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Research article

Biological control of the predominant seed-borne fungi of tomato by using plant extracts

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Abstract

Aqueous extracts from five wild traditional medicinal plants (*Achillea fragrantissima, Balanites aegyptiaca, Peganum harmala, Rumex vesicarius*, and *Urtica urens*) which were collected from different locations in Egypt were tested against the predominant fungal pathogens (*Alternaria alternata* f. sp. *lycopersici, A. solani*, *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani*) infested tomato seeds. All the aqueous plant extracts significantly inhibited the mycelial growth and spore germination of these fungi, but the extract of *A. fragrantissima* exhibited the strongest antifungal activity. The maximum seed germination, plant emergence and seedling vigor was detected after the treatment of tomato seeds with 10% *A. fragrantissima* extract. Pathogenicity testing of tomato seeds by predominant fungi indicated positive infection of tomato seeds but *A. solani* had the most aggressive infection. In greenhouse experiment, the aqueous *A. fragrantissima* extract reduced disease severity but increased total pigments, total phenolics and fruit yield.

Key words: antifungal activity, Egypt, plant extracts, seed-borne fungi, tomato seeds

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops grown in Egypt. It's grown in three seasons - winter, summer and autumn - on about 3 percent of Egypt's total planted area (Glala et al. 2005). 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. Coincidentally 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 are of considerable importance due to their influence on the overall health, germination and final crop stand in the field. The infected seeds may fail to germinate, or transmit disease from seed to seedling and/or from seedling to growing plant. Fungal pathogens may be externally or internally seed-borne, extra- or intra-embryal, or associated with the seeds as contaminants (Singh and Mathur, 2004). Other fungi, including saprophytes and very weak pathogens, may lower seed's quality causing discoloration, which reduces the commercial value of the seeds (Al-Askar et al. 2012).

Several fungi have been reported on tomato seeds as seed-borne in different countries such as *Alternaria alternata, A. solani, Aspergillus clavatus, A. flavus, A. niger, Bipolaris maydis, Cladosporium* sp., *Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, F. moniliforme, F. oxysporum, F. solani, Penicillium digitatum, Phoma destructive, Pythium* sp., *Verticillium* sp., *Rhizoctonia* sp., *Rhizopus arrhizus, R. stolonifer* and *Sclerotinia* sp. (Nishikawa, 2006; Al-Askar et al. 2014; Hamim et al. 2014).

In Egypt, reports on seed-borne mycoflora of tomato are scanty. *Alternaria alternata, A. solani, Cladosporium herbarum, Drechslera* sp., *Fusarium oxysporum, F. solani*, *Pythium aphanidermatum*, *Rhizoctonia solani* and *Verticillium alboatrum* have been reported as seed borne mycoflora of tomato (El-Wakil et al. 1998; Abdel-Kader et al. 2012). The use of some synthetic chemicals to control seed-borne fungi is restricted due to their possible carcinogenicity, high and acute toxicity, long degradation periods, and environmental pollution. There are concerns over the increasing loss of efficacy of conventional fungicides due to pathogen resistance and general unacceptability of fungicides usage because of environmental risks.

The use of biologically based compounds in plant extracts may be an alternative to currently used fungicides to control phytopathogenic fungi, because they virtually constitute a rich source of bioactive chemicals such as phenols, flavonoids, quinons, tannins, alkaloids, saponins and sterols (Burt, 2004). Since these extracts can be active against fungal pathogens, are biodegradable to nontoxic products, and are potentially suitable for use in integrated pest management programs, they could become a new class of safer disease

control agents. Some phytochemicals of plant origin have been formulated as botanical pesticides and are used successfully in integrated pest management programs (Schmutterer, 1990).

Medicinal plants in Egypt have been part of the country's natural and cultural heritage for thousands of years. Today, Egypt is home to 384 different species of medicinal plants found in the Mediterranean coastal region, in the deserts, in the oases and in the Sinai Peninsula (Boulos, 1983). The use of medicinal plants has occurred in Egypt since Pharaonic times (Manniche, 1999).

Recent and modern studies on medicinal plants proved the occurrence of active principles in the different organs of them. Their pharmacological activity has been investigated. In view of their importance as a source of extracts and active constituents used in medicine, they were embodied in different pharmacopoeias, either in Egypt and/or abroad.

The aim of present study is the evaluation of aqueous extracts from five Egyptian wild medicinal plants used in traditional medicine against the predominant fungi infested tomato seeds.

Materials and methods

Tested fungi and tomato seeds: The predominant fungal species (*Alternaria alternata* f. sp. *lycopersici, A. solani, Fusarium oxysporum* f. sp. *lycopersici*, and *Rhizoctonia solani*) infested tomato seeds were obtained from The Regional Center of Mycology, Azhar University, Egypt and tomato *(Lycopersicon esculentum* var. Giant Heirloom) seed samples were obtained from The Ministry of Agriculture, Egypt.

Plant materials and extract preparation: Five wild medicinal plants (*Achillea fragrantissima, Balanites aegyptiaca, Peganum harmala, Rumex vesicarius*, and *Urtica urens*) were collected from different locations in Egypt and used in this study (Table 1). The used parts of these plants were extracted using water. For extraction, 100 g of each air-dried medicinal plant material was separately added to1000 ml of distilled water (1:10 w/v). Extraction then took place under cold conditions for 24 h (Rivillas-Acevedo and Soriano-García, 2007). Plant extracts were filtered through two pieces of cheese cloths. Aqueous extract at a concentration of 10% (as an original concentration) and its 2-fold dilution (5%) were used in the antifungal activity experiments.

Effect of plant extracts on linear mycelial growth: The seed-borne fungi of tomato seeds (*Alternaria alternata* f. sp. *lycopersici, A. solani, Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani*) were used for antifungal activity of water extracts from different selected plants because these fungi were the most predominant species isolated from tomato seeds by many authors. To every 15 ml of sterile Potato dextrose agar (PDA) medium in Petri dishes, 5 ml of each of the aqueous extract concentrations (5 and 10%) from each plant were added. The solution in each Petri dish was gently swirled and allowed to solidify. The extract-amended

medium in the Petri dishes were inoculated each alone at the center with 5 mm inoculum-disc of each test fungus and incubated at 25 ± 2°C for 7 days. Ridomil MZ fungicide produced by SYNGENTA (Metalaxyl: 8% (Methyl N-(methoxy acetyl)-N-(2, 6-xyly)-DL-alaninate; Mancozeb: 64% (Manganese ethylene_bis (dithiocarbamate) (polymeric) complex with zinc salt and 28% inactive matter as 72% wettable powder was used as a standard. The medium with the inoculum disc, but without any extract served as the control.

Effect of plant extracts on fungal spores germination: Antifungal activity of water plant extracts on spore germination of tested fungi were evaluated according to the slide technique (Nair and Llingboe, 1962). The plant extracts were added to dried clean slides as a film. Then, 0.1 ml of spore suspensions was spread over these films. Control treatments were prepared as a film of sterilized distilled water. Three slides were used as replicates for each concentration. Each slide was placed on glass rod in Petri dish under moisten conditions and incubated for 24 h at 25oC. Four microscopic slides (x = 10 x 40) for each slide were used. The percentage of spores' germination was calculated according to the following formula: Spores germination (%) = Spores germination (no) / Total spores (no) x 100

Effect of plant extracts on seed germination: Non-treated and plant extract-treated seeds of tomato were used for laboratory, and pot experiments for evaluation of plant extracts on seed germination and seedling vigor (root and shoot lengths of germinated seedlings). The water extract of *Achillea fragrantissima* (the most effective for antifungal activity and spore germination) was used. The seeds were separately soaked in water extracts for one hour and then plated on moist blotters as well as in a sterilized soil mix in pots. The untreated seeds were soaked in distilled water for one hour and plated on moist blotters and acted as the control. A total of 100 seeds were soaked per extract. Ten seeds were plated on a blotter per Petri dish. The extract-treated and untreated seeds were incubated at 20 ± 2°C for seven days. Seeds plated on a blotter were examined for fungal growth and percentage seed germination, after 7 days of incubation. The percentage of seedling emergence was recorded in seeds sown in the sterilized soil mix after 14 days.

For the soil experiment, 20 seeds were planted per pot equidistantly, at a depth of 2.0 cm and 5 pots were used per extract treatments. All tests were replicated five times. The germination was counted when the first leaf of the seedling reached a length of 4.0 cm. The root and shoot lengths of germinated seedlings were also recorded.

Table 1: Wild medicinal plants used in the present study, their common names and their families

Scientific name	Common name	Family
Achillea fragrantissima (Forssk.) Sch. Bip	Lavender Cotton	Asteraceae
Balanites aegyptiaca (L.) Del	Egyptian balsam	Zygophyllaceae
Peganum harmala L.	Harmel	Nitrariaceae
Rumex vesicarius L.	Bladder dock	Polygonaceae
Urtica urens L.	Small nettle	Urticaceae

Pathogenicity testing of predominant fungi: Four predominant fungal pathogens, (*Alternaria alternata* f. sp. *lycopersici, A. solani, Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani*) were selected for pathogenicity testing studies and evaluated for their pathogenicity on tomato plants under greenhouse conditions. Inoculum from each of the above cultures was colonized on PDA media. Five hundred ml glass bottles, each containing 200 g of the media moistened with water, were autoclaved and the media inoculated with a 6 mm mycelial disk taken from a 7-day-old colony grown on PDA. The glass bottles were then incubated at 25^{o}C for 14 days.
Sandy clay soil 1:2 (w/w) was chemically sterilized using a 5% formaldehyde solution. After treatment with formaldehyde, the soil was covered with boards or papers and left for 24 hrs, then allowed to dry until all odor of formaldehyde has disappeared from the soil. The soil then transferred into 15 cm diameter pots, each containing 3 kg soil. Pots were inoculated with the selected fungi at the rate of 5% (w/w) and kept in the greenhouse for one week before planting tomato plants. Pots containing non-inoculated soil used as controls. Three replicates were used per treatment.
Pathogen-free seeds were surface sterilized and planted (10 seeds/pot) in both inoculated and non-inoculated soil. All pots were maintained in the greenhouse under natural conditions during the winter season and watered as needed (El-Wakil et al. 2009). Fifteen days after sowing, the disease ratios were determined by recording the number of non-emerged seeds, while post-emergence and surviving plants were recorded 30 days after sowing. The equations described by El-Wakil et al (2009) were followed:

Pre-emergence damping off % = No. of non-emerged seeds / No. of sown seeds x 100

Post-emergence damping off % = No. of dead seedlings / No. of sown seeds x 100

Surviving plants % = No. of surviving plants / No. of sown seeds x 100

Inoculation of tomato plants by *Alternaria solani*: Tomato seeds were sown in foam try field with a growing media of 1 peat: 1 vermiculite. Seedlings were transplanted into field (Experimental farm of Mansoura University, Egypt). Complete randomized blocks design with three replicates was adapted. Each plot consisted of two rows (1.5 m in wide and 5 m long (15 m^2/plot), plant spacing was 50 cm and every replicates included 20 plants. All tested medicinal plant extracts

were applied as foliar application at 3–4 leaf growth stage of plant. Tomato plants were infected by spraying of *A. solani* (30 ml of water spore suspension, containing 2.5×10^6 spores ml^{-1} with 1% Tween 80) by means of atomizer until the run-off point onto shoot of 30 days-old tomato plants. *Alternaria solani* was chosen because it is the most fungus infecting tomato plants appeared from pathogenicity testing. Thereafter, plants in each pot were left to be air-dried, sprayed with 15 ml distilled water and covered with plastic bags for two hours to maintain high humidity atmosphere around the leaves which is necessary for fungal infection.

Application of aqueous extract of *A. fragrantissima* on diseased tomato plants: Because aqueous extract *of A. fragrantissima* at the concentration of 10 % gave the strongest inhibitory effect on mycelial growth and spore germination of predominant fungi, infected tomato plants were exposed to three successive sprays within 15 days intervals by this extract. The positive control plants were sprayed only with *A. solani* spore suspension without any treatment, or sprayed with Ridomil MZ fungicide (250 g/100 L) while the negative control plants treated with the plant extract and left without any infection.

Effect of aqueous extract of *A. fragrantissima* on disease assessment: Early blight symptoms appeared 7 days after inoculation were scored as infection type and disease severity was assessed according to the 1 - 9 scale of Bernier et al. (1984).

Disease severity % = (n x v) / 9 N x 100

Where:

(n) = Number of plants in each category. (v) = Numerical values of symptom category. (N) = Total number of plants. (9) = Maximum numerical value of symptom category. Disease reduction % = Disease severity in control – Disease severity in treatment / Disease severity in control x 100

Effect of aqueous extract of *A. fragrantissima* on fruit yield: After three times of harvest, number of tomato fruits per plant was determined after the application of water plant extract.

Statistical analysis: The obtained results were statistically analyzed according to Steel and Torrie (1980). LSD test was used to compare means of treatments at the 5 % level of significance.

Results

Effect of aqueous extracts on mycelial growth of predominant fungi: The efficacy of five aqueous extracts (at 5% and 10%) against the mycelial growth of the predominant fungi (*Alternaria alternata* f. sp. *lycopersici, A. solani, Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani*) of tomato seeds are shown in Table 2. The aqueous extracts of different medicinal plants exhibited varying levels of inhibition on mycelial growth of predominant fungi when compared with the control. The synthetic fungicide at 5% and 10% concentrations recorded a minimum growth inhibition on the fungi. At the both concentrations of the extracts used in this study, *A. fragrantissima* was observed to have the highest inhibitory

effects on the four fungi followed by *P. harmala, R. vesicarius*, *B. aegyptiaca* and *U. urens*. The mycelial growth of *R. solani* was found to be more strongly inhibited by the aqueous plant extracts than growth of other fungal species.

Table 2: Inhibition of mycelial growth of predominant fungi by water plant extracts at different concentrations.

Medicinal plants	[a] Mean ± S. E. of inhibition of linear mycelial growth (mm)							
	5 %				10 %			
	AAL	AS	FOL	RS	AAL	AS	FOL	RS
A. fragrantissima	9.1± 0.4	11.2± 0.3	11.6± 0.4	8.4± 0.3	9.9± 0.3	10.1± 0.5	7.4± 0.3	7.3± 0.3
B. aegyptiaca	37.2± 0.2	36.2± 0.2	35.6± 0.5	34.5± 0.2	34.7± 0.3	34.1± 0.5	31.3± 0.3	29.4± 0.3
P. harmala	18.4± 0.1	18.2± 0.1	19.6± 0.2	18.5± 0.5	16.7± 0.3	17.1± 0.5	16.4± 0.3	16.5± 0.3
R. vesicarius	25.2± 0.3	24.2± 0.4	24.6± 0.3	23.5± 0.4	23.7± 0.3	22.1± 0.5	21.3± 0.3	22.4± 0.3
U. urens	40.1± 0.4	42.2± 0.3	44.6± 0.1	37.5± 0.3	40.7± 0.3	45.1± 0.5	35.3± 0.3	38.4± 0.3
F. (Ridomil MZ)	3.2±0.1	3.8±0.3	3.9±0.1	3.5±0.2	1.1±0.1	1.7±0.2	1.8±0.3	1.4±0.2
Control	46.3±0.5	48.2±0.4	47.5±0.6	46.3±0.4	48.2±0.3	47.5±0.4	46.3±0.5	48.2±0.4

LSD = 0.05; ± = Standard error of mean; F = Fungicide; AAL = *Alternaria alternata* f. sp. *lycopersici*; AS = *Alternaria solani*; FOL = *Fusarium oxysporum* f. sp. *lycopersici*; RS = *Rhizoctonia solani;* [a] Mean of five replicates.

Effect of plant extracts on spores' germination of predominant fungi: It was revealed from the results (Table 3) that different concentrations of plant extracts caused significant inhibition in the spore germination. However, the maximum inhibition in the spore germination was found at highest concentration (10%). It was followed by 5% concentration of plant extracts as compared to control which showed least inhibition in spore germination. The extract of *A. fragrantissima* at highest concentration was found most effective in reducing the spore germination followed by highest concentration of extract of *P. harmala*, *R. vesicarius, B. aegyptiaca* and *U. urens,* respectively. The inhibition in spore germination varied from 38.6% to 25.4%, 42.2% to 35.4%, 46.6% to 32.4%, 48.5% to 45.4% and 57.5% to 46.4% in different concentrations of *A. fragrantissima, P. harmala , R. vesicarius, B. aegyptiaca* and *U. urens,* respectively as compared to untreated control which showed least inhibition in spore germination. Fungicide exhibited the lowest the spore germination as compared to untreated control and plant extracts.

Effect of plant extracts on seed germination and seedling vigor: The effect of medicinal plant extracts on seed germination and plant emergence of tomato is recorded in Table 4. Germination of non-treated tomato seeds was low in pots (9.5% to18.9%) as compared to laboratory tests (13.7% to 27.8%). In both experiments, in laboratory and pots, treated seeds of tomato by medicinal plant extracts increased seed germination and plant emergence. The maximum germination and plant emergence were recorded in case of *A. fragrantissima* extract (39.2%, 25.0%), followed by *P. harmala* (35.5%, 19.8%), *R. vesicarius* (32.5%, 15.8%), *B.*

aegyptiaca (28.3%, 22.9%) and *U. urens* (21.4%, 12.6%), respectively, when compared with the control. Furthermore, seedling vigor (root and shoot lengths) was increased after the treatment of tomato seeds by plant extracts at the concentration of 10%. *Achillia fragrantissima* exhibited the most effective extract on root and shoot lengths (6.8cm, 14.8cm, respectively), followed by *P. harmala* (6.2cm, 14.1cm, respectively), *R. vesicarius* (5.9cm, 13.5cm, respectively), *B. aegyptiaca* (5.5cm, 12.7 cm, respectively) and *U. urens* (4.1 cm, 10.2 cm, respectively) when compared with the control (Table 5).

Table 3: Inhibition of sporulation (x 10^5) of predominant fungi by aqueous plant extracts at different concentrations.

Medicinal plants	[a] Mean ± S. E. of sporulation inhibition %							
	5 %				10 %			
	AAL	AS	FOL	RS	AAL	AS	FOL	RS
A. fragrantissima	35.1± 0.4	36.2± 0.3	38.6± 0.4	29.5± 0.3	28.7± 0.3	29.1± 0.5	30.3± 0.3	25.4± 0.3
B. aegyptiaca	47.2± 0.2	48.2± 0.2	47.6± 0.5	48.5± 0.2	45.7± 0.3	42.1± 0.5	40.3± 0.3	45.4± 0.3
P. harmala	40.4± 0.1	42.2± 0.1	43.6± 0.2	38.5± 0.5	40.7± 0.3	37.1± 0.5	39.3± 0.3	35.4± 0.3
R. vesicarius	45.2± 0.3	44.2± 0.4	46.6± 0.3	46.5± 0.4	43.7± 0.3	39.1± 0.5	40.3± 0.3	32.4± 0.3
U. urens	50.1± 0.4	52.2± 0.3	54.6± 0.1	57.5± 0.3	47.7± 0.3	45.1± 0.5	45.3± 0.3	46.4± 0.3
F. (Ridomil MZ)	23.1±0.1	23.7±0.3	23.2±0.1	23.5±0.2	21.1±0.1	21.7±0.2	21.8±0.3	21.4±0.2
Control	76.3±0.5	78.2±0.4	79.5±0.6	68.3±0.4	82.2±0.3	80.5±0.4	84.3±0.5	79.2±0.4

LSD = 0.05; ± = Standard error of mean; F. = Fungicide; AAL = *Alternaria alternata* f. sp. lycopersici; AS = *Alternaria solani*; FOL = *Fusarium oxysporum* f. sp. *lycopersici;* RS = *Rhizoctonia solani;;* [a] Mean of five replicates.

Table 4: The effect of medicinal plant extracts (at 10% concentration) on seeds germination and plant emergence of tomato

Medicinal plants	Non-treated		Plant extract-treated	
	Seed germination (%)	Plant emergence (%)	Seed germination (%)	Plant emergence (%)
A. fragrantissima	27.8±1.2	17.3±0.9	39.2±2.1	25.0±0.9
B. aegyptiaca	19.2±1.6	10.6±0.6	28.3±2.6	22.9±0.9
P. harmala	25.5±1.1	18.9±0.6	35.5±2.9	19.8±1.1
R. vesicarius	21.8±1.3	11.9±0.4	32.5±1.9	15.8±0.7
U. urens	13.7±1.1	9.5±0.3	21.4±2.6	12.6±0.6
Control	10.8±0.3	7.7±0.2	15.6±0.9	11.9±0.9

LSD= 0.05, ± = Standard error of mean

Pathogenicity testing of predominant fungal species: Results of the pathogenicity tests of of the predominant fungi (*Alternaria alternata* f. sp. *lycopersici, A. solani, Fusarium oxysporum* f. sp. *lycopersici* and

Rhizoctonia solani) infesting tomato seeds are shown in Table 6. Disease severity readings of the fungi on seeds and seedlings were achieved after 15, 30 and 45 days after sowing. *Alternaria solani* had the highest percentage of pre-emergence damping-off among all tested fungi. The percentage of infection was 45.82% on seeds compared with the control treatment which had 0% infection. The same pathogen had also the highest percentage of post-emergence damping-off among all tested fungi. The percentage of infection was 22.82% on seeds compared with the control treatment which had 0% infection. On the other hand, the same pathogen exhibited the highest percentage of dead seedlings among all tested fungi. The percentage of dead seedlings was 15.80% for infected plants compared with the control treatment which showed 6.60 % death. *Rhizoctonia solani* had the lowest percentage of pre-emergence damping-off, post-emergence damping-off and dead seedlings among all tested fungi.

Effect of aqueous extract of *A. fragrantissima* on disease severity: Disease severity (%) of early blight disease on tomato plants in the greenhouse when treated with the *A. fragrantissima* extract (10%) was shown in Table 7. Data showed that the treatment of pathogen with the extract significantly reduced the disease severity by 28.4%. Results of pathogen treated with fungicide indicated that the disease severity reduced by 39.6%. On the other hand, when the pathogen treated with both fungicide and plant extract, the disease severity was reduced by 58.0%. Generally, using of fungicide combined with the plant extract reduced the disease severity than fungicide or plant extract when they were used separately.

Table 5: The effect of medicinal plant extracts (at 10% concentration) on root and shoot lengths of tomato seedlings

Medicinal plants	Non-treated		Plant extract-treated	
	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)
A. fragrantissima	4.2±0.3	9.2±0.5	6.8±0.4	14.8±0.5
B. aegyptiaca	3.1±0.2	7.9±0.4	5.5±0.2	12.7±0.3
P. harmala	3.8±0.4	8.9±0.2	6.2±0.2	14.1±0.2
R. vesicarius	3.6±0.1	8.1±0.1	5.9±0.5	13.5±0.3
U. urens	2.9±0.1	6.2±0.2	4.4±0.1	10.2±0.2
Control	2.7±0.1	5.9±0.2	4.1±0.2	9.8±0.5

LSD = 0.05, ± = Standard error of mean

Table 6: Pathogenicity testing of predominant fungi of tomato seeds under greenhouse conditions

Fungi	Percentage of dead plants		
	Pre-emergence (15 days after sowing)	Post-emergence (30 days after sowing)	Dead seedlings (45 days after sowing)
The Control	0.00 ± 0.0	0.00 ±0.0	6.60 ±0.21
A. alternata f. sp. *lycopersici*	25.40 ± 1.10	19.50±1.75	10.20±2.20
Alternaria solani	45.82±1.02	22.82±0.80	15.80±2.92
F. oxysporum f. sp. *lycopersici*	28.30±1.91	18.36±1.42	9.70±2.75
Rhizoctonia solani	12.80 ±0.95	8.94±0.85	7..90±2.65

LSD = 0.05; ± = Standard error of mean

Table 7: Early blight disease severity (%) in tomato treated with aqueous extract of *A. fragrantissima* (at 10% concentration).

Treatment	Disease Severity %	Disease Reduction %
Infected (control)	53.8±1.8	0.0
A. fragrantissima	38.5±1.5	28.4±1.1
Fungicide (Ridomil MZ)	32.5±0.9	39.6.±2.4
A. fragrantissima + Fungicide	22.6±0.2	58.0±2.6

LSD = 0.05; ± = Standard error of mean

Table 8: Influence of aqueous extract spraying of *A. fragrantissima* extract (10%) on tomato fruit yield/plant (g)

Treatment	Average	Increase %
Control	367.5	
A. fragrantissima	454.2	23.6
A. solani	189.6	
Fungicide (Ridomil MZ)	310.8	15.4
A. solani + *A. fragrantissima*	430.6	17.2
A. solani + Fungicide	425.9	16.0
A. solani +Fungicide + *A. fragrantissima*	490.2	33.4

LSD = 0.05

Effect of aqueous extract of *A. fragrantissima* on fruit yield: In relation to control values, pathogen caused a drastic reduction ($P \leq 0.05$) in the yield components of tomato plants. Infected plants treated with the extract, led to an increase in tomato yield by 23.6%, whereas the treatment by fungicide alone gave an increase in the yield by 15.4%. Treated pathogen with extract gave an increase in yield by 17.2%, whereas, the treatment of pathogen with fungicide led to an increase in the yield by 16.0%. In general, the treatment of pathogen by extract and fungicide together increased the yield by 33.4%, which was over previousely mentioned two treatments (Table 8).

Discussion

Plant extracts can contain natural antimicrobial compounds, and these can be used for seed disinfection as an alternative to fungicide treatments. Use of plant extracts against plant pathogenic fungi and plant diseases is relatively a recent approach. All plant extracts tested in this study exhibited different degrees of antifungal activity against the predominant fungi of tomato seeds. The inhibitory effect of the tested extracts might be due to natural bioactive materials present in these extracts (Hilal et al. 1979; Oliver, 1986; Mustafa et al. 1992; Rizk and El Ghazaly, 1995). Treated seeds of tomato with extract of some medicinal plants led to a pronounced increase in seed germination and seedling vigor. These results are in agreement with those obtained by many authors (Culver et al. 2012; Norman et al. 2012; Ancuţa et al. 2013), who reported that plant extracts can be used to enhance the germination of seeds and seedling vigor. The ability of the extracts to increase seed germination and seedling emergence could be attributed to the suppression of the incidence of the seed borne fungi that could have killed the embryo of the seeds. Several studies with plant extract of many medicinal plants indicated their inhibitory effect on the mycelial growth and spore germination of several pathogenic fungi (Hasan et al. 2005; Tagoe et al. 2011; Baka, 2010, 2014; Dissanayake, 2014). *Achillea fragrantissima* extract gave the best results in reducing the mycelial growth, spore germination and disease severity and increased the fruit yield. *A. fragrantissima* has been used for many years in traditional medicine in Middle Eastern countries for the treatment of respiratory diseases, skin diseases, gastro- intestinal disturbances, high blood pressure, stomach aches and diabetes (Mustafa et al. 1992; Hamdan and Afifi, 2004). Recent reports demonstrated anti-inflammatory, antioxidant and antiproliferative capacities of *A. fragrantissima* extracts (Al-Mustafa and Al-Thunibat, 2008; Alenad et al. 2013). Aqel et al. (2012) reported that that the essential oil of *A. fragrantissima* has antibacterial and antifungal activities. The efficacy of medicinal plant species may be due to induction of the resistance mechanisms in treated plants (Yamunarani et al. 2004) or cause a delay in the development of infection in early growth stage by inhibition the mycelial growth of pathogen (Krebs and Forrer, 2001).

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