the seeds of cumin were soaked in the desired oil. The flask was shaken by hand for 5 minutes until the seeds were saturated. In control, seeds were soaked in sterile distilled water. The treated and control seeds were planted, and the irrigation process was carried out when needed.

2.5.2. Soil treatment

The same oils with the same concentrations that used in seed treatment were used for the soil treatment. The oils were added to the soil at the hole of planting until saturation (25 ml/hole). Sterile distilled water was used as a control. Then the seeds were planted. The same programs of cultivation used in case seed treatment were followed.

During the flowering periods samples were collected, plant height, plant fresh weight, root weight, number of flowers, branching and production of leaves were recorded for each treatment as indicators for degree of disease progress (Dorrance and Mc Clure, 2001).

2.5.3. Statistical analysis

Data from these studies were analyzed by one-way analysis of variance (ANOVA) and Fisher Multiple Comparison test. Results giving P values <0.05 were considered significantly different (Snedecor, 1962).