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Effect of plant extracts on morphological and pathological potential of seed-borne fungi on cucumber seeds

Eman S.H. Farrag[1], Moustafa H.A. Moharam[2] and El-Sayed H. Ziedan[3*]

[1]Department of Agricultural Botany, Faculty of Agriculture, South Valley University, Qena, Egypt, [2] Department of Plant Pathology, Faculty of Agriculture, Sohag University, Sohag, Egypt, [3] Department of Plant Pathology, National Research Centre, Dokki, Cairo, Egypt

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Seed-borne fungi of cucumber are serious problem worldwide causing damping-off, root rot and wilt diseases on cucumber plants. Several pathogenic fungal isolates were isolated from cucumber seed samples collected from commercial markets in Egypt. *Fusarium oxysporum* and *Fusarium solani* were the common fungi isolated from cucumber seeds followed by *Alternaria* sp, *Rhizoctonia solani*, *Helminthosporium* sp. and *Penicillium* spp. Pathogenicity test indicated that *F. oxysporum* was the best fungal significantly induced damping off on cucumber plants. Water extract of peppermint extract was the most effective completely inhibited spore germination and mycelial growth of *F. oxysporum* at concentration of 2%, followed by rheum and garlic extracts which completely inhibited fungal conidiospore germination and mycelial growth on agar medium by the rate of 3%. Cucumber seeds treated with 2% peppermint extract caused a highly reduction of damping off of cucumber and reduced fungal transmission from seeds to seedlings. Furthermore, vigor of cucumber seedlings raised from the treated seeds was better than that developed from the untreated ones.

Key words: Cucumber, Seed-borne fungi, *Fusarium* spp., Plant extracts, Peppermint.

Introduction

Cucumber (*Cucumis sativus* L.) is an important vegetable crop in Egypt. Seed-borne pathogens are causing various factors responsible for the crop low yield on cucumber due to damping-off and wilt caused by *F. oxysporum* (Antoniou and Tjamos, 2000; Farrag and Fatouh, 2010). Few studies have been done on the localization of seed borne pathogens on cucurbits seeds *i.e.*, on watermelon *Fusarium* spp. (Boughalleb *et al*., 2005; Boughalleb and El Mahjoub, 2006), on sorghum, *Aspergillus* sp., *Fusarium* sp. *and Penicillium* sp. (Karim, 2005; Satish *et al*., 2010). Many seed-borne fungi were generally

* Corresponding author: El-Sayed H. Ziedan; e-mail: zieadanehe@yahoo.com

managed by synthetic chemicals. Pesticide pollution of soil and water bodies is well documented (Nostro *et al*., 2000). Hence in recent time application of plant extracts for controlling plant diseases has become important of integrated pest management, as eco-friendly agents (Sahayaraj *et al*., 2009). Several investigators have screened different plant extracts and essential oil as antifungal properties (Stephan *et al*., 2005; Satish *et al*., 2010), Barrera-Necha *et al*., 2009; Belabid *et al*., 2010). The objectives of this study were to isolation and identification of seed borne fungi of cucumber seeds, then studied their pathogenic potential on cucumber as well as screening of plant extracts for controlling soil borne diseases of cucumber.

Materials and methods

Isolation of fungal associated with cucumber seeds

Seed samples of cucumber cv beta alpha were collected from the lots in commercial farms. Several fungal isolates were isolated from cucumber seeds after surface sterilized by soaking of 1 % sodium hypochlorite for 1 min. Seeds were placed in Petri plates containing 20 ml of potato dextrose agar medium (PDA). Ten seeds per plate were used. Plates were incubated at 25±2 °C for seven days. Fungal isolates were purified using single spore and hyphal tip techniques. These fungal isolates were identified based on the spore morphology and colony characters and (Ellis 1971, Domsch *et al*. 1980 and Barnett and Hunter 1998). All isolates were maintained at 5°C on PDA slants for further studies.

Pathogenicity test

Culture suspensions of fungal inocula were obtained after grown each isolate in shake culture at 25°C for 10 days. The culture suspension was filtered through one layer of cheese cloth. The concentration of suspension was determined by plate dilution technique and adjusted with sterile distillated water to 1×10^6 colony forming unit (CFU)/ ml. Cucumber seeds were surface sterilized as descried before, then soaked on fungal 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 of sterile distillated water served as control. Data were calculated as percentage of seed germination, pre- and post-emergence damping-off 10, 15 and 40 days after sowing, respectively. Also, the survival plants were recorded.

Effect of plant extracts on growth of F. oxysporum.

In vitro, plant extracts listed in (Table 1) were screened on the based on spore germination and inhibition of linear growth. Plant extracts were prepared by stirring 10 g of plant powder in 100 ml heated tap water (50°C) for one hour, followed by centrifugation at 10.000 rpm and 5°C for 10 min. 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 (10^5 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 until control plates (free plant extracts) reached the radial growth of 90 mm. Percentage of inhibition over control was also calculated.

Table 1. Lists of some plant extracts used in this study

Name of plants	Forms	Manufacturer/Distributor
Common walnut (*Juglans regia*)	Powder leaves	Alfred Galke GmbH, Gittelde, Germany
Cowslip (*Primula veris*)	Powder roots	ditto
Garlic (*Allium sativum*)	Granules	FUCHS edle Gewürze, Dissen, Germany
Goldenrod (*Solidago canadesis*)	Powder stem	Alfred Galke GmbH, Gittelde, Germany
Mugwort (*Artemisia vulgaris*)	Powder leaves	ditto
Nettle (*Urtica dioica*)	Powder leaves	ditto
Peppermint (*Mentha piperita*)	Powder leaves	Organic Herbspices, Minia, Egypt
Rheum (*Rheum rhabarbarum*)	Powder stem	Alfred Galke GmbH, Gittelde, Germany
Salvia (*Salvia officinalis*)	Powder leaves	ditto
Soapwort (*Saponaria officinalis*)	Powder stem	ditto

Effect of plant extracts on F. oxysporum infected cucumber seeds

Cucumber seeds soaked of *F. oxysporum* spore suspensions were used. Also, efficacy of the most inhibitory plant extracts was also investigated for eliminating seed-borne inocula of *F. oxysporum* from inoculated seed samples by paper towel method according to the International Seed Testing Association

Rules (Anonymous, 1996). In each treatment, 100 seeds were soaked in 100 ml of 3, 2 and 3% of garlic, peppermint and rheum extract, respectively for 15 min and then dried in shade for 24 h. Seeds soaked in sterile distilled water served as control. The treated seeds were rolled on two layers of moist blotting papers, which were placed on a polyethylene bags 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 then percentages of germination and infection was calculated. Seedling vigour index was also calculated using the formula given by Abdul Baki and Anderson, (1973). The obtained data was statistical analysed according to Snedecor and Cocharn (1980).

Results

Isolation and identification of fungi associated of cucumber seeds

Result indicated that, PDA medium employed for detecting of several seed-borne fungal infection, *i.e., Rhizoctonia* sp., *Penicillium* spp., *Alternaria* sp. and *Helminthosporium* sp. Totally, five fungal genera including both saprophytic as well as pathogenic were encountered. The results indicated the dominance of *Fusarium* spp. (28%) followed by *Rhizoctonia* sp. (12%). Other isolated saprophytic fungi included *Alternaria* sp., *Helminthosporium* sp. and *Penicillium italicum* were slightly occurred. The number of fungal colonies arising from non-disinfected seeds were larger than resulting from disinfected ones (Table 2).

Table 2. Occurrence of seed-borne fungi in seed of cucumber

Isolated fungi	**Seed treatment**	**PDA medium**	
		Colonies no. /160 seeds	Occurrence (%)
Fusarium spp.	+	31.0	28.0
	-	8.0	20.7
Alternaria alternata	+	1.8	5.3
	-	3.0	0.7
Helminthosporium oryzae	+	0.0	2.0
	-	18.0	0.0
Rhizoctonia solani	+	6.0	12.0
	-	9.0	4.0
Penecillium itllicum	+	0.0	6.0

- = Non-disinfected
+ = Disinfected

Pathogencity of fungal isolates

Results of the pathogencity test indicated that all the isolated fungi reduced seeds variably of cucumber which presented in Table 3. After ten days of infection, the minimum 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*. The pathogenic fungi *F. oxysporum*, *F. solani* and *R. solani* are transmitted from the germinated seeds to the growing seedling causing pre- and post- emergence death. The transmission rate of the tested fungi causing seed rot or pre-emergence death was higher than that causing seedling mortality. The highest percentages of seed rot or pre-emergence death (96.7%) and post-emergence death (15%), were recorded in case of *Fusarium* sp. 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 and seedlings. Symptoms on infected seedlings appeared 10 to 15 days after inoculation with *F. oxysporum* as linear cortical lesions on died seedlings or vascular wilt on the plants and ultimately caused seedling death. All the tested fungi were also re-isolated from rotted seeds and dead seedlings.

Table 3. Pathogenicity of some isolated seed-borne fungi on cucumber cv beta alpha

Tested fungi	**Inoculated seeds**			
	Germination (%)	Emergence damping-off		Survived plants (%)
		Pre-	Post-	
F. oxysporum	3.3	96.7	3.3	0.0
F. solani	46.7	6.7	15.0	31.6
A. alternata	73.3	26.7	0.0	73.3
H. oryzae	81.7	18.3	0.0	81.7
R. solani	56.7	16.7	8.3	18.3
P. italicum	78.3	26.7	0.0	78.3
Negative control*	96.7	3.3	0.0	96.7
L.S.D. 0.05	4.9	4.1	1.2	4.3

* = Soaked seeds of sterile distillated water

Efficacy of plant extracts on growth of F. oxysporum in vitro

Results indicated that water extracts of tested plants extracts significantly effect on conidiospore germination and mycelial growth of *F. oxysporum* when compared the control (Fig. 1). Different tested concentrations of plant extracts 1, 2 and 3% were tested. Peppermint extract was the most effective and

completely inhibited spore germination and mycelial growth at concentratiuon of 2%. The microscopic examination showed also the degraded and malformed conidia and mycelia caused by peppermint extract. Garlic and rheum extracts are completely suppressed spore germination and mycelial growth at rate of 3%.

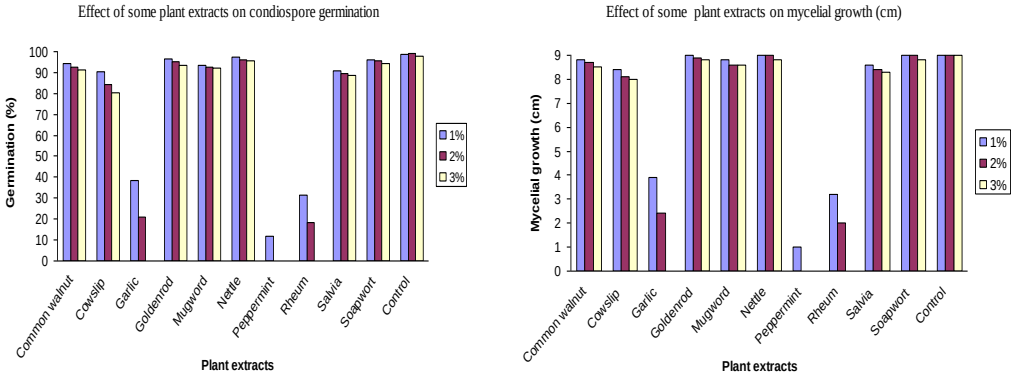

Fig. 1. Efficacy of plant extracts on spore germination and mycelial growth of *F. oxysporum in vitro* Effect of plant extracts on *F. oxysporum* infected of cucumber seeds

Effect o f plant extracts on cucumber seed germination

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% (Table 4). Garlic and rheum extracts at concentration of 3% increased also 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.

Table 4. Efficacy of some plant extracts on the seed germination, infection by *F. oxysporum* and seeding vigour of cucumber

Plant extracts	Germination (%)	Infection (%)	Vigour index (%)
Garlic	73.75	26.76	328.2
Peppermint	96.5	7.14	472.1
Rheum	78.25	15.65	383.0
Control	43.25	86.34	87.3
L.S.D. 0.05	11.41	7.17	57.1

Discussion

Seed is the most important input for crop production. Pathogen free healthy seed is urgently needed for desired plant populations and a good harvest. About 16 % annual crop losses due to plant diseases, at least 10% loss is incurred due to seed-borne diseases (Fakir, 1983). The results in this study exhibited seed-borne fungi of cucumber included that 6 genera isolated from cucumber seed including *A. alternata, F. oxysporum, F. solani, R. solani, H. oryzae* and *P. itallicum.* All these fungi reduced seed variation. *F. oxysporum* caused a highly reduction in seed germination. Similar results are in accordance with those reported by Boughalleb and El Mahjoub (2006). Also, seed-borne fungi caused damping off, root-rot and wilt diseases of plants (Basak and Woong Lee, 2002; Nasreen *et al*., 2009). Seed-borne pathogenic fungi are presented externally or internally 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 Kwasna, 1999; Khanzada *et al*., 2002). Results 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 and seedling mortality were recorded in case of *F. oxysporum* transmitted from the infected seeds. Similar results in case of seed-borne fungi of maize were reported by Basak and Woong Lee (2002). Recently, several studies have been reported to use plant extracts in controlling fungal diseases (John Sudhakar, 2002; Ja Choi *et al*., 2004; Stephan *et al*., 2005; Satish *et al*., 2010). This study showed that water extracts of the tested plants significantly varied in their effect on growth of *F. oxysporum* at all tested concentrations. Peppermint extract was the most effective and completely inhibited spore germination and mycelial growth at concentration of 2%. Similar results are in agreement with those reported by Ghorbany *et al.* (2010). Garlic and rheum extracts are also completely suppressed spore germination and mycelial growth at concentration of 3%. The obtained results indicated that seed treatment with 2% peppermint extract was the most effective and caused highly seed germination, decreased also the infection and improved seedling growth. Several studies have been tested the same or other different plants in controlling the same pathogen on other crops and found similar effects (Agbenin and Marley, 2006; Morsy *et al.,* 2009; Gorbany *et al.,* 2010).

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