Phytopathol. Mediterr. (2010) 49, 143–151

RESEARCH PAPERS

Control of tomato early blight and wilt using aqueous extract of neem leaves

NAZIHA M. HASSANEIN, MOHAMED A. ABOU ZEID, KHAYRIA A. YOUSSEF and DALIA A. MAHMOUD

Department of Microbiology, Faculty of Science, Ain-Shams University, 11566 Elkhalifa El-Mamoun street, Abbassia, Cairo, Egypt

Summary. The effect of neem (*Azadiracta indica*) leaf extract against *Alternaria solani* and *Fusarium oxysporum*, the causal agents of early blight and wilt of tomato plants (*Lycopersicon esculentum* Mill.) respectively, was studied. Concentrations (5, 10 and 20%) of aqueous neem extract suppressed mycelial growth of both pathogenic fungi and the degree of suppression gradually increased with increasing concentration. A concentration of 20% aqueous neem leaf extract sprayed on tomato plants lowered the incidence of Alternaria early blight from 53.2 to 42.5% after two weeks and from 100 to 79.2% after 4 weeks. Spraying plus irrigation with the same extract lowered the severity of Alternaria early blight from 26.8 to 11.4% after 2 weeks and from 61.7 to 17.9% after 4 weeks (control ratio of 43.71% after 4 weeks). For *F. oxysporum* wilt, germination of tomato seeds was highest in pots containing the negative control (soil free of pathogen) and in pots irrigated with the aqueous neem extract. The lowest disease incidence (19.04%) was obtained in pots treated with the pathogen and irrigated with aqueous neem extract, where an 81% control of *Fusarium* wilt was achieved. Growth parameters of tomato (shoot and root length, number of leaves, fresh and dry weight of shoots and roots) were studied 4, 6 and 8 weeks after sowing in the presence of the pathogens. There was a significant gradual increase in growth parameters when the plants were sprayed and irrigated with aqueous neem extract with the greatest improvement recorded 8 weeks after sowing.

Key words: *Azadirachta indica*, *Alternaria solani*, *Fusarium oxysporum*, tomato growth parameters, disease control.

Introduction

Tomato is the most important vegetable crop in Egypt. Some 186.000 ha is cultivated with this crop and annual production amounts to seven million tons, which are consumed either fresh or processed (Ramadan, 2008). Two common and devastating diseases of tomato are early blight and *Fusarium* wilt caused by *Alternaria solani* and *Fusarium oxysporum* f. sp. *lycopersici* respectively (Singh *et al.*, 1980, Maiero and Barksdale 1989). These two diseases cause a severe reduction in yield and high economic losses every growing season. They are controlled by chemical fungicides, long crop rotation, pasteurization of seedbeds with steam or fumigants and by breeding resistant tomato cultivars (Spletzer and Enyedi, 1999). The biocontrol of plant pathogens is currently regarded as a key practice in sustainable agriculture because it exploits a natural resource.

Nature is a source of many different biocontrol agents, including the plant- growth promoting micro-organisms (PGPM) which promote plant growth by inducing a defense response (Akköprü and Demir 2005; Siddiqui, 2006). Natural plant

Corresponding author: M.A. Abouzeid
Fax: +20 226842123
E-mail: m_abouzeid@sci.asu.edu.eg

extracts have latterly gained importance for crop protection against pests and pathogens because of their safety and target specificity. They have also been found effective against a wide range of pathogens (Manickam and Rajappan, 2001). Many reports have been published on non-chemical means to protect seed against plant pathogens. Among these means, plant extracts have proved effective in inhibiting seed-borne pathogens and in improving seed quality and the emergence of plant seeds (Nwachukwu and Umechuruba, 2001). Patil *et al*., (2001) found that neem leaf extract reduced disease incidence and increased fruit yield of tomato infected with *A. solani*, and Amadioha and Uchendu (2003) applied extracts from neem leaf to control *Fusarium solani* causing tomato fruit rot. Hosna *et al*., (2003) reported that neem extract controlled *Alternaria* blight (*A. brassicicola* and *A. brassica*) of cauliflower seed. Surender and Hari (2004) found that pure neem leaf extract completely inhibited spore germination of the chickpea wilt agent *F. oxysporum*, and Aboellil (2007) reported that trilogy, a natural product from *A. indica*, significantly retarded growth of cucumber powdery mildew, and induced resistance in cucumber plants. The objective of this study was to evaluate the effectiveness of aqueous extracts of neem leaves in controlling *A. solani* and *F. oxysporum* in tomato and to evaluate the improvement in the growth parameters of tomato plants after such treatment.

Materials and methods

Tomato plants

Seeds and seedlings of tomato (*Lycopersicon esculentum* Mill.) cv. *Castle Rock* (4 weeks old) were obtained from the Agricultural Research Center, Giza, Egypt.

Pathogens

The test strains of *A. solani* and *F. oxysporum* f. sp. *lycopersici* were obtained from the Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Neem leaf extraction

Aqueous extracts were prepared in the laboratory by the traditional Malian method. One kg of fresh neem leaves (*Azadirachta indica* A. Juss) was collected from 8-year-old trees in the Kalyoubeia governorate, Kalyoub city, Egypt. Leaves (250 g) were dried at room temperature and ground in a mortar, combining the ground leaf with water (1 L), and subsequently steeping the mixture overnight at room temperature. The next morning the extract was strained through a Miracloth (Calbiochem, San Diego, CA, USA), then centrifuged at 3500 g for 15–20 min to remove coarse material. Extracts were then sterilized by passing through a Millipore filter (0.22 μm, Millipore Corporation, MA, USA) and the desired concentration was achieved using the method described by Shetty *et al*. 1989 and Achimu and Schlösser (1992). A 20% concentration (w:v) of aqueous neem leaf extract (ANLE), gave *in vitro* the highest growth inhibition of the two test pathogens and this concentration was used in further experiments.

In vivo experiments - I. Early blight

Inoculum preparation

*Alternaria s*olani was grown on potato dextrose agar (PDA, Gibco, BRL, UK) amended with 250 μg mL^{-1} chloramphenicol (Sigma Chemical Co, St. Louis, USA) and 100 μg mL^{-1} streptomycin sulphate (Sigma), for 10 days at 25°C±2°C, and pure cultures were maintained on PDA slants and stored at 4°C for use throughout the study. To enhance sporulation, cultures were exposed to fluorescent light (80 μmol m^{-2}s^{-1}) for 6 h daily prior to use. Ten mL of sterilized water was added to each Petri-dish containing a culture of *A. solani*, and conidia were collected using a sterilized brush. The spore suspension of the fungus obtained was filtered through three layers of nylon mesh. The concentration of conidia was determined and adjusted to 10^6 conidia mL^{-1} using a haemocytometer.

Pathogenicity test

The pathogenicity test was carried out under greenhouse conditions. Twenty-five clay pots containing sterilized Nile silt soil were sown with 10 tomato seedlings. After one month, the seedlings were reduced to four per pot. After a further two weeks, the growing seedlings were sprayed with a spore suspension of *A. solani*. Five replicate pots were used for each treatment (Datar and Mayee, 1985). According to the method described by Hilaal

(1992), the inoculated plants were covered with plastic bags for 48 hours, as usual, to maintain high relative humidity and favour fungal infection. Disease incidence was estimated after 2 and 4 weeks of inoculation as mentioned by Horsfall and Barratt (1945).

Effect of ANLE on early blight of tomato

As mentioned above 10 seedlings of tomato were sown in pots (20 cm high, 25 cm diameter) filled with sterilized soil. After one month seedlings were reduced to 4 per pot. Treatments were: T1, 30-day-old seedlings sprayed with water (-ve control); T2, 30-day-old seedlings sprayed with ANLE; T3, 30-day-old seedlings sprayed with *A. solani* spore suspension (+ve control); T4, one-month-old seedlings sprayed at 8-day-intervals with ANLE and when they were 45 days old, sprayed with the *A. solani* spore suspension; T5, 30-day-old seedlings sprayed and irrigated at 8-day-intervals with ANLE and when they were 45 days old, sprayed with the *A. solani* spore suspension. Five pots were used per treatment, and inoculated plants were covered with plastic bags for 48 h to maintain relative humidity favouring infection. Plants were grown for 2 months, fertilized weekly with inorganic liquid fertilizer (Thrive®, Arthur Yates & Co Limited, Milperra, Australia) and watered as needed. Disease incidence, severity and control were determined after 2 and 4 weeks of inoculation as described by Horsfall and Barratt (1945).

***In vivo* experiments - II. *Fusarium* wilt**

Inoculum preparation and soil infestation

Fusarium oxysporum was grown on PDA amended with 250 μg mL^{-1} chloramphenicol and 100 μg mL^{-1} streptomycin sulphate, for 8 days in the dark at 25±2°C. Pure cultures were maintained on PDA slants at 4°C for the study. Flasks (250 mL) containing 25 g seeds of maize (*Zea mays* L.) in 100 mL distilled water were maintained overnight, then autoclaved at 121°C for 30 min on three consecutive days. Each flask was inoculated with eight agar plugs (2 mm) from actively growing margins of a 2-week-old colony of the *F. oxysporum* test strain and was incubated at 25±2°C in the dark for 2 weeks. Flasks were occasionally shaken to ensure uniformity of colonization. Controls consisted of non-inoculated sterile maize seeds. Prior to use, some inoculated and non-inoculated (control) maize seeds were plated on PDA to ascertain whether the pathogen was present or not (Wong *et al.,* 1984). *F. oxysporum* inoculum (1% weight of maize seeds-based inoculum/weight of steam-pasteurized soil) was thoroughly dispersed into the steamed soil of two sets of pots (3 kg/pot). The first set contained the pathogen while the second set received the same quantities of non-inoculated maize seeds and served as the control. All pots were watered as needed and left for a week to ensure inoculum distribution.

Preparation of seeds for cultivation and pathogenicity test

Tomato seeds were surface-sterilized by immersion in 70% ethanol for 2 min. then in 0.2% sodium hypochlorite (NaOCl) for 3 min, and washed several times with sterile distilled water. Ten seeds per pot were placed in 30 cm diameter free draining pots, each filled with about 3 kg of soil. The seeds were sown at 3 cm depth and when emergence was complete (10 days) the seedling density was reduced to 4–5 seedlings/pot. Five replicates were used per treatment. The pots were placed in a greenhouse, fertilized weekly with inorganic liquid fertilizer (Thriveâ) and watered on alternate days. To prevent nutrient deficiency, pots were replenished twice weekly with 1/4 strength Hoagland's modified salt mixture (MP Biochemicals, Irvine, CA, USA). Tomato plants were inspected daily for two months, and foliar symptoms were checked 4 weeks after inoculation, at which time the percentage of disease incidence and the seed germination were also determined.

Effect of ANLE on Fusarium wilt of tomato

According to the method described by Ganapathy and Narayanasamy (1990), surface-sterilized tomato seeds were subjected to the following treatments: T1, seeds soaked for 3 h in tap water (3HTW), sown in soil free of *F. oxysporum* and irrigated with water (-ve control); T2, 3HTW seeds sown in soil free of *F. oxysporum* and irrigated with ANLE; T3, 3HTW seeds sown in soil infested with *F. oxysporum* irrigated with water (+ve control); T4, 3HTW seeds sown in soil infested with *F. oxysporum* and irrigated with ANLE; T5, seeds soaked for 3 h in ANLE then sown in soil infested with *F. oxysporum* then irrigated with water. Each treatment was replicated 5 times and

pots were watered on alternate days. Wilt disease started to appear four weeks after sowing and the percentage of seed germination, disease incidence and the degree of wilt control were determined.

Effect of ANLE on growth parameters of tomato

Some growth parameters of tomato plants (shoot and root length, number of leaves, shoot and root f wt and d wt) were determined after 4, 6 and 8 weeks, at the same time as the effect of ANLE on early blight and wilt was recorded.

Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA) and significant differences between the means were determined using Fisher's Protected LSD Test at P=0.05. Superanova® (Abacus Concepts, Inc., Berkeley, CA, USA) was used for all analyses.

Results

***In vivo* experiments - I. Early blight**

Pathogenicity test

Pathogenicity tests of *A. solani* revealed that the percentage of early blight incidence was 53.2% after 2 weeks and reached 100% after 4 weeks of inoculation compared with the uninfected control plants.

Effect of ANLE on early blight of tomato

The incidence and severity of *Alternaria* early blight and the percentage of control achieved 2 and 4 weeks after inoculation showed that after 2 weeks when tomato were both sprayed and irrigated with ANLE, blight incidence was substantially reduced to 39.5% and that the percentage of disease severity was reduced to 11.4%. The percentage of disease control was 13.7%. After 4 weeks, the values recorded were 71.7%, 17.9% and 28.3% for these parameters respectively (Table 1).

In vivo experiments - II. Fusarium wilt

Pathogenicity test

Pathogenicity tests of *F. oxysporum* showed a reduction of 30% in the germination of tomato seeds in fungus-infested soil, compared with 100% germination in the control soil free of the pathogen. Also, the percentage of *Fusarium* wilt incidence on tomato in infested soil was 94.3%.

Table 1. Effect of ANLE on disease incidence and severity and on control of tomato early blight 2 and 4 weeks after inoculation.

Treatment	Two weeks after inoculation						Four weeks after inoculation					
	Disease incidence (%)	±SE	Disease severity (%)	±SE	Disease control (%)	±SE	Disease incidence (%)	±SE	Disease severity (%)	±SE	Disease control (%)	±SE
Plant + pathogen (+ve control)	53.20 b	0.81	26.81 a	0.63	-	-	100.00 a	0.89	61.66 b	0.38	-	-
Plants sprayed with ANLE +pathogen	42.54 c	0.28	16.75 b	0.09	10.66 b	0.06	79.20 c	0.81	26.19 c	0.28	20.80 a	0.63
Plants sprayed and irrigated with ANLE + pathogen	39.49 a	0.92	11.45 c	0.05	13.71 a	0.12	71.67 b	0.85	17.95 a	0.67	28.33 b	0.44

N.B. Values are means of 5 replicates of each treatment from three independent experiments. Values with the same letter within a column are not significantly (P>0.05) different according to Fisher's protected LSD test.

Effect of ANLE on Fusarium wilt

Table 2 summarizes the effect of ANLE on seed germination, disease incidence and the percentage of control of the wilt. When soil infested with *F. oxysporum* was irrigated with ANLE, there was a 100% germination of tomato seeds and the incidence of *Fusarium* wilt was 19%. Irrigation with the same concentration of ANLE gave the highest percentage (81%) of control in tomato wilt, compared with only 5.7% for the +ve control.

Effect of ANLE on growth parameters of tomato

In the case of early blight, ANLE significantly improved plant growth parameters after 4, 6 and 8 weeks (Table 3). However, this improvement was more pronounced in tomato plants grown in the presence of *A. solani* and sprayed as well as irrigated with ANLE, especially after 8 weeks of sowing. Moreover, uninfected tomato plants that received ANLE had the best growth parameters. In the case of *Fusarium* wilt, ANLE also produced significant gradual increase in tomato growth parameters with the treatments run for the same periods (Table 4). The greatest improvement in *F. oxysporum*-infested soil occurred when tomato seeds were first soaked for 3 hours in water and then were irrigated with ANLE. As with early blight, the growth parameters were optimum after 8 weeks of sowing and especially in those plants grown in pathogen-free soil and irrigated with ANLE.

Discussion

Aqueous extracts from the leaves of the neem tree were studied *in vivo* for their effectiveness against *A. solani* and *F. oxysporum*. The extracts substantially reduced the incidence of both *Alternaria* and *Fusarium* and the growth of these pathogens. In this study, four concentrations of ANLEs effectively suppressed mycelial growth of the two pathogens and the 20% concentration highly suppressed growth of *F. oxysporum*. This is consistent with Dwivedi and Shukla (2000) who found that the inhibition of mycelial growth increased with plant extract concentration, and that ANLE at a concentration of 100% completely inhibited spore germination of different *Fusarium* spp. Treating the plants or seeds, or irrigating the soil with a given concentration of ANLE slightly decreased the incidence and severity of both early blight and *Fusarium* wilt of tomato, whereas spraying or soaking, followed by irrigation with the same concentration, was more effective against *A. solani* and *F. oxysporum* respectively. These results were in agreement with Chattopadhyay (1999), who found that foliar spraying of *A. indica* leaf extract and azadirachtin, reduced mycelial growth of *A. alternata*, a fungus causing loss of sunflower and tomato, decreased the severity of disease caused by this fungus and increased the yield as compared with the control. Babu *et al.,* (2000) reported that spraying tomato pot cultures with 3% neem oil reduced disease incidence by

Table 2. Effect of ANLE on percentage of seed germination, disease incidence and on control of tomato *Fusarium* wilt [a].

Treatment	Seed germination (%)		Disease incidence (%)		Disease control (%)
	Mean ± SE	%	Mean ±SE	%	%
Seeds + H_2O (-ve C)	10.0 c 0.85	100	0 0	0	0
Seeds + ANLE	10.0 c 0.62	100	0 0	0	0
Seeds + pathogen + H_2O (+ve C)	7.0 a 0.08	70	6.60 a 0.09	94.29	5.71
Seeds + pathogen + ANLE	8.4 e 0.19	84	1.60 b 0.01	19.04	80.96
Seeds soaked in ANLE + pathogen + H_2O	8.2 a 0.15	82	2.60 b 0.03	31.70	68.29

[a] Values are means of 5 replicates for each treatment from three independent experiments. Values with the same letter within a column are not significantly (P>0.05) different according to Fisher's protected LSD Test.

Table 3. Effect of ANLEs on some growth parameters of tomato 4, 6 and 8 weeks after sowing (early blight disease)[a].

Parameter	Treatment[b]	Time after sowing					
		4 weeks		6 weeks		8 weeks	
		Mean	SE	Mean	SE	Mean	SE
Shoot length	1	15.50[d]	0.09	20.86[d]	0.98	27.60[d]	0.62
	2	21.11[a]	0.26	35.42[a]	0.92	51.68[a]	0.81
	3	13.70[e]	0.08	18.68[e]	0.28	23.81[e]	0.67
	4	16.52[c]	0.18	23.25[c]	0.52	35.57[c]	0.10
	5	18.02[b]	0.17	29.82[b]	0.44	45.32[b]	0.28
Root length	1	2.68[d]	0.88	10.50[d]	0.82	15.80[d]	0.09
	2	5.20[a]	0.17	13.86[a]	0.77	21.20[a]	0.18
	3	2.51[d]	0.87	9.40[e]	0.85	13.86[e]	0.75
	4	3.64[c]	0.70	11.31[c]	0.89	16.60[c]	0.26
	5	4.43[b]	0.15	12.30[b]	0.28	19.32[b]	0.02
Number of leaves	1	4.00[d]	0.72	7.23[d]	0.34	9.70[d]	0.08
	2	6.50[a]	0.81	12.20[a]	0.25	14.50[a]	0.23
	3	3.00[e]	0.91	7.30[d]	0.36	9.60[d]	0.29
	4	4.50[c]	0.40	8.32[c]	0.23	10.53[c]	0.08
	5	5.80[b]	0.62	10.61[b]	0.18	13.22[b]	0.12
Shoot fresh weight	1	3.82[b c]	0.67	6.12[d]	0.14	21.82[d]	0.13
	2	5.90[a]	0.88	15.40[a]	0.22	36.44[a]	0.27
	3	3.21[c]	0.72	5.51[e]	0.80	13.60[e]	0.62
	4	4.11[bc]	0.52	7.78[c]	0.32	26.81[c]	0.85
	5	4.61[b]	0.61	13.12[b]	0.48	31.21[b]	0.77
Shoot dry weight	1	0.56[c]	0.01	0.88[d]	0.12	3.30[d]	0.27
	2	0.95[a]	0.02	2.31[a]	0.06	5.50[a]	0.21
	3	0.43[d]	0.87	0.68[e]	0.08	2.08[e]	0.31
	4	0.58[c]	0.09	1.13[c]	0.07	4.23[c]	0.12
	5	0.64[b]	0.06	1.43[b]	0.08	5.32[b]	0.31
Root fresh weight	1	0.50[d]	0.21	1.08[d]	0.91	2.28c	0.31
	2	1.55[a]	0.11	2.05[a]	0.87	5.18[a]	0.81
	3	0.34[c]	0.21	0.42[e]	0.32	0.63[d]	0.36
	4	0.64[c]	0.86	1.25[c]	0.77	2.68[c]	0.87
	5	0.88[b]	0.08	1.43[b]	0.32	3.52[b]	0.21
Root dry weight	1	0.073[d]	0.87	0.183[d]	0.01	0.352[d]	0.08
	2	0.277[a]	0.65	0.377[a]	0.02	0.804[a]	0.09
	3	0.051[e]	0.78	0.062[e]	0.00	0.087[e]	0.52
	4	0.105[c]	0.79	0.175[c]	0.18	0.421[c]	0.32
	5	0.127[b]	0.88	0.215[b]	0.02	0.552[b]	0.38

[a] Values are means of 5 replicates of each treatment from three independent experiments (4 plants pot^{-1}). Values with the same letter within a column are not significantly ($P>0.05$) different according to Fisher's protected LSD Test.

[b] 1, Uninfected plants (-ve control); 2, Plants sprayed with ANLEs; 3, Plants + pathogen (+ve control); 4, Plants+ pathogen (sprayed with ANLEs); 5, Plants +pathogen (sprayed and irrigated with ANLEs).

Table 4. Effect of ANLEs on some growth parameters of tomato plants 4, 6 and 8 weeks after sowing (wilt disease).

Parameter	Treatment [b]	Time after sowing					
		4 weeks		6 weeks		8 weeks	
		Mean	SE	Mean	SE	Mean	SE
Shoot length	1	15.16 [bc]	0.08	21.26 [d]	1.40	27.80 [d]	0.78
	2	21.12 [a]	0.93	37.38 [a]	0.23	52.18 [a]	0.62
	3	12.52 [c]	0.09	16.74 [e]	1.12	23.58 [e]	0.23
	4	18.04 [b]	0.08	30.58 [b]	1.37	45.66 [c]	1.75
	5	16.22 [b]	0.12	23.36 [c]	1.86	36.56 [c]	0.65
Root length	1	2.68 [d]	0.21	10.30 [d]	0.10	16.10 [d]	0.17
	2	5.10 [a]	0.11	14.84 [a]	0.24	21.52 [a]	0.19
	3	2.42 [d]	0.17	9.40 [e]	0.04	14.30 [e]	0.13
	4	4.44 [b]	0.13	12.32 [b]	0.12	19.60 [b]	0.09
	5	3.84 [c]	0.10	11.50 [c]	0.10	17.28 [c]	0.12
Number of leaves	1	4.00 [c]	0.32	7.20 [b c]	0.37	9.80 [d]	0.37
	2	6.20 [a]	0.37	11.60 [a]	0.51	13.40 [a]	0.40
	3	3.00 [c]	0.32	6.80[c]	0.37	9.40 [c]	0.24
	4	5.40 [a]	0.40	10.40 [a]	0.51	12.00 [b]	0.55
	5	4.20 [b c]	0.20	8.20 [b]	0.37	10.40 [c]	0.24
Shoot fresh weight	1	3.55 [c]	0.05	6.18[d]	0.09	22.42 [d]	0.24
	2	5.91 [a]	0.01	15.52 [a]	0.13	36.54 [a]	0.60
	3	3.06 [d]	0.15	4.28[e]	0.12	13.50 [e]	0.49
	4	4.24 [b]	0.09	12.50 [b]	0.13	30.92 [b]	0.42
	5	3.82 [c]	0.13	8.42 [c]	0.15	26.78 [c]	0.46
Shoot dry weight	1	0.540 [d]	0.006	0.948 [d]	0.005	3.500 [d]	0.031
	2	0.924[a]	0.005	2.412[a]	0.004	5.700[a]	0.031
	3	0.464 [e]	0.004	0.652[e]	0.002	2.060[e]	0.025
	4	0.652 [b]	0.004	1.944 [b]	0.004	4.790 [b]	0.023
	5	0.572 [c]	0.004	1.346 [c]	0.002	4.174 [c]	0.004
Root fresh weight	1	0.504 [d]	0.103	1.062[d]	0.048	2.480 [c]	0.156
	2	1.454[a]	0.019	2.070[a]	0.023	5.396[a]	0.204
	3	0.277 [e]	0.03	0.428[e]	0.01	0.660[d]	0.049
	4	0.900 [b]	0.014	1.436 [b]	0.06	3.724 [b]	0.134
	5	0.730 [c]	0.011	1.260 [c]	0.04	2.880 [c]	0.156
Root dry weight	1	0.074 [d]	0.001	0.158 [d]	0.775	0.371 [d]	0.124
	2	0.218 [a]	0.099	0.311 [a]	0.741	0.809 [a]	0.162
	3	0.042 [e]	0.449	0.064 [e]	0.717	0.098 [e]	0.348
	4	0.135 [b]	0.449	0.214 [b]	0.071	0.557 [b]	0.745
	5	0.109 [c]	0.001	0.188 [c]	0.449	0.433 [c]	0.002

[a] See Table 3.
[b] 1, Seeds + water (-ve control); 2, seeds + ANLEs; 3, seeds + pathogen+ water (+ve control); 4, seeds + pathogen+ ANLEs; 5, seeds soaked in ANLEs + pathogen+ water.

53% over the control, while Patil *et al.* (2001) found that the incidence of Alternaria early blight of tomato was reduced by neem seed extract that also increased fruit yield from 15,643 to 16,856 kg ha^{-1}. Similar results were also obtained by Amadioha and Uchendu (2003) who concluded that extracts from different parts of the neem tree, especially the bark, could be used by farmers to control the rot of tomato fruits caused by *F. solani* during storage. *In vitro* tests carried out by Chaudhary *et al.,* (2003) using different plant extracts, including those from *A. indica*, against *A. alternata* causing early blight of potato, revealed that extracts of *A. indica* gave the second highest inhibition of *A. alternata* (54%). Sanjeet *et al.* (2005) found that *A. indica* extracts provided good control of leaf spot of faba beans caused by *A. alternata* under both laboratory and field conditions. The marked reduction in the severity of Alternaria early blight and wilt when tomato plants were treated with ANLE may be related to the toxic antifungal substances the extracts contained and/or to the change of soil pH due to the alkaline nature of the neem leaves (National Research Council, 1992).

Aqueous neem leaf extracts significantly increased shoot and root length, the number of leaves and also the shoot and root fresh and dry weights of tomato plants. The highest growth rates occurred in the presence of *A. solani* on tomato plants that received ANLE by spraying and irrigation, followed by plants that were only sprayed with ANLE, whereas the lowest growth rates were recorded for the untreated control (+ve). Similar growth parameters were recorded when tomato seeds were irrigated with ANLE in the presence of *F. oxysporum*. These results confirm Vats and Nandal (1993) who found that the growth parameters of tomato plants were greatly improved when seedlings were dipped for 2–4 hours in neem leaf extract, and with Walia *et al*. (1994) who recorded a significant increase in the growth of tomato plants in soil amended with neem leaves, as compared with the control in unamended soil.

The improvement in the growth parameters of tomato plants following treatment with ANLE was explained by Avenimelech (1986) as due to changes in the physical, chemical and biological characteristics of the soil, which in turn increased plant growth and improved productivity. Taheruzzaman and Kushari, 1995 attributed these changes to enrichment of the soil with nutrients such as N, P and K and other minerals, while Kumar *et al.,* 1998 related the improvement in growth to an increase in the organic carbon content. Other investigators have reported that ANLE protects plants from pests and/or increase the population of soil micro-organisms that promote growth (Krishnan *et al.,* 1995) or favour antibiotic producing micro-organisms such as actinomycetes and biological control agents (Dohroo and Gupta, 1995).

In conclusion, the study confirms that natural extracts from leaves of neem trees which are widely cultivated in Egypt are very effective against two common fungal pathogens that affect some of the most important vegetable crops in Egypt. This study found a positive effect on both the incidence and the severity of the two diseases studied, in addition to an increase in the growth parameters of the tomato plants. Further field investigations should now be undertaken into the possible application in agriculture of either natural extracts from plants such as neem trees or the biologically active constituents of these extracts, which may enhance plant growth and increase yield.

Literature cited

Aboellil A.H., 2007. Trilogy, a product of neem (*Azadirachta indica*) induces resistance in cucumber against *Podoshaera xanthii*. *Research Journal of Microbiology* 2, 402–414.

Achimu P. and A. Schlösser, 1992. Effect of neem seed extracts (*Azadirachta indica* A. Juss) against downy mildew (*Plasmopara viticola*) of grape vine. *Medical Faculty Landbouww University Gent* 57, 423–431.

Akköprü A. and S. Demir, 2005. Biological control of *Fusarium* wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF *Glomus interadices* and some rhizobacteria. *Journal of Phytopatholgy* 153, 544–550.

Amadioha A.C. and P.N. Uchendu, 2003. Post harvest control of tomato fruit rot caused by *Fusarium solani* with extracts of *A. indica*. *Discovery and Innovation*. 15(1–2), 83–86.

Avenimelech Y., 1986. Organic residues in modern agriculture. In: *The Role of Organic Matter in Modern Agriculture* (Y. Chen, Y. Avenimelech, ed.), *Dordecht Martinus Nijhoff Publishers*, Dordrecht, Netherlands, 10 pp.

Babu S., K. Seetharaman, R. Nandakumar and I. Johnson, 2000. Effect of selected plant extracts/oils against tomato leaf blight. *International Journal of Tropical Agriculture* 18(2), 153–157.

Chattopadhyay C., 1999. Yield loss attributable to

Alternaria blight of sunflower (*Helianthus annuus* L.) in India and some potentially effective control measures. *International Journal of Pest Management* 45(1), 15–21.

Chaudhary R.F., R.L. Patel, S.M. Chaudhari, S.K. Pandey and S. Brajesh, 2003. *In vitro* evaluation of different plant extracts against *Alternaria alternata* causing early blight of potato. *Journal of the Indian Potato Association* 30, 141–142.

Datar V.V and C.D. Mayee, 1985. Chemical management of early blight of tomato. *Journal of Maharashtra Agriculture University* 3, 278–280.

Dohroo N.P. and S.K. Gupta, 1995. Neem in plant disease control. *Agriculture Review Karnal* 16, 133–140.

Dwivedi B.P. and D.N. Shukla, 2000. Effect of leaf extracts of some medicinal plants on spore germination of some *Fusarium* species. *Karnataka Journal of Agricultural Sciences* 13(1), 153–154.

Ganapathy T. and P. Narayanasamy, 1990. Effect of plant extracts on the incidence of major diseases of groundnut. *International Arachis Newsletter* 7, 20–21.

Hilaal M.R., 1992. *Epidemiological Study on Early Blight of Tomatoes in Relation to Fungicidal Resistance*. M.Sc. Thesis, Faculty of Agriculture, Ain Shams University, Egypt, 167 pp.

Horsfall J.G. and R.W. Barratt, 1945. An improved grading system for measuring plant disease. *Phytopathology* 55, 655 (abstract).

Hosna K., S.K. Khoda and I.H. Mian, 2003. Foliar spray of fungicides and botanicals to control *Alternaria* blight of cauliflower seed crop. *Bangladesh Journal of Plant Pathology* 19, 63–67.

Krishnan N., D. Ravi and M.K. Gajapathy, 1995. Effect of neem litter addition in free nitrogen fixers and fungi in soil and on plant (fenugreek) growth. *Advances in Plant Science* 8, 65–70.

Kumar A., M.S. Hooda Raj-Bahadur, A. Kumar and R. Bahadur, 1998. Impact of multipose tree on productivity of barley in arid ecosystems. *Annals of Arid Zone* 37, 153–157.

Maiero M.T. and T.H. Barksdale, 1989. Combining ability estimates for early blight resistance in tomato. *Journal of the American Society of Horticultural Science* 114, 118–121.

Manickam K. and K. Rajappan, 2001. Field efficacy of plant extracts and chemicals against greengram leaf curl disease. *Indian Journal of Virology* 15, 35–37.

National Research Council, 1992. *Neem; a tree for solving global problems. Report of an Ad Hoc Panel of the Board of Science and Technology for International Development*. National Academy Press, Washington, Washington DC, USA, 152 pp.

Nwachukwu E.O. and C.I. Umechuruba, 2001. Antifungal activities of some leaf extracts on seed-borne fungi of African yam bean seeds, seed germination and seedling emergence. *Journal of Applied Science and Environmental Management* 5, 29–32.

Patil M.J., S.P. Ukey and B.T. Raut, 2001. Evaluation of fungicides and botanicals for the management of early blight (*Alternaria solani*) of tomato. *PKV-Research Journal* 25, 49–51.

Ramadan M.A., A.M. El-Bassiony and A.M. Hoda, 2008. Behaviour of some micronutrients in soil and tomato plant organs under different levels and types of fertilizers. *Australian Journal of Basic and Applied Sciences* 2, 288–295.

Sanjeet K., J.P. Upadhyay and K. Sanjeev, 2005. Evaluation of plant extracts for control of *Alternaria* leaf spot of *Vicia faba*. *Annals of Plant Protection Sciences* 13(1), 258–259

SAS, 1988. *SAS/STAT User's Guide*. Release 6.03 Edition, SAS Institute. Cary, NC, USA, 1028 pp.

Shetty S.A., H.S. Prakash and H.S. Shetty, 1989. Efficacy of certain plant extracts against seed-born infection of *Triconiella padwickii* in paddy (*Oryza sativa*). *Canadian Journal of Botany* 67, 1956–1958.

Siddiqui Z.A., 2006. A proteomics perspective on bicontrol and plant defense mechanism. In: *PGPR Biocontrol and Biofertilization*. *Springer Publisher, Dordrecht, Netherlands*, 233–255.

Singh U.P., H.B. Singh and R.B. Singh, 1980. The fungicidal effect of neem (*Azadirachta indica*) extracts on some soil-borne pathogens of gram (*Cicer arietinum*). *Mycologia* 72, 1077–1093.

Spletzer M.E. and A.J. Enyedi, 1999. Salicylic acid induces resistance to *Alternaria solani* in hydroponically grown tomato. *Phytopathology* 89, 722–727.

Surender S. and C. Hari, 2004. Effect of extracts of some medicinal plants on spore germination of chickpea wilt pathogen (*Fusarium oxysporum* f. sp. *ciceri* [Pad.] Snyd. and Hans.). *Indian Journal of Plant Protection* 32, 162–163.

Taheruzzaman Q. and D.P. Kushari, 1995. Effect of leaf-leachate enriched water of neem (*Azadirachta indica*) on the growth of *Azolla pinnata*. *Environment and Ecology* 13, 589–594.

Vats R. and S.N. Nandal, 1993. Optimization of dipping periods of tomato seedlings in plant extracts for the management of root-knot nematode, *Meloidogyne javanica*. *Current Nematology* 4, 161–164.

Walia K.K., N. Mehata and D.C. Gupta, 1994. Effect of Green manuring on *Rhizoctonia* and root -knot nematode complex on tomato. *Nematologia Mediterranea* 22, 131–133.

Wong D.H., M.J. Barbetti and K. Sivasithamparam, 1984. Effects of soil temperatures and moisture on the pathogenicity of fungi associated with root rot of subterranean clover. *Australian Journal of Agriculture Research* 35, 675–684.

Accepted for publication: December 17, 2009