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Effects of Two Plant Extracts on the Damage of *Meloidogyne incognita* in Tomato Plants

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Abstract
The effects of the aqueous extracts of *Euphorbia myrsinites* (Euphorbiaceae) and *Urginea maritima* (Liliaceae) on the damage of *Meloidogyne incognita* (Nematoda: Heteroderidae) to tomato plants was evaluated. The trials were conducted in two series in climatized rooms and in greenhouse conditions. The results of both the climatized rooms and greenhouse trials are parallel each other. All dilutions (1:100, 2:100 and 4:100 v v^{-1}) of the extracts applied to the soil reduced the root galling caused by *M. incognita* and enhanced tomato yield compared to an untreated control. Efficacy of the highest concentration (4:100 v v^{-1}) of both extracts on the control of *M. incognita* was similar to the positive chemical control (Oxamyl). The highest concentration of *U. maritima* was superior to the extracts of *E. myrsinites*. Aqueous extracts from these two plants reduced the damage of root-knot nematodes.
Keywords: *Euphorbia myrsinites*, *Meloidogyne incognita*, plant extract, tomato, *Urginea maritima*.

İki Bitki Ekstraktının Domates Bitkilerinde *Meloidogyne incognita*, Chitwood (Nematoda: Heteroderidae) Zararına Etkileri

Özet
Euphorbia myrsinites (Euphorbiaceae) ve *Urginea maritima* (Liliaceae)'nin su ekstraktlarının domates bitkilerinde *Meloidogyne incognita* (Nematoda: Heteroderidae) zararını kontrol altına alma etkileri araştırılmıştır. Denemeler, iklim odalarında ve sera koşullarında olmak üzere iki aşamada gerçekleştirilmiştir. Hem laboratuar hem de sera denemelerinin sonuçları birbiriyle paralellik göstermektedir. Uygulama yapılmayan pozitif kontrol ile kıyaslandığında, toprağa uygulanan ekstraktların tüm konsantrasyonları (1:100, 2:100 ve 4:100 hacim $hacim^{-1}$), köklerde *M. incognita*'nın neden olduğu urlanmayı azaltmış ve serada domates verimini arttırmıştır. Her iki ekstraktın en yüksek konsantrasyonlarının (4:100 hacim $hacim^{-1}$) *M. incognita*'yı kontrol etme etkililiği, kimyasal pozitif kontrole (Oxamyl) yakın değerlere sahip olmuştur. *U. maritima*'nın en yüksek konsantrasyonu *E. myrsinites* ekstraktlarından daha üstün bulunmuştur. Bu iki bitkinin su ekstraktları kök-ur nematodlarının zararını azaltmışlardır.
Anahtar Kelimeler: Bitki ekstraktı, domates, *Euphorbia myrsinites*, *Meloidogyne incognita*, *Urginea maritima*.

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INTRODUCTION

Plant-parasitic nematodes decrease agricultural production by approximately 11% worldwide (Agrios 2005), reducing production by millions of tones every year. Among the plant-parasitic nematodes, root-knot nematodes (*Meloidogyne* spp. (Nematoda: Heteroderidae)) cause crop losses of around 15% alone in tropical countries (Sasser 1979). Moreover yield losses of 50-80% caused by these nematodes in vegetable crops are common (Siddiqi 2000). Root-knot nematodes have great importance both in their harm to crops and in the difficulties of their control in terms of diseases and pests seen in greenhouse-grown tomatoes in Turkey. The controls of these pests have little chance in success and are uneconomical because these nematodes live in the soil and feed on the internal plant tissue. In addition high dosages and repeated uses of nematicides bring about risks as environmental pollution, pesticide residues, resistance to them and health problems in human beings.

In recent years, there has been increased interest in natural plant products in order to develop new

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insecticides (Addor 1995). In addition, research in this area has accelerated due to the negative effects of synthetic pesticides (Ujvary 2002). The discovery of active compounds which are more selective and less persistent would be beneficial both for the environment and agricultural product consumers although they can not automatically be assumed to be of no risks (Pascual and Robledo 1999). Two plants that might contain such valuable plant products are *Euphorbia myrsinites* (Euphorbiaceae) (Myrtle spurge) and *Urginea maritima* (Liliaceae) (Red squill) (Oksuz et al. 1995, Pascual and Fernandez 1999).

E. myrsinites (Myrtle Spurge, also known as Creeping Spurge or "donkey tail"), is a succulent species of spurge, native to southeastern Europe and Asia Minor from Italy east through the Balkans to the Crimea and Turkey. It is a herbaceous perennial plant with sprawling stems growing from 20-40 cm in length. The leaves are spirally arranged, fleshy, pale glaucous bluish-green, 1-2 cm long. The flowers are inconspicuous, but surrounded by bright sulphur-yellow bracts (tinged red in the cultivar 'Washfield') and are produced in the spring. Although sometimes grown as a decorative plant in gardens, particularly valued in xeriscaping in dry areas, myrtle spurge is often considered noxious, and is invasive in some regions (Davis 1984). Leaf extracts of *Euphorbia hirta* influenced the emergence of larvae from their cysts and caused toxicity to the second-stage larvae of the cyst nematodes (*Heterodera avenae* and *Heterodera cajani*) (Bhatti et al. 1997).

The plant *U. maritima* has the common names red squill, sea squill, sea onion, ein sit, and ada sogani. The bulb can get quite large, reaching over five pounds in weight. This squill is native to the sandy coastal regions of the Mediterranean and is widely cultivated. In Turkey, this plant grows naturally and widely in the Western Anatolia and Mediterranean Regions. The plant has been used as a rodenticide and may show promise as an insecticide. The most active compounds in the plant are scillirosides, especially proscillaridine A. In Israel the sea squill has gained an almost iconic status, and is popularly known as the "harbinger of autumn", due to the fact that the flowers pop out all over the country at the end of the dry summer, some time before the first rain (Davis 1984). The plant extracts of *U. maritima* were topically applied to the 25-day old larvae of *Tribolium castaneum* (Coleoptera: Tenebrionidae), with a resultant larval mortality of 60-100 % (after 24 h) at doses over 10 g insect^{-1} (Pascual and Fernandez 1999). Also it was reported that the bulbs of *U. maritima* showed contact toxicity in the pupae of *T. castaneum*, resulting in 100% mortality (Pascual and Robledo 1999).

Some growers in Ortaca (Mugla-Turkey) put *E. myrsinites* and *U. maritima* into their irrigation tanks in the greenhouses, thus creating a crude extract, whereas other growers boil the plants in large cauldrons then add the solution to the irrigation tanks. They use these solutions against the root-knot nematodes and some insect pests, with this observation, this study was planned. The objective of this study was to investigate the effectiveness of the aqueous extracts of *E. myrsinites* and *U. maritima* in reducing the damage of *Meloidogyne incognita*.

MATERIAL AND METHODS

Source Plants and Preparation of Their Extracts

Plants in the blooming phase were collected from the wild areas in the Ortaca and Dalyan (Mugla-Turkey) districts based on the seasons and our requirements. Only the white bulbs of *U. maritima* were collected from October to January and whole plants of *E. myrsinites* were collected from January to April and all plant parts to be extracted were taken to the laboratory where Voucher specimens of each plant were stored in Mugla University, Faculty of Technical Education, Laboratory of Extraction, Mugla, Turkey.

Plants were washed thoroughly to remove any soil or debris. Those not immediately used were dried and stored in paper bags at 8°C until needed. The bulbs of *U. maritima* were initially cut into pieces and then placed in a blender to slice them into tiny pieces. All sections (flowers, leaves, stems and roots) of *E. myrsinites* were homogeneously processed into smaller granules. Plant samples of 2 g were extracted for 4 h in 100 mL of water using a soxhlet hot water extractor (PILZ, Heraus-Witmann, Heidelberg, Germany). The final volume of this extract was 100 mL and the extract wasn't concentrated further. Extracts were sealed and maintained in a bottle at 4°C until use. Fresh extracts were prepared weekly.

Laboratory Trials

Laboratory trials were carried out between April 2005 and August 2006 under the conditions of

24±2°C, 65±5% RH and a photoperiod of 12:12 (L:D) h in climatized rooms (Ege University, Faculty of Agriculture, Department of Plant Protection, Izmir, Turkey). Trials with 9 treatments, each replicated 10 times, were arranged in a randomized parcel design and repeated two times. Laboratory trials were 4-months in duration.

Tomato seeds cv. Astona F1 (Nunhems®) sown in vials of sterilized torf were grown until four true leaves appeared then transplanted into plastic pots. The mixed soil and pots (18cm by 22cm) had been sterilized using 10% formaldehyde. The sandy soil was blended with sand and dried goat-sheep manure in equal proportions and 1100 g of the mix placed into each pot.

The second-stage juveniles (J2) and eggs of M. incognita used in the trials were obtained from tomato roots with galls from the experimental greenhouse in Ortaca (Mugla-Turkey). Eggs of *M. incognita* were obtained by the blender-sieve method in order to infect the tomato seedlings with root-knot nematodes. Nematode-inoculated tomato seedlings were inoculated with 20 000 eggs of *M. incognita* per plant during transplanting.

Concentrated extracts of *E. myrsinites* and *U. maritima* were diluted to 1:100; 2:100; 4:100 (vol vol^{-1}) in distilled water immediately before used. When the seedlings were being transplanted, ten plants were treated with each concentration of both extracts by applying 100 mL of diluted extract to each pot. Treatment was repeated once a week for 14 weeks. There was a total 15 applications including the transplanting application, and the final volume of extract applied was 1500 mL. Additional treatments included, Oxamyl a.i. (Vydate® L, DuPont, applied at the recommended rate of 30 L ha^{-1} or 1 mL $plant^{-1}$) by soil application one time during transplanting as a positive chemical control for comparison with *M. incognita* inoculated plants untreated, and uninoculated plants untreated.

The effect of treatments on the damage of *M. incognita* was assessed using a 0-5 gall mass index (Sasser et al. 1984) where 0 represented a root with no galls and 5 as a maximal degree of galling (root functioning is loose and decaying) at the end of trial. The plants were uprooted without bruising the roots, especially the capillary ones. The soil was then removed from the exposed roots, which were then washed carefully and rated. Also, the roots were weighed and then dried in an oven at 70°C for two days, and then reweighed.

Greenhouse trials

Greenhouse trials were carried out in a commercial (Şükrü KILIÇ's) greenhouse located in the Ortaca (Mugla-Turkey) district. The greenhouse (2,500 m^2) was made of an iron framework covered with a solid polyethylene. Trials were arranged in randomized block design with 8 treatments replicated 5 times in the 2006 and 2007 winter growing season. Tomato seedlings cv. Astona F1 (Nunhems®) were planted on 06 October 2005 and 12 October 2006. They were watered and fertilized according to local grower practices using a drip irrigation system.

At the beginning of the greenhouse trials to determine the numbers of *M. incognita* in 100 g of soil, the J2 were extracted by using the "Improved Baermann-Funnel Methods" (Hooper 1986) and then counted under a microscope. There were 600 J2 100g of $soil^{-1}$ as an initial population.

Within the greenhouse, 30 m^2 plots (42-44 plants) were randomly assigned to be treated manually with one of the plant extracts starting from transplanting and repeating once a week for 15 weeks or the Oxamyl a.i. (Vydate® L, DuPont, applied at the recommended rate of 30 L ha^{-1} or 1 mL $plant^{-1}$) only during transplanting. An untreated control was also included. There was a nontreated buffer zone of 1 m between each. The concentrated plant extracts were diluted to 1:100; 2:100; 4:100 (vol vol^{-1}) in distilled water just before use. Fifty mL of the appropriate plant extract dilution was applied once a week for 7 weeks to the base of each plant in soil. When the plant reached 1 m in height, 100 mL was applied for 5 weeks. From a height of 2 m, 200 mL of extract was applied for 3 weeks. A total of 16 applications including the transplanting application, were applied, with the final volume of extract applied as 1500 mL. All tomatoes were harvested and weighed once a week from the middle of January to the end of the harvest each year. The sum total of the values of weekly weighed tomatoes was calculated as total yield and the yields were given as ton ha^{-1} for each year separately. The trials were terminated after 32-33 weeks when the plants stopped producing fruits.

Ten plants in each plot were randomly chosen and uprooted at the end of the growing season. Then, the effects of treatments on the damage levels of *M. incognita* were studied using the galling index scale of 0 to 5 (Sasser et al. 1984).

Statistical Analyses

SPSS 12.0 software (SPSS Inc., Chicago, IL.) was used for the analyses of variance (Anonymous 2003). Root galling index and tomato yields were analyzed following standard procedures for analysis of variance (ANOVA). Comparison of means was made according to Duncan's Multiple Range Test at P<0.05 levels.

RESULTS

Laboratory Trials

In the 2005 trial, a significant amount of root galling was determined in all of the treatments including Oxamyl compared to the 2006 trials. The reduced efficacy of Oxamyl and other plant extracts was attributed to the fact that the root-knot nematode eggs and juveniles used in the 2005 trial were more infective than those of 2006. The highest concentrations of each plant extract applied as a soil drench was statistically similar to the Oxamyl treatment, in reducing the root galling in the tomatoes (P<0.05) (Table 1). The least damage in the roots was evaluated from the treatments of Oxamyl and the highest concentration (4:100) of *U. maritima* at the same significance level, followed by *E. myrsinites* at 4:100 (P<0.05). Root galling indices of these two treatments were less than all the other plant extract treatments. However, the highest root galling indices were found in the untreated positive control pots and differences between the untreated control plots and other treatments were significant (P<0.05).

In the 2006 trial, the least damage in the roots was evaluated from the Oxamyl treatment, followed by the highest concentration (4:100) of *E. myrsinites* and which placed them, statistically, in the same group (P<0.05) (Table 1). Also in all treatments of the 2006 trials had lower root galling than those of the 2005 trial. Root galling indices of the highest concentration (4:100) of *E. myrsinites* were less than all of the other plant extract treatments. However, the highest root galling indices were found in the untreated positive control pots and differences between untreated control plots and other treatments were significant (P<0.05).

The results from the fresh and dried weights of the roots of plants are not presented here, since there is no statistical significance among all the treatments (P<0.05).

Greenhouse Trials

Table 1. The effect of the treatments on the root galling of tomato plants caused by root-knot nematodes (*Meloidogyne incognita*) in the years 2005 and 2006 in climatized rooms.

Treatments	Galling index (0-5)*			
	2005		2006	
1. Control (+)	4.00±0.00**	a***	3.11±0.26	a
2. *Euphorbia myrsinites* 1:100	3.00±0.37	bc	1.56±0.24	b
3. *Euphorbia myrsinites* 2:100	3.50±0.22	ab	1.11±0.26	bc
4. *Euphorbia myrsinites* 4:100	2.83±0.40	bc	0.78±0.28	c
5. *Urginea maritima* 1:100	4.00±0.00	a	1.67±0.24	b
6. *Urginea maritima* 2:100	3.67±0.21	ab	1.11±0.35	bc
7. *Urginea maritima* 4:100	2.33±0.33	c	1.67±0.17	b
8. Oxamyl	2.33±0.42	c	0.44±0.18	c
9. Control (-)	0.00±0.00		0.00±0.00	

* Data are mean values of ten replicates.
** Values are given with ±SE.
*** Values in the same column with different letters show significant differences at P<0.05 according to Duncan's Multiple Range test. The data of "Control (-)" didn't included in mean separation.

Effects of Treatments on Root Galling

In the 2006 trial, the highest concentration (4:100) of *U. maritima* plant extract applied as a soil application was statistically similar to the Oxamyl treatment, reducing dramatically root galling in the tomato as in the laboratory pot trials (P<0.05) (Table 1 and 2). The least damage in the roots was found from the treatments of Oxamyl and the highest concentration (4:100) of *U. maritima* were at the same significance level, followed by *E. myrsinites* at 4:100 (P<0.05). The root galling indices of the two highest concentrations of the plant extracts were less than those of all other extract treatments. However, the highest root galling indices were found in the untreated positive control plots, with significant differences between the untreated control plots and the other treatments (P<0.05).

In the 2007 trial, the two highest concentrations of the plant extracts applied as a soil application were statistically similar to the Oxamyl treatment, reducing dramatically the root galling in the tomato as in the laboratory pot trials (P<0.05) (Table 1 and 2). The least harm in the roots was observed from the Oxamyl treatments, followed by the highest concentrations (4:100) of *U. maritima* and *E. myrsinites* at the same amount and significance level (P<0.05). Root galling indices of the two highest concentrations of the plant extracts were less than those of all other extract treatments as in the 2006 trial. However, the highest root galling indices were found in the untreated positive control plots, with significant differences between untreated control plots and other treatments (P<0.05).

Yield Assessment

Table 2. The effect of the treatments on the root galling of tomato plants caused by root-knot nematodes (*Meloidogyne incognita*) in the years 2006 and 2007 in Muğla, Turkey.

Treatments	Galling index(0-5)*			
	2006		2007	
1. Control	3.10±0.47**	a***	2.00±0.35	a
2. *Euphorbia myrsinites* 1:100	0.68±0.11	b	0.56±0.09	b
3. *Euphorbia myrsinites* 2:100	0.60±0.09	bc	0.28±0.05	cd
4. *Euphorbia myrsinites* 4:100	0.32±0.06	cd	0.16±0.04	de
5. *Urginea maritima* 1:100	0.60±0.09	bc	0.64±0.10	b
6. *Urginea maritima* 2:100	0.56±0.09	bc	0.48±0.08	bc
7. *Urginea maritima* 4:100	0.24±0.04	d	0.16±0.04	de
8. Oxamyl	0.20±0.04	d	0.00±0.00	e

* Data are mean values of five replicates at different four blocks.
** Values are given with ±SE.
*** Values in the same column with different letters show significant differences at P<0.05 according to Duncan's Multiple Range test.

Table 3. The effects of the treatments on the yield assessment of tomato plants in the years 2006 and 2007 in Muğla, Turkey.

Treatments	Yield (ton ha^{-1})*			
	2006		2007	
1. Control	59.47±3.26**	d***	74.47±0.59	d
2. *Euphorbia myrsinites* 1:100	70.33±1.89	c	76.07±0.88	d
3. *Euphorbia myrsinites* 2:100	92.67±1.55	ab	91.80±1.96	b
4. *Euphorbia myrsinites* 4:100	95.13±1.90	ab	90.13±0.83	b
5. *Urginea maritima* 1:100	74.53±2.67	c	84.13±0.89	c
6. *Urginea maritima* 2:100	91.60±1.58	b	90.13±2.98	b
7. *Urginea maritima* 4:100	98.67±0.94	a	91.87±0.76	b
8. Oxamyl	99.00±1.57	a	100.40±1.89	a

* Data are mean values of five replicates at different four blocks.
** Values are given with ±SE.
*** Values in the same column with different letters show significant differences at P<0.05 according to Duncan's Multiple Range test.

In the 2006 trial, all treatments significantly increased the tomato yield compared to the untreated control (P<0.05) (Table 3). The highest yield was recorded from the Oxamyl treatment and the highest concentration (4:100) of *U. maritima* at the same significance level, followed by *E. myrsinites* at 4:100 and 2:100 (P<0.05). However, the least tomato yield was recorded from the untreated control plots.

In the 2007 trial, all treatments significantly increased the tomato yield compared to the untreated control same as the 2006 trial (P<0.05) (Table 3). The highest yield was recorded from the Oxamyl treatment placing it in a different statistical group. The yields of the plots of *U. maritima* at 4:100 and 2:100 and *E. myrsinites* at 4:100 and 2:100 followed this treatment. The least tomato yield was recorded from the untreated control plots.

DISCUSSION

This study is one of the initial works on the effects of these plants extracts in reducing the damage of nematodes in Turkey. Unfortunately it is impossible to adequately compare the results of the study with those of specific works made on this matter by the previous scientists. Therefore the outcome of this study could only be discussed with those of similar works on the matter.

The results of both laboratory and greenhouse trials are almost in agreement with each other. All dilutions of the extracts applied reduced the root galling and enhanced the tomato yield in the greenhouse as compared with the positive control. The positive effects of the highest concentrations of both extracts on the reducing the damage of root-knot nematode in tomato are similar to the positive chemical (Oxamyl) control and the highest concentration of *U. maritima* being superior to other plant extracts. The chemistry of *U. maritima* is complex and also well studied, due to its cardiac glycoside (medicinal) and raticide activity. Unfortunately little is known about its insecticidal effect and nothing has been determined in terms of its nematicidal characteristic. The active compounds of *U. maritima* are L-azetidine-2-carboxylic acid (AZA) (Hegnauer 1970), the bufadienolides scilliroside, scilla glycoside and aglycones (Verbiscar et al. 1986). Hassid et al. (1976) were the first to examine the insecticidal properties of the extracts of *U. maritima*. They showed that the foliage of *U. maritima* was highly toxic to the lepidopterous larvae, such as *Spodoptera littoralis*, and methanol extracts of the dried leaves (which contained AZA). And when added to an artificial diet, caused 100% mortality as well. Subsequently, Adeyeye and Blum (1989) found that AZA incorporated into the artificial diet of another lepidopteran species, *Helicoverpa zea* disrupted growth and development. Pascual and Fernandez (1999) observed that ethanol extracts of the red and white *U. maritima* bulbs of different ploidy differed in their topical and dietary effects on the stored food pest, *Tribolium castaneum*. Their studies showed that the white bulbs of higher ploidy (tetra- and hexaploids) had more activity than that of white triploids or red bulbs. Whereas this was sufficient to show insecticidal properties in general, but it could not be used in nematicidal effects. In our study, the aqueous extracts of pentaploid (n= 50) white bulbs from *U. maritima* were used, and we applied these extracts as a nematicide to be used against root-knot nematodes through the irrigation system. There was no significant difference in terms of the root galling index between the highest concentration (4:100) of *U. maritima* and the treatments of Oxamyl in the 2005 laboratory trial and in the 2006 greenhouse trial. The same results were gained in terms of tomato yield in the 2006 greenhouse trial. These results show that the highest concentration of *U. maritima* has positive effects on reducing the damage of root-knot nematode, and is similarly effective as the Oxamyl treatments and probably its active compounds of AZA has a systemic or fumigant action. Some previous studies have indicated that *U. maritima* have such insecticide effects. For instance, Pascual (2002) concluded that the effects of the extracts of *U. maritima* on *T. castenaeum* were harmful in stored products and were so high as to be an alternative to Methyl Bromide. It was also reported that the aqueous extracts from *E. myrsinites* and *U. maritima* exhibited both translaminar and systemic activity and are potential candidates as new organic insecticides especially in greenhouses against larval leafminer *Liriomyza trifolii* (Civelek and Weintraub 2004).

Besides this, *E. myrsinites* is native to Eurasia but has invaded other continents and is known to cause mild skin irritation (Spoerke and Temple 1979, Eberle et al. 1999). The recent work on an acetone extract of the plant from Turkey revealed four new diterpene esters in addition to the previously known cycloartane-type triterpenoids and betulin (Oksuz et al. 1995). It is unknown whether these chemicals also are found in the aqueous extracts of *E. myrsinites* and whether they are responsible for the nematicidal effects. In our study, there was no significant difference in terms of the root galling index between the highest concentration (4:100) of *E. myrsinites* and the treatments of Oxamyl in the 2006 laboratory trial. These results show that the highest concentration of *E. myrsinites* has a positive effect on reducing the damage of root-knot nematode and is similarly as effective as Oxamyl treatments and probably systemic in action. Because previous studies have indicated that some other *Euphorbia* spp. have such nematicide effects. For example, the leaf extracts of *E. hirta* influenced the emergence of larvae from their cysts and caused toxicity to the second stage juvenile of the cyst nematodes (*Heterodera avenae* and *Heterodera cajani*) (Bhatti et al. 1997).

This study started with the observations that some growers used these plants solutions in order to reduce the nematode problem in their greenhouses. These plants inhabit Turkey and could serve as an inexpensive and organic alternative to commercial pesticides available. The study was the first to find that these two plant extracts would hopefully reduce the damage of nematodes in the future. Therefore, these treatments could be used for root-knot nematode control in IPM programs for tomato in the greenhouses in Turkey. The research showed that one should investigate the nematicidal effects in vitro condition and identify the active ingredients in the aqueous fractions and also compare the nematicidal activity of the aqueous and ethanol extractions of *U. maritima*.

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