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Orijinal araştırma (Original article)

Effect of some plant extracts on *Meloidogyne arenaria* Neal, 1889 (Tylenchida: Meloidogynidae) and tomato[1]

Bazı bitki ekstraktlarının *Meloidogyne arenaria* Neal, 1889 (Tylenchida: Meloidogynidae) ve domatese etkisi

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Summary

Some plant extracts that have toxic effects to nematodes might be used as an alternative control method of plant parasitic nematodes. For this purpose; two different preparation types (cold and hot) of extracts of *Viscum album, Rosmarinus officinalis, Circium arvense, Myrtus communis, Ocimum basilicum, Capsella bursa-pastoris, Melisa officinalis, Taraxacum officinale, Matricaria chamomilla, Chelidonium majus, Humulus lupulus, Mentha pulegium* were searched the effects on *Meloidogyne arenaria* Neal, 1889 (Tylenchida: Meloidogynidae) in petri and pot experiments. Cold extracts were exhibited lower egg hatching and higher juvenile mortality than hot extracts except *R. officinalis*. Cold extract of *C. arvense* was completely inhibited egg hatching. The pot experiment conducted in a completely randomized block design with four replications on greenhouse condition at 27 ±2°C. Cold extract of *V. album* had the lowest root galling on tomato by followed hot extract of *M. communis* and *C. bursa-pastoris*. The most effective cold extract was *M. pulegium* and hot extract was *H. lupulus* on nematode reproduction. The highest stem length, root weight, fresh and dry stem weight were recorded by the treatment with cold extracts of *T. officinale* and *R. officinalis*, hot extracts of *O. basilicum* and *V. album*, respectively.

Key words: *Meloidogyne arenaria*, plant extracts, tomato, toxic effect

Özet

Nematodlara toksik etkilere sahip bazı bitki ekstraktları bitki paraziti nematodların mücadelesinde alternatif olarak kullanılabilir. Bu amaçla, *Viscum album, Rosmarinus officinalis, Circium arvense, Myrtus communis, Ocimum basilicum, Capsella bursa-pastoris, Melisa officinalis, Taraxacum officinale, Matricaria chamomilla, Chelidonium majus, Humulus lupulus, Mentha pulegium*'un iki farklı (soğuk ve sıcak) şekilde hazırlanmış ekstraktlarının *Meloidogyne arenaria* Neal, 1889 (Tylenchida: Meloidogynidae)'a etkisi petri ve saksı denemeleriyle araştırılmıştır. *R. officinalis* hariç soğuk ekstratlar, sıcak ekstraktlardan daha yüksek larva ölümü ve daha düşük yumurta açılımına neden olmuştur. *C. arvense*'nin soğuk ekstraktı yumurta açılımını tamamen engellemiştir. Saksı denemesi 27 ±2°C'deki sera koşullarında, 4 tekerrürlü tesadüf parselleri deneme desenine göre oluşturulmuştur. *V. album*'un soğuk ekstratı domates bitkisindeki en düşük ur indeksine sahiptir, bunu *M. communis* ve *C. bursa-pastoris*'in sıcak ekstraktları takip etmektedir. Nematod üremesine en etkili soğuk ekstrakt *M. pulegium*; sıcak ekstrakt ise *H. lupulus*'dur. En yüksek gövde uzunluğu, kök ağırlığı, yaş ve kuru gövde ağırlığı sırasıyla *T. officinale* ve *R. officinalis*'in soğuk ekstratları, *O. basilicum* ve *V. album*'un sıcak ekstraktlarından oluşan uygulamalarda kaydedilmiştir.

Anahtar sözcükler: *Meloidogyne arenaria*, bitki ekstraktları, domates, toksik etki

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Introduction

Root-knot nematodes (*Meloidogyne* spp. Goldi) are capable of severally damaging on horticultural and field crops. *Meloidogyne incognita* Kofoid and White, *Meloidogyne arenaria* Neal and *Meloidogyne javanica* Treub (Tylenchida: Meloidogynidae) causes serious yield losses in vegetables-growing areas of Turkey (Elekçioğlu et al., 1994; Mennan & Ecevit, 1996; Kaşkavalcı & Öncüer, 1999; Devran & Söğüt, 2009). Their infection is damaged root system that resulted in weak and poor plant growth (Abad et al., 2003). Nematode feeding causes injuries on root tissue which enable entry soil-borne pathogens as fungi and bacteria. The incidence and severity of some soil-borne pathogens are increased in nematode presence (Javed et al., 2007).

These nematodes are among the most difficult pests to control because they have much reproduction capability. Chemical nematicides have been proved as a powerful tool to prevent yield losses caused by root knot nematodes, but these chemical compounds used have adverse effect on environment. Therefore, use of some broad-spectrum nematicides is limited and alternative control methods have been considerable interest dealing with control of plant parasitic nematodes. Current agricultural systems depend on large amounts of pesticides and fertilizers. However, sustainable agricultural methods have become widespread in organic agriculture and good agricultural practices with a minimum use of chemical compounds. Addition of organic materials can change physical and biological properties of soils and improve the plant resistant to soil-borne diseases and nematodes affecting plants. Toxicity effects from plant against nematodes may also occur (Chitwood, 2002).

Plants that have nematicidal effects are an alternative management for nematode suppression. There are many researches related with control of plant parasitic nematodes (Ferris & Zheng, 1999; Chitwood, 2002; Hatipoğlu & Kaşkavalcı, 2007). Some aqueous plant extract were evaluated with laboratory and pot experiments for their nematicidal potentials. They can reduce ability of egg hatching and cause juvenile mortality, and also can improve plant growth (Adegbite & Adesiyan, 2005, Natarajan et al., 2006; Kaşkavalcı & Civelek, 2009). The objective of this study was evaluated some plant extracts for effects on *M. arenaria* and tomato.

Material and Methods

Plant extracts preparation

Twelve dry plants used in this study were purchased from herb seller (Table 1). Extracts were prepared two different types as cold and hot. To prepare the hot extract, ten grams of plant material was soaked in 200 ml of distilled water at 90°C for an hour. The extracts were allowed to cool for 30-45 minutes. The cooled extracts were then squeezed through a cotton cloth and stored at 4°C for overnight. Each extract was filtered through a Whatman filter paper (No. 2). Cold extracts were prepared by soaking ten grams of plant material in 200 ml distilled water in dark bottle. After 1 day, the solution and plant material were squeezed through cotton cloth and aqueous was then filtered through a Whatman filter paper No. 2. All extracts were kept at -20°C in dark bottle until used.

Nematode culture

Meloidogyne arenaria population was maintained on tomato plants cv. Falcon. Species identification was confirmed by isozyme phenotypes (Esbenshade & Triantaphyllou, 1985).

Eggs were extracted from infected tomato roots with 5% commercial bleach solution (Hussey & Barker, 1973). Eggs released from the roots were collected on a 450 mesh sieve and transferred into distilled water. Second juveniles were obtained by placing egg masses into hatching vessel that were put in petri dishes adding 3 ml water. After 1 day, second juveniles were collected from water.

Petri experiments

Effect of plant extract on egg hatching

Three ml of each plant extract was added to each petri dish containing 30 eggs in a 100 µl of distilled water. Controls were established by adding 3 ml distilled water to nematode suspension. Each treatment was replicated four times. The petri dishes were kept at 27°C and the total number of hatched juvenile was counted after 1, 3 and 5 days. Results were expressed as mean per cent hatch related to actual hatch in control for 5 days.

Effects of plant extracts on juvenile mortality

A 25 µl of suspensions containing 20 hatched juveniles were put into a 96-well Elisa Plate and 150 µl of each plant extract was added to each well. Distilled water served as controls. All treatment was replicated four times. Elisa plate was kept at 27°C. Percent mortality was calculated after 6, 12, 24 and 48 hours. Mortality of nematodes was confirmed by touching the juvenile with fine needle at each period of observation. After 48 hours, each extract was removed with a pipet into petri dishes and immobile juveniles were transferred to distilled water for 24 hours to check for revival.

Pot experiment

Forty-five days old tomato seedlings cv. Falcon were transplanted into plastic pots containing 250 cm^3 of autoclaved soil. Experiment was set up to assess effect of plant extract on nematode and tomato plant growth. To see the effect of plant extract on nematode, pots were inoculated by pipetting 1 ml of a suspension containing 1000 eggs. Ten milliliter of extract was poured near roots as soil drench immediately following nematode inoculation. To see the effect of plant extract on plant growth, 10 ml of extract were applied pots without nematode. Distilled water and nematicide (240 g/l Oxamyl) were served as negative and positive controls, respectively. Experiment was designed random in a completely randomized block design with four replicates in greenhouse at 27°C. Eight weeks after inoculations, plants were uprooted and plant stem length and fresh weight were measured. Roots were carefully washed with tap water and weighted. Roots were rated on a 0-10 scale for gall index (GI) (Bridge & Page, 1980). Eggs were extracted from roots of each plant with 10% commercial bleach solution (Hussey & Barker, 1973). Eggs were counted, and nematode reproduction was assessed by calculating the reproductive index (RI) in which RI=Pf/Pi, where Pi= the initial inoculum level and Pf= final egg recovery (Sasser et al., 1984).

Statistical analysis

Data were analyzed by ANOVA and means were separated by Tukey test at $P \leq 0.05$ using SAS statistical program (SAS Institute, 1985).

Results and Discussion

Petri Experiments

All extracts effected egg hatching and juvenile mortality of *M. arenaria* in petri experiments (Table 1 and Table 2). Table 1 shows the effect of plant extracts on cumulative egg hatches of *M. arenaria*. Cold extracts were exhibited lower egg hatching than hot extracts of each plant except cold extract of *R. officinalis* that was showed highest egg hatching percent (97%) in related to actual hatching in control. Cold extract of *C. arvense* was completely inhibited egg hatching. Cold extracts of *C. bursa-pastoris, O. basilicum* and *H. lupulus* had also lowest egg hatching but *H. lupulus* hot extract was exhibited highly significant egg hatching (Table 1). Similar to egg hatching test, cold extracts were showed higher juvenile mortality than hot extracts at 6 and 12 hour after treatment (Table 2). Cold extract of *R. officinalis* were also not affected on juvenile mortality. Juvenile mortality was occurred at 6 hours on most of extracts in this experiment. In some plant extracts, the mortality rates of juveniles increased with an increase in exposure time. Hot extracts of *M. communis, M. officinalis, M. chamomilla, H. lupulus, M. pulegium* were not exhibited toxicity toward juveniles of *M. arenaria* after 12 hour.

Table 1. Effect of plant extracts on egg hatching of *Meloidogyne arenaria*

Treatment		Egg hatch (%)*, at 27 ± 1°C, after		
Extraction method	Plant species	I. Day	III. Day	V. Day
Hot extract	*Viscum album*	30	41	43
	Rosmarinus officinalis	35	35	49
	Circium arvense	3	3	24
	Myrtus communis	14	27	76
	Ocimum basilicum	19	19	19
	Capsella bursa-pastoris	11	11	16
	Melisa officinalis	24	27	32
	Taraxacum officinale	14	24	30
	Matricaria chamomilla	27	27	27
	Chelidonium majus	32	32	43
	Humulus lupulus	19	57	95
	Mentha pulegium	22	35	51
Cold extract	*Viscum album*	3	8	14
	Rosmarinus officinalis	22	46	97
	Circium arvense	0	0	0
	Myrtus communis	3	16	35
	Ocimum basilicum	3	5	5
	Capsella bursa-pastoris	0	3	3
	Melisa officinalis	8	8	19
	Taraxacum officinale	11	11	24
	Matricaria chamomilla	0	3	8
	Chelidonium majus	3	5	14
	Humulus lupulus	5	5	5
	Mentha pulegium	27	27	27

* Percentage egg hatch in relation to actual hatch in control after five days.

When the comparing the efficacy of these extract preparation methods, present study showed that cold extracts generally were more effective than hot extracts in petri experiments. Hatching tests are useful in screening extracts for nematicidal activity, because counting hatched juveniles is more accurate than counting juveniles in a particular J2 population (Oka et al., 2000). In this study, cold extract of *R. officinalis* showed highest egg hatching and did not observe nematicidal activity against juveniles of *M. arenaria*. These results agree with findings by Zouhar et al. (2009). They did not observe nematicidal activity of essential oils from *R. officinalis* on *Ditylenchus dipsaci*. These results are not consistent with the result presented by Oka et al. (2000).

Table 2. Effect of plant extracts on juvenile mortality of *Meloidogyne arenaria*

Treatment		Juvenile mortality (%),at 27 ± 1°C, after			
Extraction method	Plant species	6 h	12 h	24 h	48 h
Hot extract	*Viscum album*	100	100	100	100
	Rosmarinus officinalis	2	100	100	100
	Circium arvense	100	100	100	100
	Myrtus communis	0	0	0	100
	Ocimum basilicum	21	21	100	100
	Capsella bursa-pastoris	100	100	100	100
	Melisa officinalis	0	0	100	100
	Taraxacum officinale	100	100	100	100
	Matricaria chamomilla	0	0	100	100
	Chelidonium majus	100	100	100	100
	Humulus lupulus	0	0	16	100
	Mentha pulegium	0	0	26	71
Cold extract	*Viscum album*	100	100	100	100
	Rosmarinus officinalis	0	0	0	0
	Circium arvense	100	100	100	100
	Myrtus communis	100	100	100	100
	Ocimum basilicum	100	100	100	100
	Capsella bursa-pastoris	100	100	100	100
	Melisa officinalis	100	100	100	100
	Taraxacum officinale	100	100	100	100
	Matricaria chamomilla	17	100	100	100
	Chelidonium majus	95	100	100	100
	Humulus lupulus	100	100	100	100
	Mentha pulegium	100	100	100	100
	Control	0	0	0	0

Pot Experiment

Phytotoxicity was not observed on experimental plants during the pot experiment. Application of extracts to inoculated plant had a significant reduction of reproduction index and gall index compared to water (Table 3). Cold extract of *V. album* had the lowest root galling on tomato by followed hot extract of *M. communis* and *C. bursa-pastoris*. The most effective extracts were cold extract of *M. pulegium* and hot extract of *H. lupulus* on nematode reproduction (Fig. 1).

Results of pot experiment demonstrated cold extract of *V. album* were suppressed gall formation inflected by *M. arenaria*. Hot extract of *H. lupulus* and cold extract of *M. pulegium* was exhibit in reducing the nematode reproduction. Ntalli et al. (2010) tested nematicidal activity of the essential oils from some Lamiaceae plants including *M. officinalis*, *O. basilicum* and *M. pulegium* against *M. incognita*. These authors observed high nematicidal activity of *M. pulegium* and *M. officinalis*. Similar results were shown that different species of the genus *Mentha* (*M. rotundifolia*) exhibit excellent nematicidal activity in vitro and pot experiments (Oka et al., 2000).

Table 3. Effect of plant extracts on tomato inoculating with *Meloidogyne arenaria*

Treatment		Stem Length (cm)	Fresh Stem Weight (g)	Root Weight (g)	Dry Stem Weight (g)	Gall Index (GI)	Reproduction Index (RI)
Hot Extract	*Viscum album*	28,62 bc	25,72 ab	2,19 a	3,79 a	8,25 ab	31,25 d-i
	Rosmarinus officinalis	36,00 a-c	24,37 ab	0,90 bc	3,64 a	7,25 a-e	52,50 b-d
	Circium arvense	37,12 a-c	24,42 ab	1,28 bc	3,40 a	7,25 a-e	28,75 d-i
	Myrtus communis	36,62 a-c	22,82 ab	1,19 bc	3,34 a	6,00 ef	63,75 ab
	Ocimum basilicum	36,37 a-c	22,80 ab	1,18 bc	3,09 a	8,25 ab	48,75 b-e
	Capsella bursa-pastoris	39,62 ab	26,80 a	1,16 bc	3,67 a	6,00 ef	15,00 h-j
	Melisa officinalis	37,75 ab	22,70 ab	0,91 bc	2,99 a	7,25 a-e	52,50 b-d
	Taraxacum officinale	39,87 ab	22,75 ab	1,04 bc	3,07 a	7,50 a-e	32,50 c-i
	Matricaria chamomilla	38,50 ab	22,77 ab	0,93 bc	3,27 a	7,25 a-e	28,75 d-i
	Chelidonium majus	38,62 ab	17,32 b	0,89 bc	2,34 a	6,25 d-f	15,00 h-j
	Humulus lupulus	40,00 ab	21,90 ab	0,71 bc	3,10 a	6,75 b-e	13,75 ij
	Mentha pulegium	40,25 ab	22,60 ab	0,98 bc	2,81 a	6,25 d-f	27,50 d-j
Cold Extract	*Viscum album*	37,00 a-c	24,75 ab	1,08 bc	3,69 a	5,00 f	22,50 e-j
	Rosmarinus officinalis	35,87 a-c	22,95 ab	1,15 bc	3,73 a	6,75 b-e	18,75 f-j
	Circium arvense	37,37 a-c	24,75 ab	1,58 ab	3,93 a	7,50 a-e	46,25 b-f
	Myrtus communis	36,25 a-c	21,52 ab	1,13 bc	3,29 a	7,25 a-e	51,25 b-d
	Ocimum basilicum	36,87 a-c	23,37 ab	1,06 bc	3,28 a	8,25 ab	43,75 b-g
	Capsella bursa-pastoris	40,12 ab	25,02 ab	1,10 bc	3,32 a	7,75 a-d	30,00 d-i
	Melisa officinalis	36,75 a-c	21,22 ab	1,02 bc	3,00 a	8,25 ab	60,00 a-c
	Taraxacum officinale	36,25 a-c	22,62 ab	1,02 bc	3,40 a	8,25 ab	42,50 b-h
	Matricaria chamomilla	38,87 ab	19,70 ab	0,82 bc	2,83 a	8,00 a-c	38,75 b-i
	Chelidonium majus	41,12 a	21,62 ab	0,90 bc	2,88 a	6,75 b-e	33,75 c-i
	Humulus lupulus	40,00 ab	23,70 ab	0,85 bc	3,32 a	6,25 d-f	17,50 g-j
	Mentha pulegium	37,37 a-c	19,42 ab	0,90 bc	3,17 a	6,50 c-f	13,75 ij
Control (-)	Water	26,00 c	22,77 ab	2,26 a	3,02 a	8,75 a	82,50 a
Control (+)	Nematicide	35,62 a-c	18,57 ab	0,56 c	2,45 a	0,00 g	0,00 j

*Means within a column followed by a common letter are not different according to Tukey (P≤0.05).

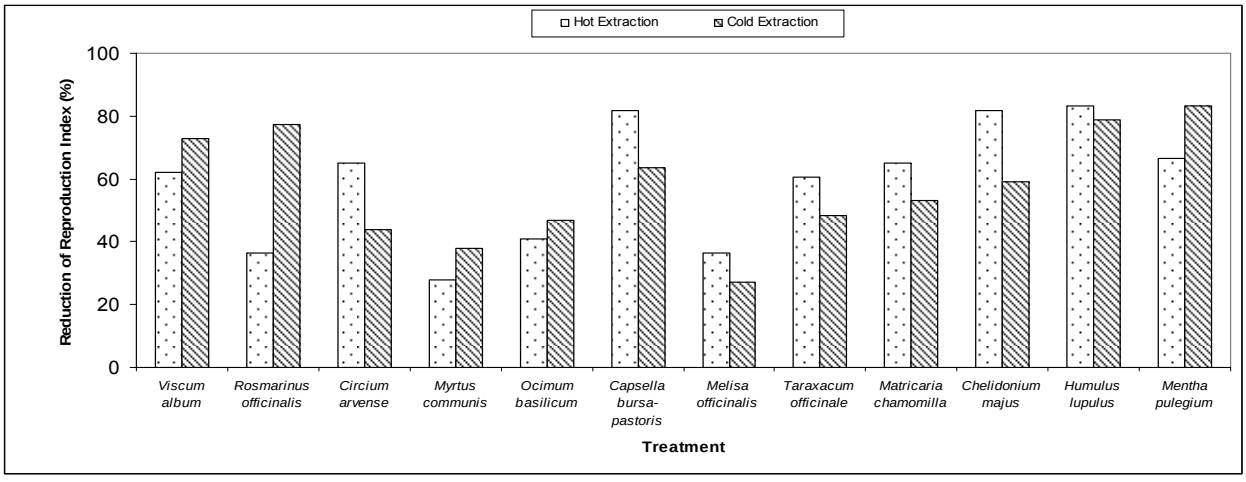

Fig. 1. Effect of plant extracts on reduction of reproduction index (%) of *Meloidogyne arenaria*.

Extracts had a significant impact on plant growth criteria of tomato in uninoculated plants (Table 4). In particular stem length was significantly increased at treated tomato plants with extract compared to water. The highest stem length, root weight, fresh and dry stem weight were recorded by the treatment with cold extracts of *T. officinale*, *R. officinalis*, hot extracts of *O. basilicum* and *V. album*, respectively.

Table 4. Effect of plant extracts on tomato without *Meloidogyne arenaria*

Treatment		Stem Length (cm)	Fresh Stem Weight (g)	Root Weight (g)	Dry Stem Weight (g)
Hot Extract	*Viscum album*	33,75 d	26,70 ab	1,24 b-d	4,60 a
	Rosmarinus officinalis	36,37 b-d	23,92 ab	1,82 a-d	3,60 a-c
	Circium arvense	41,50 a-c	26,00 ab	2,68 a	3,95 ab
	Myrtus communis	38,75 a-c	23,10 ab	2,43 a-c	3,35 ab
	Ocimum basilicum	40,50 a-c	27,02 a	2,62 ab	3,16 bc
	Capsella bursa-pastoris	40,37 a-c	25,67 ab	2,22 a-d	3,14 bc
	Melisa officinalis	44,00 ab	22,52 ab	1,45 a-d	3,35 a-c
	Taraxacum officinale	44,25 ab	22,22 ab	1,48 a-d	3,33 bc
	Matricaria chamomilla	41,75 a-c	25,15 ab	1,51 a-d	3,38 a-c
	Chelidonium majus	40,87 a-c	22,10 ab	1,44 a-d	2,86 bc
	Humulus lupulus	40,87 a-c	22,42 ab	1,22 b-d	2,55 c
	Mentha pulegium	41,75 a-c	24,90 ab	1,10 cd	3,43 a-c
Cold Extract	*Viscum album*	36,25 b-d	23,32 ab	1,43 a-d	3,89 ab
	Rosmarinus officinalis	39,00 a-c	24,97 ab	2,82 a	3,76 a-c
	Circium arvense	39,87 a-c	24,77 ab	2,42 a-c	3,52 a-c
	Myrtus communis	39,00 a-c	25,25 ab	2,21 a-d	3,69 a-c
	Ocimum basilicum	39,00 a-c	26,50 ab	2,10 a-d	3,56 a-c
	Capsella bursa-pastoris	44,62 ab	24,62 ab	2,03 a-d	3,59 a-c
	Melisa officinalis	45,75 a	22,42 ab	1,62 a-d	2,89 bc
	Taraxacum officinale	46,87 a	21,70 ab	1,04 cd	3,08 bc
	Matricaria chamomilla	41,62 a-c	22,77 ab	1,17 cd	2,87 bc
	Chelidonium majus	40,62 a-c	23,02 ab	1,56 a-d	3,05 bc
	Humulus lupulus	42,37 a-c	22,90 ab	1,21 b-d	3,19 bc
	Mentha pulegium	38,75 a-c	20,52 b	0,93 d	3,32 bc
Control (-)	Water	27,75 d	24,42 ab	1,63 a-d	3,78 a-c
Control (+)	Nematicide	36,12 b-d	21,02 ab	1,84 a-d	3,29 bc

*Means within a column followed by a common letter are not different according to Tukey (P≤0.05).

The study showed that amending the soil with some plant extracts, namely *V. album*, suppressed the populations of *Meloidogyne arenaria* both in the soil and on the roots of tomato with a concomitant increase in the growth and yield of tomato. No previous study, to our knowledge, has demonstrated effect of *V. album* extract on phytoparasitic nematodes. *Viscum album* is used medicinally, for example to treat various forms of cancer (Vicaş et al., 2011). Major compounds extracted from *Viscum* are the thionins, termed viscotoxins that are toxic against a varied number of cell types. They belong to plant thionins, and are produced from the leaves and stems of the *V. album* (Giudici et al., 2003). The biological activity of viscotoxins might be related to plant defense since its high expression gives enhanced resistance to pathogens (Holtorf et al., 1998). Reports about induced resistance with used extracts of *V. album* against plant bacterial diseases have been reviewed by Zeller (2006). Chandrashekhara et al. (2010) tested that

seeds of pearl millet were treated with different concentrations of aqueous extract of the plants to examine their efficacy in controlling downy mildew (*Sclerospora graminicola* [Sacc.] Schroet.). Among the plant extracts tested, *V. album* treatment was found to be more effective in enhancing seed quality parameters and also in inducing resistance against downy mildew disease. Cold extract of *V. album* were reduced gall index but hot extract were not. Probably, compounds in *V. album* might be inactive with heat. Park et al. (1999) showed that the cytotoxic effect of the components decreases after heat treatment, which is important for a few medicaments in traditional therapy.

Root-knot nematodes are the serious enemy of tomato growers and these pathogens are becoming a more significant damage on tomato plants. Using the resistant tomato plant carrying Mi-gene is one of the most effective control methods for major root-knot nematodes including *M. arenaria*. Nevertheless, the Mi-gene is unable to inhibit the reproduction of selected and naturally Mi-virulent populations (Abad et al., 2003). Chemical nematicides are commonly used for control of nematodes. However, these compounds have been restricted due to environmental pollution. Therefore, growers are need to alternative control strategies (Mennan & Melakeberhan, 2010). In recent years, there has been considerable interest in plant extracts including nematicidal activity (Chitwood, 2002; Meyer et al., 2008; Douda et al., 2010). These extracts can effect plant growth as well as nematode reproduction. The development of nematicidal phytochemicals has not yet enough power for root knot nematodes and most of research related them have largely consisted of basic (Elbadri et al., 2009).

Results also showed that tomato plant development influenced as a positive after applying extracts. This may be due to the higher organic amendments coming from extracts. Akhtar & Malik (2000) concluded that the availability of more nitrogen enhances the availability of the organic amendment to control nematodes. In conclusion, this study has shown that some plant extracts is very beneficial in the management of root knot nematodes in tomato production. These materials are common and are found in abundance easily. They are almost cheap with no negative effects on the environment. Therefore, they can be used as efficient means of suppressing nematode problems with attendant yield increases. However, more work is needed to determine the actual contributions of the amendments towards controlling these nematodes which may contain toxic compounds released by the decomposing organic soil amendments, increase the populations of nematophagous fungi and release nutrients by decomposing organic matter. These factors were not investigated in this study and further studies are needed to understand the causes of these effects as a physiochemical and ecological basis research.

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