EFFECT OF CASTOR BEAN [*RICINUS COMMUNIS* L. (EUPHORBIACEAE)] AND DIEFFENBACHIA [*DIEFFENBACHIA MACULATA* (ARACEAE)] OF ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) ON GREENHOUSE TOMATOES

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ABSTRACT: One of the major pests of the vegetables is root-knot nematodes (*Meloidogyne* spp.) (RKNs) which cause economic losses in production by causing knots in the roots. RKNs are generally seen in the greenhouse vegetable growing areas of the coastal regions of Turkey. In this study, the efficacy of pieces of leaves of castor bean [*Ricinus communis* L. (Euphorbiaceae)] and dieffenbachia [*Dieffenbachia maculata* Schott (Araceae)] were evaluated at two different applications (10 and 15 g) against *Meloidogyne incognita* on tomatoes. Experiments were carried out in pots under greenhouse conditions using tomatoes cv. Seyran F1 as assay plants that are commonly cultivated in Turkey. The efficacy of pieces of leaves of *R. communis* and *D. maculata* were evaluated at two different applications (10 and 15 g pieces of leaves pot^{-1}) against *M. incognita* on tomatoes. As a result of this experiment, *R. communis* and *D. maculata* were found to be effective against *M. incognita*. Of these plants especially *R. communis* demonstrated to have a quite high effect.

KEY WORDS: Root-knot nematode, *Meloidogyne incognita*, *Ricinus communis, Dieffenbachia maculata,* parted leaves, tomato

Tomato is the vegetable that is grown the most in the world and in our country (Turkey). It becomes necessary to control many pests in tomatoes that are generally grown in fields or greenhouse cultivation. Plant-parasitic nematodes (PPNs), particularly *Meloidogyne* spp. root-knot nematodes (RKNs), are widely distributed and cause significant yield losses in a wide range of crops (Sasser & Freckman, 1987; Davis, 2005; Luc et al., 2005; Javed et al., 2006a,b). Major root-knot nematode species are *M. arenaria*, *M. exigua*, *M. graminicola*, *M. hapla*, *M. incognita*, *M. javanica* and *M. mayaguensis* (Luc et al., 2005). In Turkey, the species *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla* are the most commonly found, with *M. incognita and M. javanica* which causes serious problems to a number of economically important agriculture and greenhouse crops (Kepenekci, 2012).

The most destructive species is *Meloidogyne incognita* (Kofoid & White) Chitwood, which causes serious problems to a number of economically important agriculture and greenhouse crops (Tsay et al., 2004). Nematode control is largely based on synthetic nematicides, which besides being costly present potential risk to non-target organisms. In the search for more benign acceptable alternatives to chemicals, the possibilities are being investigated of exploiting nematode-antagonistic plants for the management of plant parasitic nematodes (Chitwood, 2002; Akhtar, 2004). Current management of nematodes has been done by using plant resistance, crop rotation, cultural practices or chemical nematicides (Chitwood, 2002). Chemical control is expensive and is economically viable only for high value crops, and could create a potential hazard to the environment and human health. Because of these inconveniences scientists identified natural product with nematicidal activity such as plant extract, root exudates, plant volatiles etc. It is necessary to look for other less harmful management options to control RKNs as well as other pests. In recent years broad spectrum nematicides have been used to control this pest in Turkey. Control is difficult mainly due to resistance to conventional pesticides. The use of plant extracts as alternative pesticides for control of RKNs is becoming important. A great deal of research has been carried out in this area in recent years. Linford et al. (1938) were the first to study the nematicidal effect of chopped pine-apple (*Annanas comosus*) leaves used as organic amendment against *Meloidogyne* spp., while a review of phytochemical strategies for the control of nematodes was given by Chitwood (2002). Numerous plant species, representing 57 families, have been shown to contain nematicidal compounds (Sukul, 1992).

Many plants including Brassicaceae, Asteraceae, Myrtaceae and Rutaceae families' member plants can contain nematicidal compounds (Sukul, 1992; Andres et al., 2012). The use of plant extracts as an alternative to synthetic pesticides for control of RKNs is becoming important and in recent years, studies on plant extracts have accelerated (Lee, 2011, Ntalli et al., 2011; Andres et al., 2012; Oka, 2012). *Azadirachta indica* is well known as a pesticide and controlling insect, mite, nematode and plant diseases (Agbenin et al., 2005; Bashir, 2013; Anwar, 2015; Benelli, 2015). *Tagetes* spp. includes a-Terthienyl and this content shows highly nematicidal activities against plant-parasitic nematodes especially RKNs (Ploeg, 1999). Nematicidal effect of garlic has been studied against RKNs (Bekhiet et al., 2010; El-Nagdi & Youssef, 2013).

In this study, the efficacy of pieces of leaves of castor bean [*Ricinus communis* L. (Euphorbiaceae)] and dieffenbachia [*Dieffenbachia maculata* (Araceae)] were evaluated at two different applications (10 and 15 g pieces of leaves pot^{-1}) against RKN, *M.incognita* on tomatoes. Experiments were carried out in pots under greenhouse conditions using tomatoes cv. Seyran F1 as assay plants that are commonly cultivated in Turkey.

MATERIALS AND METHODS

The study was carried out in the climate chamber, greenhouse and laboratory of Atatürk Science and Art Centre (ASAC) and Atatürk Central Horticultural Research Institute (ACHRI), Yalova, Turkey.

Plant material. The castor bean (*Ricinus communis* Linn) (Euphorbiaceae), whose nematicidal activity is investigated in the study, was picked from a garden in Yalova (Turkey) and the other plant dieffenbachia (*Dieffenbachia maculata* Schott) (Araceae) was procured from a company that grows ornamental plants in Yalova (Turkey).

Inaculum material (infected tomato roots). The RKN eggs that were used in the study were obtained from the roots of the tomato (Rio Grande variety susceptible to RKNs) (*Solanum lycopersicum* L.) that was grown in the ACHRI laboratory. Seeds of Rio Grande variety tomato were seeded in pots containing sterile soil and sand in the climate chambers with a temperature of 25±1ºC to be used in creating pure nematode cultures. The seedlings of tomato plants were infected with RKN, *Meloidogyne incognita* that was used in the study. 3000 eggs ml^{-1} of *M.incognita* were applied to 2 cm depth of soil surface (Melakeberhan, 1997). Nine weeks after inoculation, plants were uprooted and their roots gently washed under non-pressurized tap water to cleanse the root system from soil thoroughly. In the trials, 10 and 15 g infected tomato roots were used (two different applications).

Greenhouse pot experiments. Experiments were carried out in pots under greenhouse conditions using tomatoes cv. Seyran F1 as assay plants that are commonly cultivated in Turkey. Since temperatures would drop in the fall, tomatoes were grown in the laboratory in climate chamber conditions (25±2 C° temperature and 16 hours of light, 8 hours of darkness). Each experimental unit consisted of the plastic pot (17×17 cm) containing sterilized loamy sand/pot (sterile moist loamy soil, 85% sand and 15% soil) (approximately 255-265 g) and a seedling tomato (cv. Seyran F1).

The prepared soil-sand mixture was autoclaved twice at 121°C for 15 minutes and the interval between the two treatments was 24 hours (Nakasone et al., 2004). Before they were put in the pots, paper ties were placed under the soil mixtures to prevent the roots from getting out and the soil from spilling. In the trials, tomato seedlings (cv. Seyran F1) susceptible to RKNs in their 7-8 leaves period were placed in pots, one seedling per pot. Seedlings that were either smaller or larger than average were not included in the trials. The galled roots of tomato plants of Rio Grande variety infected with *M. incognita* were cut into 1-2 cm pieces and placed near the roots of the seedling in each pot. Pieces of *D. maculata* and *R. communis* plants were also placed in the pots with the galled roots in two different treatments of 10 and 15 gr (Hatipoğlu & Kaşkavalcı 2007).

The negative control pots received only with galled root pieces infected with *M. incognita* and the positive control pots received only water. The chemical control group was treated with nematicide (Nemacur 400 EC 2 cc pot^{-1}) after being infected with *M. incognita*. Each experiment was arranged in a randomized block design (RBD) with 7 factors and 5 replications. Trials were conducted between November 2016 and January 2017.

Nine weeks after the application, the plants were removed from the pots together with the soil and washed under non-pressurized tap water to completely clean the root system from the soil. After the washing process; Zeck scale was used to assess the galling levels of the roots (infestation level) in order to determine the efficacy of the applications on nematodes (Zeck, 1971) (Table 1).

The height of the tomato plant in each pot was measured from the base to the terminal bud in the development period (2nd, 4th and 9th weeks).

Later, to determine the end of harvest *M. incognita* populations, the soil remaining in the pots after the plants were removed was subjected to nematological extraction in the laboratory and the juveniles (second stage juveniles, J2s) of the *M. incognita* were counted. The nematodes were separated from the soil by the "Modified Baermann-Funnel Technique" (Hooper, 1986).

The root of plants was measured and later weighed on a precision scale and recorded. The same process was repeated after 48 hours of drying at 70°C (Mohammad et al., 2007).

Statistical analysis. Variance analysis (ANOVA) of the data obtained was performed in SPSS software (SPSS, 1999). The impacts were found by comparing them to the control groups. The comparison of the means was performed by the Duncan test at the level of $P<0.05$. Statistical analyses were conducted after log10

(X+1) and square-root transformations were performed on the root-knot scale values. In order to measure the percentage effects the % Abbott formula was used.

RESULTS

The efficacy of pieces of leaves of castor bean, *Ricinus communis* and dieffenbachia, *Dieffenbachia maculata* were evaluated at two different applications (10 and 15 g) against *Meloidogyne incognita* on tomatoes. As a result of this experiment, *R. communis* and *D. maculata* were found to be effective against RKN, *M.incognita*. Of these plants especially *R.communis* demonstrated to have a quite high effect (Table 2).

Effect of applications on leaf count. Tomato plants used in the trials had 7-8 leaves, and the leaves were counted at the end of week 2, 4 and 9 after the treatments. At the end of week 2, they were in the same statistical group as the control groups [Negative control (treated only with water), Positive control (treated only with root-knot nematode, *M. incognita*) and Chemical control (treated with nematicide)] and plants had on average 9.6 leaves. In the treatments with 10 g plant pieces, *D. maculata* (10.2±0.20) and *R. communis* (10.4±0.24) were occured the same group. The highest leaf formation was observed in the treatments with 15 g of *R. communis* (11.6±0.24) ($F= 5.863$; $df= 3.52$; $P<0.01$). In week 4, the highest leaf formation was again in the treatments with 15 g of *R. communis* (16.4±0.24) which was followed by 10 g treatments of the same plant and 15 g treatment of *D. maculata* (13.8±0.49 and 14.2±0.58, respectively). The lowest leaf formation was observed in the plants in the positive and chemical control groups (11.8±0.20 and 12.2±0.58, respectively) ($F= 13.166$; $df= 3.52$; $P<0.01$). At the end of week 9, the highest leaf formation was in the 15 g treatment with *R. communis* (20.8±0.37), followed by treatments with *R. communis* 10 g, *D. maculata* 15 g and 10 g and negative control treatments which fall into the same statistical group (17.8±0.49, 17.4±0.75, 17.0±0.32, 17.0±0.32, respectively). Like in week 4, in week 9 the lowest leaf formation in the treated plants was observed in the positive and chemical control groups (14.2±0.37 and 14.4±0.51, respectively) ($F= 23.26$; $df= 3.52$; $P<0.01$) (Table 2).

Effect of applications on plant height. Tomato plants used in the trials were 15-16 cm high, and the height of the plants were measured after 2, 4 and 9 weeks after the treatments. At the end of week 2, all three control groups (Negative, Chemical and Positive control) fell into statistically different groups (29.8±0.73, 28.8±1.15 and 26.2±1.01, respectively). The plants with the greatest height were in the treatments with 15 g *R. communis* (11.6±0.24) followed by 10 g treatment with the same plant and 15 g treatment with *D. maculata* (31.6±0.50 and 30.6±0.39, respectively) ($F= 9.158$; $df= 3.52$; $P<0.01$). At week 4, the greatest plant height was measured again in the treatments with 15 g *R. communis* (16.4±0.24) followed by 10 g treatment with the same plant (14.2±0.58). In the *D. maculata* treatments plant height was measured as 43.2±0.37 for 15 g and 41.0±0.95 for 10 g. At week 4 evaluations, lowest plant height was observed in the positive control treatments with root-knot nematode (*M. incognita*) only (38.2±1.02) ($F= 12.711$; $df= 3.52$; $P<0.01$). At the end of week 9 evaluations there was no difference and in the treatments with 15 g *R. communis* plants had the greatest height (62.8±0.37). This was followed by *R. communis* 10 g (59.4±0.68), *D. maculata* 15 g (57.2±0.37), negative control (55.4±1.69) and *D. maculata* 10 g (52.6±1.16) treatments. As in week 4, in week 9 the lowest plant height was observed in the positive control group (49.2±1.02) ($P<0.05$) ($F= 15.852$; $df= 3.52$; $P<0.01$) (Table 2.).

Effect of applications on plant root weight. At the end of the trial, roots of the plants were weighed and evaluated statistically. According to wet root weight, the greatest root weight was observed in 15 g treatments with *R. communis* (35.78±1.84) followed by 10 g treatments of the same plant (28.29±1.51). Next, rank *D. maculata* 15 and 10 g treatments, and chemical control group which fall into the same group statistically (24.20±0.57, 21.90±0.53 and 22.90±0.57, respectively). Plants with the lowest root weight fall into the positive control group (11.00±0.50) ($F= 66.944$; $df= 3.52$; $P<0.01$). Similar findings were obtained when dry root weights were evaluated. The greatest root weight was observed in treatments with *R. communis* (for 15 g 5.77±0.22 and for 10 g 4.75±0.17). Plants with the lowest root weight were in the positive control group (1.53±0.36) ($F= 44.587$; $df= 3.52$; $P<0.01$) (Table 2.).

Effect of applications to the juveniles (J2s) of *M. incognita* in the soil and gall formation (gall index scale) in the plant roots. The highest nematode population in the soil were obtained in the *D. maculata* 10 g and chemical control treatments (720±14.12 and 664±29.21, respectively). This was followed by 15 g treatment of the same plant with 720±14.12. The number of nematodes in *R. communis* treatments, which were observed to be highly effective, was recorded as 80±14.12 for 15 g and this was followed by 10 g of the same plant as 216±14.67. The nematode population in the soil where the plants that were only treated with *M. incognita* and nothing else (positive control) were grown was recorded as 1372±26.49 ($F= 658.86$; $df= 3.52$; $P<0.01$). 15 and 10 g treatments with the *R. communis* plant were proven to reduce the nematode population in the soil by 94.2% and 84.3%. This effect was found by comparing the J2s count in the soil in the group treated with *M. incognita* only. When damage caused by *M. incognita* in plant roots was evaluated based on gall index scale; rather low scale values (1.6±0.24 and 2.6±0.24, respectively) that is low galling/damage were detected in treatments of 15 and 10 g of *R. communis* and it was observed that *R. communis* decreased knots in plant roots by 79.5% in treatments of 15 g and 66.7% in treatments with 10 g ($F= 109.549$; $df= 3.52$; $P<0.01$) (Table 2.).

DISCUSSION

In an experiment carried out in Turkey, it is reported that 30 g/2.5 kg soil of castor bean is effective and reduces the galls in the tomato plant by 67.06% (Hatipoğlu & Kaşkavalcı, 2007). Reddy et al. (1993) have found that crushed leaves of the castor bean plant have reduced the galling caused by RKN in the papaya [*Asimina triloba* L. (Annonaceae)] plant in India. Mukhtar et al. (1994) have found that plant parts of *R. communis* are effective against *M. incognita* at the rate 25 g/kg soil in Pakistan.

In experiments done by Hatipoğlu & Kaşkavalcı (2007), in the soil of the pots treated with leaves of the castor bean plant against *M. incognita*, J2s was not present at all and were found to be 100% effective. Alvarez et al. (1998) have evaluated the knot formation in the roots by *M. hapla* in Colombia after growing the carrot plant together with some plants including the castor bean plant and have found no significant difference. However, they have detected the lowest J2s population in the treatment with castor bean plant.

In the experiments done by Hatipoğlu & Kaşkavalcı (2007), among the treatments without RKN the greatest plant height was detected in the treatment with castor bean leaves (45.20±3.47) and was reported to be statistically different form the other treatments. Among the treatments infected with RKN the greatest plant height was detected again in the treatment with castor bean leaves (28.50±1.6). As a result of evaluations of plant height, among treatments with or without RKN, treatments with the leaves of the castor bean plant were found to have an increasing effect by far. In an experiment done in Pakistan the leaf extract of the castor bean plant were used, with *Verticillium chlamydosporium*, against RKN, *M. javanica* and was found to have a positive effect on plant development (Mukhtar, 2002).

In experiments in Hatipoğlu & Kaşkavalcı (2007) according to fresh root weight, among the treatments without RKN leaves of the castor bean plant were found to increase fresh root weight compared to the negative control.

As a result of leaf count in the experiments Hatipoğlu & Kaşkavalcı (2007) performed, treatments were found to have no significant effect on compound leaf count. The highest leaf counts were obtained, in treatments with RKN in the positive control; in the treatments without RKN treatment with leaves of castor bean, treatment with roots of marigold and negative control.

CONCLUSION

In this experiment; *Ricinus communis* and *Dieffenbachia maculate* treatments were found to be effective against RKN, *M.incognita*. Experiments showed that the plant parts used had nematicidal activity to differing degrees. *R. communis* plant parts are observed to be more effective than *D. maculata*. Data obtained have shown that plant parts used are effective in suppressing RKN.

In addition to these findings it is concluded that similar experiments could be made in coastal regions of Turkey in areas where protected cultivation is widespread and based on the results obtained *R. communis* could be used in organic farming and in integrated control against RKN. As a result of these experiments, it has been found that *R. communis* exhibits nematicidal activity against RKN, *M. incognita* and that it is crucial to evaluate data that will be obtained from experiments on other RKNs.

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Table 1. Index showing degree of infestation with root-knot nematode (RKN) (Zeck, 1971).

Infestation category	**Degree of Infestation.**
0	The root system is completely healthy and no galls.
1	Very few galls are observed when roots are inspected very carefully.
2	Galls are small but quite a few compared to category 1.
3	The root system has a large number of galls, some galls have grown larger by merging but the roots work well.
4	In addition to a large number of galls, large galls are present but most of the roots continue to work.
5	About 25% of the roots do not work due to galls.
6	50% of the root system does not work.
7	75% of the root system has gallsi there is yield loss.
8	No healthy roots, the nutrition of the plant is affected but the plant is still green.
9	The root system is completely covered with galls, the root rots.
10	Root and plant dies.

Table 2. Effectiveness (or efficacy) of extracts of Castor Bean [*Ricinus communis* Linn (Euphorbiaceae)] and Dieffenbachia [*Dieffenbachia maculata* (Araceae)] against Root-knot Nematode (*Meloidogyne incognita*) on Greenhouse Tomatoes (leaf count, plant height, wet and dry root weights, juvenile (J2) populations in the soil, knot scale values and % effects).

Treatments*	Leaf count (number)			Plant height (cm)		
	Week 2	Week 4	Week 9	Week 2	Week 4	Week 9
Neg. control	9.6±0.24c	13.6±0.24b	16.6±0.24	29.8±0.73bc	42.4±1.47	55.4±1.69cd
Poz. control	9.6±0.40c	11.8±0.20c	14.2±0.37	26.2±1.01d	38.2±1.02	49.2±1.02e
Ch. control	9.6±0.40c	12.2±0.58c	14.4±0.51	28.8±1.15c	40.6±1.32	49.8±2.26e
D.m 10 g pot^{-}	10.2±0.20b	13±0.32bc	17.0±0.32	28.8±0.85c	41.0±0.95	52.6±1.16de
D.m 15 g pot^{-}	10.8±0.37a	14.2±0.58b	17.4±0.75	30.6±0.39bc	43.2±0.37	57.2±0.37bc
R.c 10 g pot^{-1}	10.4±0.24b	13.8±0.49b	17.8±0.49	31.6±0.50ab	46.6±0.51	59.4±0.68ab
R.c 15 g pot^{-1}	11.6±0.24a	16.4±0.24a	20.8±0.37	33.8±0.58a	48.6±0.51	62.8±0.37a

	Plant root weight (g)		J2 population in the s		Gall index value	
	Wet weightt	Dry weight	J2 count	Effect (%)	Index valu	Effect (%)
Neg. control	17.82±0.92d	3.19±0.27d	0±0.00g		0±0.0f	
Poz. control	11.00±0.50e	1.53±0.36e	1372±26.4a	100	7.8±0.37a	0.0
Ch. control	22.90±0.57c	4.64±0.18b	664±29.21c	51.6	4.2±0.20b	46.2
D.m 10 g pot^{-}	21.90±0.53c	3.78±0.09cd	720±14.12b	47.5	4.4±0.24b	43.6
D.m 15 g pot^{-}	24.20±0.57c	4.17±0.11bc	592±18.51d	56.9	3.6±0.24c	53.8
R.c 10 g pot^{-1}	28.29±1.51b	4.75±0.17b	216±14.67e	84.3	2.6±0.24d	66.7
R.c 15 g pot^{-1}	35.78±1.84a	5.77±0.22a	80±14.12f	94.2	1.6±0.24e	79.5

* Negative (Neg.) control (treated with only water), positive (Poz.) control (treated only with galled root parts infected with *M. incognita*), chemical (Ch.) control (treated with nematicide, Nemacur 400 EC 2 cc/pot after being infected with root-knot nematode). *R.communis* (*R.c*) and *D.maculata* (*D.m*).