Pathogenic Fungi Transmitted Through Cucumber Seeds and Safely Elimination by Application of Peppermint Extract and Oil

Eman S. H. FARRAG1, Moustafa H.A. MOHARAM2*1

1South Valley University, Faculty of Agriculture, Agricultural Botany Depart, Qena, Egypt
2Sohag University, Faculty of Agriculture, Plant Pathology Depart, El-Kawther, Sohag P.O. 82786, Egypt; moharam@hotmail.com (*corresponding author)

Abstract

Diseases induced by Fusarium, like damping-off and wilt on cucumber, are serious problems around the world. Samples of cucumber seeds were collected from commercial markets in Egypt and tested for seed-borne fungi. In order to detect the maximum number of internal and external seed-borne fungi, agar plate examination of disinfected and non-disinfected seeds were used. Two species of Fusarium were the most frequent and predominant fungi. Facultative parasites of the genera Alternaria, Rhizoctonia, Helminthosporium and Penicillium were also found. A total 33 isolates of Fusarium spp. were obtained using Komada’s selective medium. Fusarium oxysporum and F. solani were highly frequent. Pathogenicity test indicated that, F. oxysporum isolate (Fem8) was the main causal organism of pre- and post-emergence damping off. Furthermore, it occurred in all seed parts tested. Some infected seeds germinate, but they were either rapidly overgrown by F. oxysporum or they developed into a diseased seedling. The water extract of garlic, peppermint and rheum completely inhibited the conidiospore germination and mycelial growth of F. oxysporum at tested conc. 3, 2 and 3%, respectively. Soaked seeds in 2% peppermint extract and evaporated seeds by vapor of peppermint oil caused a highly reduction in the infection and reduced transmission of the referred fungi from seeds to the growing seedlings. The vigor of cucumber seedlings raised from the treated seeds was better than that developed from untreated ones.

Keywords: cucumber, elimination, Fusarium spp., peppermint oil, plant extracts, seed-borne fungi, transmission, vapour phase

Introduction

Seed is the most important input for crop production. Pathogen free healthy seeds are essential for desired plant populations and a good harvest. Of the 16% annual crop losses due to plant diseases, at least 10% loss occurs due to seed-borne diseases (Fakir, 1983). Coincidently important or devastating crop diseases are seed-borne and caused by fungi. In addition, it has demonstrated that seed borne fungi are responsible for poor quality seeds in many crops (Neergaard, 1979). Seed-borne fungi causing plant diseases include Macrophomina phaseolina on soybean and maize (Raut, 1978; 1987; Singh and Kaiser, 1995), F. oxysporum on sesame (Patel and Patel, 1990), Aspergillus flavus, Alternaria tenuis and Rhizopus arrhizus on sunflower (Prasad and Kulshreshtha, 1999), Fusarium spp. on watermelon (Boughallab et al., 2005; Boughalleb and El Mahjoub, 2006), Aspergillus sp., Fusarium sp. and Penicillium sp. on sorghum (Karim, 2005; Satish et al., 2010), Al. alternata, ds. flavus, Curvularia lunata, F. oxysporum and F. solani on eggplant (Habs et al., 2007), and M. phaseolina on cucumber (Nasreen et al., 2009). Cucumber (Cucumis sativus L.) is an important vegetable crop ranking fourth after potato, tomato and onion in Egypt. Approximately 2351.16 tons of cucumber fruits are produced from about 1199.52 hectares in Egypt through 2009 (FAO). Among severe fungal diseases, seed-borne pathogens are causing various factors responsible for the crop low yield. Important fungal diseases recorded on cucumber are damping-off and wilt caused by F. oxysporum (Antoniou and Tjamos, 2000; Farrag and Fatouh, 2010). Studies are limited on seed-borne diseases of cucumber in Egypt. The seed borne fungal diseases are transmitted by seeds, where the fungi can survive as conidia or mycelia on the seed coat or surface (Blancart et al., 1991; Champion, 1997; Gargouri et al., 2000; Martyn and Bruton, 1989). Analysis of seed infection level is a valid investigation tool to foresee the disease development transmitted by seeds (Taylor et al., 2001). Few studies also have localized these pathogens on the seeds (Michail et al., 1989; 2002). Many seed-borne fungi are generally managed by synthetic chemicals, which are considered both efficient and effective. However, the continuous use of these fungicides started unraveling non-biodegradability and is known to have residual toxicity causing pollution (Pimentel and Levitan, 1986). Pesticide pollution of the soil and water bodies is well documented (Nostro et al., 2000). Hence, in recent time application of plant extracts as well as plant metabolites for plant disease management has become important viable component of Integrated Pest Management, as plant metabolites are eco-friendly where botanicals place an important role (Sahayraj et al., 2009). Several investigators have screened dif-
different plants for their antifungal properties (Ja Choi et al., 2004; Stephan et al., 2005; Satish et al., 2010), which are due to oil compounds synthesized by these plants. During the regular screening, a highly significant antifungal activity of some water extracts or essential oils of plants was recorded. Since some plants are already known to possess several biological activities (Amin et al., 2009; Barrera-Necha et al., 2009; Belabid et al., 2010; Farshabaf Moghadam et al., 2002), the objectives of this study were: (1) to isolate and identify Fusarium spp. from cucumber seeds, study its location in the seed and to elucidate its effect on seed germination and disease development. (2) to determine the Fusarium spp., load in each seed (3) to investigate in vitro the antifungal activity of some plant extracts on growth of F. oxysporum and (4) to introduce also the manual seed cleaning with these plant extracts and treating with their essential oil vapors for safely elimination of seed-borne transmitted fungi and reducing infestation level.

Materials and methods

The present investigation was conducted through 2010-2012 in the Depart. of Agric. Botany, Fac. of Agric., South Valley Univ. and Plant Pathol. Depart, Sohag Univ., Egypt. Seed samples of cucumber were collected from the commercial lots that used for sowing in the farms. Seeds were stored at room temperature (approx. 28°C and 50-60% relative humidity, dry storage) until use and further they were subjected to initial seed health testing.

Seed infection evaluation

Whole seed test

Seeds were divided into two equal parts, the first: non-surface sterilized seeds, the second: disinfected ones by soaking in 1% sodium hypochlorite for 1 min. The seeds were used in two experiments as follow. Initial seed health testing was conducted by agar plate method. Ten seeds were placed in Petri plates containing 20 ml of Potato Dextrose Agar medium (PDA) and incubated at 25 ± 2°C for seven days. After incubation, the Petri plates were examined for fungal growth under stereo-binocular microscope. Roots of 15-day-old plants were washed, trimmed to 5 cm length, and then dipped in the spore suspension for pathogenicity test in two ways. The first way: cucumber seeds were sown in an autoclaved soil mixture (peat, compost and clay, respectively, 60:20:20 v/v/v) in plug trays and maintained at 25°C, with 12 h/day of fluorescent light. Roots of 15-day-old plants were washed, trimmed to 5 cm length, and then dipped in the spore suspension for 10 min. Inoculated plants were transplanted in pots filled with steamed soil (1.5 L volume). Control plants were prepared similarly but soaked in SDW. Six replicates were used and each replicate consisted of five plants. The experiment was carried out under glasshouse conditions.

Location of the pathogen in the seed

Location of the pathogen F. oxysporum in the cucumber seeds was studied by employing component plating technique (Maden et al., 1975). Isolation was done on non-disinfected seeds or disinfected ones as mentioned above (fifteen seeds each). Seeds were washed four times with tap water and then surface sterilized as mentioned above. After that, the seeds were washed again with sterile distilled water (SDW) and soaked in water for 30 min. On the other hand, another seed sample (non-disinfected) was soaked only in sterile distilled water for 30 min. Seeds were disected aseptically by sterile needles and forceps. The separated seed parts viz., seed coat, cotyledon and embryo were plated separately before drying on plates containing Komada selective medium and incubated at 25 ± 2°C for seven days. The seed components were examined under stereo-binocular microscope for presence of the Fusarium in different seed parts. The number of contaminated parts was enumerated every 3 days for a period of 15 days. The infection level in each seed part was evaluated according to the following formula:

\[ \text{Infection level (\%)} = \frac{\text{total number of infected seed parts}}{\text{total number of seed parts}} \times 100 \]

The fungal colonies that appeared identical to Fusarium spp. around every seed part were transferred to PDA medium supplemented with 40 μg/mL streptomycin sulphate.

Identification of the isolated fungi and the used isolates

The isolated fungi from seeds were purified using single spore isolation technique and hyphal tip method and then were numbered. Identification of the numbered isolates was done based on the spore morphology and colony characters referring to the ‘Illustrated genera of Imperfect fungi’ (Barnett and Hunter, 1972) and ‘Demataceous hyphomycetes’ (Ellis, 1971) and (Domsch et al., 1980). The pure cultures belonging to Alternaria, Fusarium, Helminthosporium, Penicillium and Rhizoctonia isolates were authenticated at Assiut University Mycology Centre (AUMC). Furthermore, the obtained pathogenic isolate F. oxysporum f. sp. cucumerinum (Farrag and Fatouh, 2010) was used as positive control. All isolates were maintained at 5°C on PDA slants for further studies.

Pathogenicity tests

The fungal inocula of the different isolates were prepared in shake culture at 25°C for 10 days. The culture suspension was filtered through one layer of cheese cloth. The concentration of spores was determined by hemacytometer and adjusted with SDW to 1 × 10⁶ colony forming unit (CFU)/ml. The spore suspension was used as inoculum for pathogenicity test in two ways. The first way: cucumber seeds were sown in an autoclaved soil mixture (peat, compost and clay, respectively, 60:20:20 v/v/v) in plug trays and maintained at 25°C, with 12 h/day of fluorescent light. Roots of 15-day-old plants were washed, trimmed to 5 cm length, and then dipped in the spore suspension for 10 min. Inoculated plants were transplanted in pots filled with steamed soil (1.5 L volume). Control plants were prepared similarly but soaked in SDW. Six replicates were used and each replicate consisted of five plants. The experiment was carried out under glasshouse conditions.
conditions at temperature ranging between 30-34°C. Plants were checked daily and the typical symptoms of *Fusarium* wilt in positive control that started to be visible 10 days after inoculation. The wilting plants were counted and the percentage of dried plants were calculated after 30 days of inoculation.

The second way: cucumber seeds were surface sterilized as described before. After washing seeds with sterile distilled water, seeds were soaked in the fungal spore suspension for 5 min. After that, seeds were air dried immediately and then were cultivated in pots filled with steamed soil. Six seeds were sown per pot and ten pots were used. Soaked seeds in the SDW served as control. Data were calculated as percentage of germination, pre- and post-emergence damping-off 10, 15 and 40 days after sowing, respectively. In addition, the number of plants survived was also recorded. The data obtained were statistically analyzed using complete randomized design and means were compared using L.S.D. test according to Gomez and Gomez (1984).

**Paper towel (Rolled towel) method**

Paper towel (Rolled towel) method was employed to know the effect of seed-borne inoculum on seed quality parameters of cucumber i.e. to carry out germination and vigor tests. In addition, to investigate the efficacy of seed treatments against seed-borne fungal inoculum according to the International Seed Testing Association Rules (Anonymous, 1996). Randomly selected 100 seeds were placed on two layers of moist papers, which were placed on a polythene page, and ten seeds per row were used. The seeds were covered with another moist paper and rolled carefully to avoid any excess pressure on seeds. These towels were incubated at 25°C under 12 h light and 12 h darkness for ten days. Seed germination was recorded after 4 and 10 days of incubation. All the morphologically normal seedlings were counted and the percentage of germination was calculated. To find out the seedling vigor, ten normal seedlings were taken from the germination test at random. The root length (cm) was measured from the collar region to the tip of the primary root and the mean root length was calculated. The same seedlings were used for the measurement of shoot length. In addition, the shoot length (cm) was measured from the collar region to the point of junction of the cotyledons. The mean shoot length was recorded. Vigor index was calculated using the following formula, given by Abdul Baki and Anderson (1973):

\[
\text{Vigor index} = \text{Seed germination} \times \text{Seedling Length (Shoot + Root Length)}
\]

**Overcoming seed-borne fungi of cucumber**

**A-Efficacy of some plant extracts on the condiospore germination and mycelial growth of *F. oxysporum* in *vitro**

The efficacy of some plant extracts listed in Tab. 1 was screened on the base of spore germination and inhibition of linear growth *in vitro*. Plant extracts were prepared by stirring 10 g of the plant powder in 100 ml heated tap water (50°C) for an hour, followed by centrifugation at 1453 g and 5°C for 10 min. The supernatant was added to warm (45°C), sterilized PDA medium before solidification to obtain final concentrations of 1, 2 and 3%. The controls were PDA medium amended with sterile distilled water instead of plant extracts. The plates were inoculated with 1 ml of spore suspension (10⁶ conidia mL⁻¹) and then incubated at 20°C for 24 hours. Following spore staining with lactophenol blue, the germination was checked microscopically. Four replicates for each treatment were used.

Fifty spores per each replicate were examined and the percentage of germinated spores was calculated. Other plates were inoculated with fungal disc (6 mm in diameter) and then incubated at 25 ± 2°C till control plates (free plant extracts) reached the radial growth of 90 mm, the mycelium was checked microscopically. Percentage of inhibition over control was also calculated.

**B-Elimination of *F. oxysporum* from infected seeds of cucumber**

Efficacy of the most inhibitory plant extracts was also investigated for eliminating seed-borne inocula of *F. oxysporum* from infected seed samples by paper towel method as described before. In each treatment, 100 seeds were soaked in 100 ml of 3, 2 and 3% of garlic, peppermint and rhizome extract, respectively for 15 min and then shade dried for 24 h. Seeds soaked in sterile distilled water served as control. The treated seeds were tested in four replications, then incubated at 25 ± 2°C for ten days under 12 h light and 12 h darkness. The germinated seeds were counted and percentages of germination and infection were computed. Seedling vigour index was also calculated.

**C-In *vitro* evaluation of peppermint oil by paper towel method**

For extraction peppermint crude oil, steam distillation of wilted leaves was used and the oil was distilled using a Clevenger type apparatus (Gunter, 1948). Seeds were soaked in 100 ml of oil emulsions (1, 2 and 3%) for 15 min and then dried in shade for 1 h. Seeds soaked in sterile distilled water served as control. The treated seeds were tested in four replications of 100 seeds by employing paper towel method, and then incubated at 25 ± 2°C for ten days. The percentages of germination and infection were calculated after ten days of incubation. Seedling vigour index was also calculated.

**D-In *vitro* evaluation the vapour phase of peppermint oil**

To evaluate the vapor phase of peppermint oil for eliminating *F. oxysporum* from infected seeds, doses of 5, 10 and 15 μl oil were applied to a filter paper (100 mm in diameter). After that, the filter paper was mounted on the inverted lid in a Petri dish (140 mm × 23 mm, which offers 400 ML air space). The tested seeds (100 per each treatment) were placed in each dish. The dishes were sealed with vinyl tape and left at 30°C for 48 h. Seeds not exposed to oil vapors were served as control. The treated seeds were
sown in pots filled with steamed soil and the pots were left at 25°C. Six seeds per pot and ten pots per each treatment were used. Percentages of pre- and post-emergence damping-off were calculated 15 and 40 days after sowing, respectively. The percentage of survival plants was also calculated at the end of the experiment.

Results

**Evaluation of seed health testing methods**

The results presented in Tab. 2 indicate that among the two different media employed for detecting seed-borne fungal infection, PDA medium was found to be very good for detecting *Rhizoctonia* sp., *Penicillium* spp., *Alternaria* sp. and *Helminthosporium* sp. On the other hand, Komada selective medium was found better than PDA for detecting *Fusarium* spp. in the majority of seed lots (25 and 32% occurrence), where the growth was more sensitive than of PDA medium (28 and 20.7% occurrence). Finally, PDA medium exhibited good results in detecting the external seed-borne fungal infection of cucumber. Totally, five genera were identified. These were *A. alternata* (Fr.) Keissler, *F. oxysporum* (Schlechtendahl), *F. solani* (Mart.) Sacc., *P. italicum* (Wehmer), *Rhizoctonia solani* (Kohn) and *Helminthosporium oryzae* (Tab. 2 and 4). Furthermore, the most predominant fungi detected were *F. oxysporum*, *F. solani* and *R. solani*.

**Identification and pathogenicity tests of isolated fungi**

From the total population of fungi encountered in this study, only five genera were identified. These were *A. alternata* (Fr.) Keissler, *F. oxysporum* (Schlechtendahl), *F. solani* (Mart.) Sacc., *P. italicum* (Wehmer), *Rhizoctonia solani* (Kohn) and *Helminthosporium oryzae* (Tab. 2 and 4). Furthermore, the most predominant fungi detected, were *F. oxysporum*, *F. solani* and *R. solani*.

Results of the pathogenicity test presented in Tab. 5 indicate that all the isolated fungi reduced seeds variably.
F. oxysporum transmitted from the infected seeds. The lowest ones were in case of H. oryzae and P. italicum. Finally, all isolates of Fusarium spp. collected from seed revealed to be pathogenic to cucumber seeds (Fig. 1A) and seedlings (Fig. 1B). Symptoms on infected seedlings appeared 10 to 15 days after inoculation with F. oxysporum (isolate Fem8) as linear cortical lesions on died seedlings (Fig. 1B) or vascular wilt on the plants and ultimately caused seedling death. Isolate Fem8 could be then classified as F. oxysporum f. sp. cucumerinum (positive control). All the tested fungi were also re-isolated from rotted seeds and dead seedlings.

Overcoming seed-borne fungi of cucumber

A-Efficacy of some plant extracts on the conidiospore germination and mycelia growth of F. oxysporum in vitro

Data for conidiospore germination and mycelial growth of F. oxysporum under control and effect of some plant extracts on these processes are summarized in Table 3. The lowest germination was recorded in case of F. oxysporum treated pots (3.3%) as compared with non-treated pots of negative control (96.7%), followed by F. solani and R. solani.

Tab. 3. Location of F. oxysporum in the different seed parts of cucumber

<table>
<thead>
<tr>
<th>Seed treatment</th>
<th>Seed component</th>
<th>Seed coat</th>
<th>Cotyledon</th>
<th>Embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-disinfected</td>
<td>26</td>
<td>4.1</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Disinfected</td>
<td>7</td>
<td>1.3</td>
<td>0.17</td>
<td></td>
</tr>
</tbody>
</table>

Tab. 4. Identification of isolated fungi from cucumber seeds and their location

<table>
<thead>
<tr>
<th>Genera</th>
<th>Species</th>
<th>Seed location</th>
<th>Isolate No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium</td>
<td>F. oxysporum (Schlecht.)</td>
<td>Coat, cotyledon, embryo</td>
<td>Fco2, Fco5, Fem8</td>
</tr>
<tr>
<td></td>
<td>F. solani (Mart. Succ.)</td>
<td>Coat</td>
<td>Fco17</td>
</tr>
<tr>
<td>Alternaria</td>
<td>A. alternata (Fr.) eissler</td>
<td>Coat</td>
<td>Aco11</td>
</tr>
<tr>
<td>Helminthosporium</td>
<td>H. oryzae</td>
<td>Coat</td>
<td>Hco9</td>
</tr>
<tr>
<td>Rhizoctonia</td>
<td>R. solani (Kohn)</td>
<td>Coat</td>
<td>Rco13</td>
</tr>
<tr>
<td>Penicillium</td>
<td>P. italicum (Wehmer)</td>
<td>Coat</td>
<td>Pco32</td>
</tr>
</tbody>
</table>

Tab. 5. Pathogenicity of some isolated seed-borne fungi of cucumber under greenhouse conditions

<table>
<thead>
<tr>
<th>Tested fungi</th>
<th>Inoculated seeds</th>
<th>Inoculated seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Germination (%)</td>
<td>Emergence damping-off (%)</td>
</tr>
<tr>
<td>F. oxysporum (Fem8)</td>
<td>3.3</td>
<td>96.7</td>
</tr>
<tr>
<td>F. solani (Fco17)</td>
<td>46.7</td>
<td>6.7</td>
</tr>
<tr>
<td>A. alternata (Aco11)</td>
<td>73.3</td>
<td>26.7</td>
</tr>
<tr>
<td>H. oryzae (Hco9)</td>
<td>81.7</td>
<td>18.3</td>
</tr>
<tr>
<td>R. solani (Rco13)</td>
<td>56.7</td>
<td>16.7</td>
</tr>
<tr>
<td>P. italicum (Pco32)</td>
<td>78.3</td>
<td>26.7</td>
</tr>
<tr>
<td>Positive control</td>
<td>5.0</td>
<td>91.7</td>
</tr>
<tr>
<td>Negative control</td>
<td>96.7</td>
<td>3.3</td>
</tr>
<tr>
<td>L.S.D. 0.05</td>
<td>4.9</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Tab. 6. Efficacy of some plant extracts on the seed germination, infection by F. oxysporum and seeding vigor of cucumber

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Germination (%)</th>
<th>Infection (%)</th>
<th>Vigor index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic</td>
<td>73.75</td>
<td>26.76</td>
<td>328.2</td>
</tr>
<tr>
<td>Peppermint</td>
<td>96.5</td>
<td>7.14</td>
<td>472.1</td>
</tr>
<tr>
<td>Rheum</td>
<td>78.25</td>
<td>15.65</td>
<td>383.0</td>
</tr>
<tr>
<td>Control</td>
<td>43.25</td>
<td>86.34</td>
<td>87.3</td>
</tr>
<tr>
<td>L.S.D. 0.05</td>
<td>11.41</td>
<td>7.17</td>
<td>57.1</td>
</tr>
</tbody>
</table>

Tab. 7. Effect of seed treatment with peppermint oil on the seed germination, infection by F. oxysporum and seedling vigor of cucumber

<table>
<thead>
<tr>
<th>Oil concentration</th>
<th>Germination (%)</th>
<th>Infection (%)</th>
<th>Seedling vigor index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>42.34</td>
<td>83.36</td>
<td>88.9</td>
</tr>
<tr>
<td>1</td>
<td>72.18</td>
<td>20.43</td>
<td>252.6</td>
</tr>
<tr>
<td>2</td>
<td>76.95</td>
<td>16.35</td>
<td>323.2</td>
</tr>
<tr>
<td>3</td>
<td>83.74</td>
<td>11.41</td>
<td>468.9</td>
</tr>
<tr>
<td>L.S.D. 0.05</td>
<td>14.23</td>
<td>7.12</td>
<td>43.54</td>
</tr>
</tbody>
</table>
extracts are presented in Fig. 2. Results indicate that the water extract of tested plants significantly varied in their effect at all tested concentrations 1, 2 and 3%. Peppermint extract was the most effective and completely inhibited spore germination and mycelial growth at conc. 2%. Garlic and rheum extracts at 3% completely suppressed spore germination and mycelial growth.

B-Elimination of F. oxysporum from infected seeds of cucumber

Seed treatment with 2% peppermint extract was the most effective, where it caused a highly seed germination (96.5%) and decreased the infection by *F. oxysporum* to 7.14% (Tab. 6). Garlic and rheum extracts at conc. 3% increased the germination to 73.75 and 78.75%, respectively. Moreover, they decreased the infected plants to 26.76 and 15.56%, respectively as compared with control.

C-In vitro evaluation of peppermint oil by rolled towel method

The results presented in Tab. 7 indicate that, there is a high increase in seed germination, seedling vigor and a decrease in the infection by *F. oxysporum* at all tested concentrations of peppermint oil. Seeds treated with 1, 2 and 3% peppermint oil exhibited an increase in germination by 72.18, 76.95 and 83.74%, respectively, a decline in infection by 20.43, 16.35 and 11.41%, respectively and increase in seedling vigor to 252.6, 323.2 and 468.9%, respectively compared to control (88.9%).

D-In vitro evaluation the vapour phase of peppermint oil

Highly significant reduction was occurred in the percentage of pre-emergence damping-off when cucumber seeds were evaporated by the vapor phase of peppermint oil (10 and 15 µl /400 ml air) before sowing (Tab. 8). Seeds, which failed to germinate, were found to be covered with the fungal mycelium (Fig. 1A). On the other hand, the same treatments caused complete protection against post-emergence damping-off. The survival rate of the plants was also increased by 81.7 and 86.7%, respectively compared with control.

Discussion

Many important diseases of plants are caused by seedborne fungi (Basak and Woong Lee, 2002; Littke, 1996; Nasreen et al., 2009; Neergaard, 1977). These diseases have shown variable trends over the years with huge loss in total crop production. Another adverse effect of these
pathogens is that they were spreading to new areas. These emphasize the need for viable, healthy and pathogen free seeds. In order to increase the production of cucumber quantitatively and qualitatively, farmers require healthy and quality seeds with a high germination and purity. Hence, it is imperative that the seeds must be tested before sowing in the field. Further, it necessitates seed health and free from seed-borne diseases are constantly desired with the eradication of seed-borne inocula through various seed treatments. Occasionally, seedling loss may originate from poor germination or pre-emergence disease. An understanding of the origin and nature of seed-borne fungi may be helpful in reducing losses and improving yields. Therefore, seed health testing for presence of these seed-borne pathogens is an important step in the management of crop diseases. Due to climate change, emerging, re-emerging and endemic plant pathogens continue to be a challenge in safeguarding plant health in Egypt. Therefore, early and accurate diagnoses and pathogen surveillance will allow time for development and application of seed treatments. Several methods have been developed to detect the seed-borne microflora in seed lots (Begum and Momin, 2000; Boughalleb and El Mahjoub, 2006; Jeffrey et al., 1985; Kunwar et al., 1986; Krishnappa and Shetty, 1990; Moore, 1984; Neergaard, 1956; 1977; Raut, 1987; Sadashiviah et al., 1986; Shahda et al., 1995;). The emphasis has been on methods, which are simple, easy, economic, sensitive, reproducible and efficient, but some methods such blotter and deep freezing suppress seed germination (Limonard, 1968). The results in this study indicate that PDA medium exhibited good results in detecting the seed surface pathogens of cucumber, whereas, Komada selective medium was better in detecting Fusarium spp., on seed samples. The results of the present study are in accordance with those reported by Boughalleb and El Mahjoub (2006) where the dominance of Fusarium spp. followed by R. solani in all tested seed lots have been reported.

The seed-borne pathogen may be present externally or internally or also associated with the seed as contaminant. This pathogen may cause a seed abortion and rot, necrosis, reduction and elimination of germination capacity as well as seedling damage at later stages of plant growth resulting in development of the disease as systemic or local infection (Bateman and Kwasa, 1999; Khanzada et al., 2002). The seed-borne fungi are known to be located in different parts of the seeds. Results obtained in this study showed that F. oxysporum occurred mainly in both seed coat and cotyledon, but at low levels in embryo of cucumber seeds. These results are in agreement with those obtained by Boughalleb and El Mahjoub (2006).

The results of the present study also indicates the incidence of six fungi in cucumber seeds including A. alternata, F. oxysporum, F. solani, R. solani, H. oryzae and P. italicum. All these isolated fungi reduced seeds viability and only F. oxysporum caused a highly reduction in seed germination. Fusarium oxysporum, F. solani and R. solani are transmitted from seeds to seedlings and caused pre- and post-emergence death. Kabeere et al. (1997) reported that the transmission of Fusarium subglutinans from maize seeds to seedlings from non-surface-disinfect- and surface-disinfected seeds, respectively, which were similar to the respective seed-borne inoculum levels, suggesting that under favorable conditions the fungus may be effectively transmitted from seeds to seedlings. The present study showed that the transmission rate from seeds to seedlings of the tested fungi which causing pre-emergence death was higher than that causing seedling mortality. The highest percentages of pre-and post-emergence death and seedling mortality were recorded in case of F. oxysporum transmitted from the infected seeds. Basak and Lee (2002) also reported similar results in case of seed-borne fungi of maize.

Recently, several studies have reported the use of plant extracts in controlling fungal pathogens (Ja Choi et al., 2004; John Sudhakar, 2002; Satish et al., 2010; Stephan et al., 2005). The present study showed that water extracts of the tested plants significantly varied in their effect on controlling F. oxysporum at all tested concentrations. Peppermint extract was the most effective in completely inhibiting spore germination and mycelial growth at conc. 2%. These results are in agreement with those reported by Ghorbany et al. (2010) where garlic and rheum extracts completely suppressed spore germination and mycelial growth of Fusarium at conc. 3%. Yi et al. (2009) reported that that garlic bulb extract at 0.625% completely controlled F. oxysporum f.spp. cucumerinum and Pythium aphanidermatum and significantly enhanced seed germination, seedling growth and vigor of cucumber. For safe elimination of F. oxysporum from seeds of cucumber, application of these extracts, paper towel method was used. The results in this study indicated that seed treatment with 2% peppermint extract was the most effective and caused high seed germination, decreased infection rate and improved seedling growth. Several studies have tested the same or different plants in controlling Fusarium pathogen on other crops and found similar effect (Aghenin and Marley, 2006; El-Mougy Nehal and Alhabeb, 2009; Gorbany et al., 2010; Morsy et al., 2009). Cucumber seeds treated with 1, 2 and 3% peppermint oil exhibited higher seed germination and a marked decrease in the infection incidence resulting in and increased seedling Vigor. Kritzinger et al. (2002) reported that peppermint oil significantly inhibited the storage fungi including F. oxysporum on the white cowpea seeds thereby increasing the percentage germination and emergence. Results also showed that evaporated seeds by vapors of peppermint oil before sowing caused a marked reduction of pre-emergence damping-off and complete protection against post-emergence damping-off. The survival plants were also increased. Finally, seed treatment may not only protect seeds but may also colonize and protect roots and increase the plant growth. Further research into these extracts will identify the active compounds responsible for their fungicides activity.
Acknowledgement

Authors are thankful to all members of experimental farm, faculty of agriculture, Sohag University and south Valley Univ, for their financial support to carry out the research work.

References


INRA, (in French).


