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Nematicidal effects of olive pomace and green walnut husk on root-knot nematode *Meloidogyne incognita* on tomato

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ABSTRACT

We studied the nematicidal effects of olive pomace (OP) and green walnut husk (GWH) on root-knot nematode (*Meloidogyne incognita*) in tomato. We determined under *in-vitro* conditions, the efficacy of OP and GWH extracts (0, 25, 50, 75 and 100%) against the second stage juvenile of *M. incognita*. Then, GWH and OP were mixed with sandy loam soil at rates of 0 %, 0.5 %, 1 %, 1.5 % and 2 % w/w under controlled atmosphere conditions. Tomato seedlings (*Solanum lycopersicum* L. cv Troy) were transplanted in pots and inoculated by second stage juveniles of *M. incognita*. Gall index (GI), egg mass index, root and shoot development of tomato plants were determined 60 days after inoculation. GWH (2 %) application reduced the GI of tomato seedlings by 85 % while OP (2 %) reduced it by 53 % than unamended control. Mortality rates of J2 were significantly affected by type of material, concentrations and the exposure time. GWH had higher juglone (5-hydroxy-1,4- naphthoquinone) concentration which suppressed the root knot nematodes as compared to OP. Higher rates of GWH and OP added into the soil resulted into healthy and much longer root systems. Plant fresh and dry weight increased in all treatments than control. Use of GWH and OP suppressed the root-knot nematodes without causing phytotoxicity to tomato plants.

Keywords: Allelopathy, green walnut husk, Juglone, *Meloidogyne incognita*, mortality, olive pomace, root-knot nematode, soil, Tomato.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is major vegetable crop in temperate and tropical regions of the world. Tomato is also important crop in Turkey (13). Many pests and diseases adversely affect the tomato production, plant parasitic root knot nematodes (*Meloidogyne incognita*) cause economic losses in tomato (3).

Nematode infestation damages the plant by disrupting the uptake and transport of water and nutrients (26,33). The control of *M. incognita* is difficult because of their wide host range and high rates of reproduction (28). Synthetic nematicides are expensive and toxic to beneficial soil microorganisms (44). Carbofuran commonly used for nematode control persists in soil, with half life of 30-60 days (43) and very toxic to other organisms. There is a need for effective non-chemical alternatives to methyl bromide fumigation of soil (10,19,36). Now many chemical and non-chemical approaches are available for nematode control [soil amendments, cover crops, flooding, solarization, or bare fallowing (30)]. Soil solarization (covering moist soil with clear plastic), controls many soil-borne pests and weeds, enhances the physical and chemical properties of soil and increases the yield of subsequent crops (20). However it works well in loamy and clay soil with quick drainage but not in sandy soils (32).

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In recent years, interest on plant based nematicidals have increased due to health, environmental and economical concerns. Organic amendments offer an alternative control of nematodes. African marigold (*Tagetes erecta*) extract, from 40 and 70 days old plants reduced the root gall index of tomato roots and results are comparable with carbofuran (28). Kaşkavalcı (19) investigated the effects of soil solarization, chicken manure (CM), olive processing waste (OPW), and soil solarization with CM, OPW or half doses of Dazomet and Dazomet alone on *M. incognita*. CM (10 t ha^{-1}) and OPW (30 t ha^{-1}) alone did not affect the root-knot nematodes, where root galling indices were similar to untreated control. Influence of *Pseudomonas fluorescens*, *Azotobacter chroococcum*, *Azospirillum brasilense* and composted organic fertilizers alone and in combination have been evaluated on *M. incognita* . The poultry manure with *P. fluorescens* was the best combination to control *M. incognita* in tomato (40). Applications of dry cork compost suppressed the root-knot diseases of tomato caused by *M. incognita race 1* and *M. javanica*, and olive pomace and rice husk composts suppressed only *M. incognita race 1* (29). Researches reported suppression of root-knot nematodes with composted agricultural wastes (31), however the application rates to soils were too high for economical use.

One of the best available agricultural waste for Mediterrenean Countries is the olive pomace (OP) [olive pulp, stones, residual oil and vegetative waters]. Approximately, 50-60% of olive by volume is solid waste (OP). Walnut is important tree grown throughout the Turkey (13,18).

OP is used in agriculture to supply plant nutrients and organic matter in soil. It also improves the soil physical and chemical properties (soil N, total organic carbon, aggregate stability and water holding capacity) of coarse textured soil (21,22) which are also favourable to *M. incognita.*

Juglone (5-hydroxy-1,4- naphthoquinone) an important phenolic compound in walnut, is antimicrobial and has allelopathic effects. Besides, there are also other secondary metabolites (phenolic acids, flavonoids, amines and alkaloids) in juglans which are involved in allelochemical interactions (46). Juglone is toxic due to formation of naphthosemiquinone and reactive oxygen species (8). Additionally, unripe green walnut husks also contain methyl palmitate, which caused 97.9 % mortality of adult *T. cinnabarinus* (45). These agricultural wastes are cheap and widely available in our area.

This study aimed to determine the nematicidal activity of green walnut husk (GWH) and olive pomace (OP) against root knot nematode (*M. incognita*) and tomato growth.

MATERIALS AND METHODS

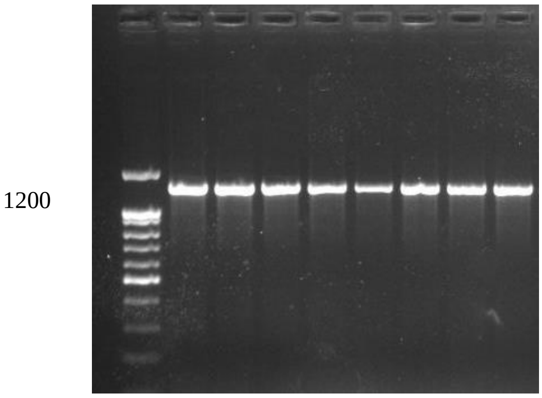
The study was conducted during 2015-2016 in climate controlled room at Canakkale Onsekiz Mart University, Turkey (Latitude 40°6’36”N, Longitude 26°24’59”E, Altitude 8 m). Mean annual temperature, max and min temperatures between 1928-2017 was 15 °C, 41.7 °C and -11.5 °C respectively. The mean total annual rainfall between 1928-2017 was 616.3 mm. (27).

1. Collection of pure cultures of *Meloidogyne Incognita*.

The root-knot nematode species (*M. incognita*) used in the study was collected in 2015 from the tomato production areas in Umurbey province and cultured in tomato plants under controlled atmosphere conditions. A population of *M. incognita* was purified from a single eggmass. *M. incognita* populations collected from tomato fields were reproduced from a single egg from the roots of tomato plants grown in climate controlled room (26^{o}C, 16 h light and 8 h dark, humidity: 70 %). The *M. incognita* populations was confirmed by perineal pattern study of mature females.

In addition to the morphological diagnosis, species identification was also confirmed with species-specific primers. *M.incognita* was confirmed by PCR with *M. incognita* specific Finc and Rinc Primers. Amplification of rDNA extracted from an adult female with *M. incognita*-specific SCAR primers Finc/Rinc and Inc-K14F/Inc-K14R yielded a 1200 bp. (47). DNA isolation was done by taking female individuals (using Qiagen DNA extraction kit) from a population of root-knot nematode cultured in pure culture.

The PCR reaction contained 20 ng DNA, 2.5 μliters PCR buffer, 1.5 mM MgCl2, 200 μM dNTP (1 μliter), 10 μmolar Primer Finc (1 μliter), 1 unit Taq DNA polymerase (0.25 μliters) and distilled water (12.25 μliters) (Total of 25 μliters). The PCR amplification conditions were as under: denaturation for 30 sec at 94 ºC, annealing for 30 sec at 56 ºC, extension for 60 sec at 72 ºC and repeated 35 cycles. PCR products were seperated by electrophoresis on 2% agarose gels and visualized with UV illumination. DNA bands of about 1200 bp in length were obtained only in samples with *M. incognita* as a result of the PCR, and confirmed to be *Meloidogyne incognita* (9) (Picture 1).



Picture 1. PCR products of *M. incognita.*

2. Collection of olive pomace and green wallnut husk

Olive pomace (OP) and green walnut husk (GWH) were obtained from the Altindamla olive oil factory, Çanakkale Ezine district and from walnut farmers in Ezine. OP and GWH were air dried and ground in a mill. To use in *in-vitro* testing, 120 g of GWH and OP were mixed with 600 ml hot water then filtered through filter paper and centrifuged at 8000 rpm for 10 min.

3. *In vitro* testing for *M. incognita* mortality

The efficacy of OP and GWH extract against the second stage juvenile of *M. incognita* was investigated. Two ml extracts of OP and GWH of different concentrations (0, 25, 50 and 75 % and 100 %) were added separately to each compartment. Twelve well plates were used for each treatment and replicated thrice. Within the compartment, 100 pieces of second stage J2 of *M. incognita* (within 3-days after emergence from the eggs) were released. The number of dead and J2 in each well were determined using an invert Leica microscope 12, 24, 48 and 72 h after the larvae were added in the solutions. These plates were kept in climate cabinet at 26 ^oC in dark. In control, 2 ml ultra pure water was added in each compartment and 100 pieces of J2 (as above) were released. In control group, the number of dead and living J2 in each well were determined after 12, 24, 48 and 72 h using an invert Leica microscope.

4. Green walnut husk (GWH) and olive pomace (OP) analysis

One hundred g sub-sample of dry GWH and OP was ground in grinder for 5 min. For elemental analysis the extract of GWH and OP were prepared separately by dissolving 0.5 g of ground material in 16 ml aqua regia (12 ml hydrochloric acid and 4 mL nitric acid) . After wet combustion and cooling the digests were filtered through filter paper and diluted with ultrapure water to 25 ml. Blank samples were proceeded for each extraction procedure. Inductively coupled plasma optical emission spectrometer ICP-OES were used for elemental detection. Air dry GWH and OP samples were used for the determination of electrical conductivity and pH in a 1:10 (v/v) aqueous extract (21). Total C and N analysis was carried out using a LECO Truspec 2000 CN-analyser (23). Juglone content of OP and GWH determined by UV-Vis spectroscopy (15). Aqueus extract was prepared by mixing powdered GWH and OP (12 g) with boiling water (120 mL). Mixtures were shaked for 30 minutes, centrifuged at 8000 rpm and supernatant fraction was removed. Then 0.1 mL of extraction was diluted with approximately 1000 times using ultra pure water. The juglone (5-hydroxy-1,4-naphthoquinone) standard (Sigma Aldrich, 97%) dissolved in 990 ml water and 10 ml ethanol mixture. The samples were read against juglone standard at 254 nm wavelength by using UV-VIS spectroscopy. The results were multiplied by the dilution factor.

Additional phenolic compounds of OP and GWH extracts were determined by a Shimadzu-HPLC equipped with a PDA detector and an Inertsil ODS-3 (5 µm; 4.6 x 250 mm) column. 2% acetic acid in water and acetonitrile mixture were used as mobile phases. The flow rate of the mobile phase was 1 ml min^{-1} at 30°C and the injection volume was 20 µl. The total running time for each sample was 60 min. The peak records were carried out at 280, 320 and 360 nm. Phenolic compounds were determined according to the retention time and absorption spectra of peaks of standard compounds. The total area under the peak was used to quantify the phenolics (16).

5. Nematicidial effects of GWH and OP on *M. incognita* infection (Growth chamber experiment)

The study was done in growth chamber [26 ± 2˚C, 16 h light and 8 h dark and humidity : 70 %]. Sandy soil was used for better nematode growth, easy separation of roots and low in organic matter and plant nutrients. Soil sample was sieved through 2 mm sieves and autoclaved at 121°C for 90 min prior to inoculation of eggs. Each pot (11 cms dia and 14 cms depth, 2 kg soil) received dried and ground either OP or GWH at rates of 0 %, 0.5 %, 1 %, 1.5 % and 2 % (w/w). The treatments were replicated four times in randomised block design. Additionally pots were used as control without nematode infection. The maximum dose was kept at 2%, based on our previous studies results (22) that when OP application to the soil was > 2%, it inhibited tomato growth (21). Sensors capable of measuring soil moisture control were installed in each pot. HOBO data recorders (Onset Com.) were used to continuously monitor the soil moisture content data measured from the sensors. Soil water content was constantly monitored by FDR sensors. Water and Hoagland sollution was added to the pots, when available moisture content decreased by 50%. The three weeks old tomato seedlings (Troy variety) germinated in sterile medium were transplanted to pots in December 28, 2015. Each plant was inoculated with 20 ml water containing 1000 *M. incognita* J2 from the hole drilled around the root, the day after transplantion.

Pot experiment was conducted in winter 2015-2016. The Hoagland solution was prepared to fertilize the tomatoes (17). After 60 days of inoculation, plants were cut 1 cm above soil surface. The roots were pulled and removed from the sandy soil and placed in a container filled with water and the soil settled to the bottom, the floating root samples were filtered through 0.163 mm sieve and the remaining root samples on each sieve were collected with the help of a tweezer. This process was continued several times. Gall index rate and egg mass index analyzes were completed and then root parameters (root length and root surface area) were determined using WinRhizo Basic 2007 (Regent Inst) program. The whole root system was rated for galling on a 0 to 5 scale (34, 42) where 0 = no gall or and 5 = >100 galls per root system. Egg mass index was on a scale of 0 to 5 as described for gall index 1 (42). Data on shoot length and weight, root length and weight were also recorded.

6. Statistical analysis

Effects of exposure times, extract concentrations and materials on nematode mortality were determined by analysis of variance (ANOVA). Effects of different application rates of GWH and OP on egg mass index, gall index and plant development were also determined by analysis of variance. Tukey's Test was used to compare the means (39).

RESULTS AND DISCUSSION

Properties of GWH and OP used in the experiments are presented in Tables 1-3. The C/N ratio of GWH was 41.44 and the OP was 31.03 (Table 1). GWH and OP have high levels of minerals such as K (48.32 g kg^{-1} and 11.28 g kg^{-1}) , Mg (3.96 g kg^{-1} and 2.01 g kg^{-1}) and Ca (30.64 g kg^{-1} and 11.21 g kg^{-1}) and is low in Na (0.66 g kg^{-1} and 0.39 g kg^{-1}) respectively (Table 1). Macro and some micro element contents of GWH are greater than those of OP. On the other hand especially Fe and Al contents of OP were greater than those of GWH (Table 2). Considering revised European Ecolabel limits (37) for soil improver and growing media criteria, all microelements are below limits (Table 2).

Table 1. Plant materials contents of green walnut husk (GWH) and olive pomace (OP).

Plant material	N	C	C/N	Mg	Na	Ca	K
	(%)				(g kg^{-1})		
GWH	0.85	35.20	41.41	3.96	0.66	30.64	48.32
OP	1.45	45.00	31.03	2.01	0.39	11.21	11.28

OP : Olive pomace , GWH: Green Walnut husk

Table 2. Trace elements (mg kg^{-1}) in green walnut husk (GWH) and olive pomace (OP).

Plant material	Zn	Pb	Co	Cd	Ni	Ba	B	Mn	Cr	Fe	Al	Cu
	(mg kg^{-1})											
GWH	32.11	7.31	0.11	0.0	3.01	31.1	81.7	45.6	11.98	918	4.12	21.93
OP	23.31	1.81	1.37	0.0	10.02	21.8	21.4	67.2	1.37	727	3.89	15.54

OP : Olive pomace , GWH: Green Walnut husk

pH of GWH and OP were 9.87 and 7.25 respectively (Table 3). The soil pH significantly increased after incorporation of the GWH and OP over a period of 8 weeks. Compared with non-amended soil with a pH of 7.71, the highest pH (7.93) was observed in soil amended with 2% GWH and OP (7.84), BET surface area, cation Exchange capacity (CEC), pH and EC values of GWH were greater than those of OP (Table 3).

Table 3. Chemical properties of green walnut husk (GWH) and olive pomace (OP).

Plant material	BET surface area	CEC	pH	EC
	(m^2 g^{-1})	(cmol kg^{-1})		(dS m^{-1})
OP	0.545	80.9	7.25	1.00
GWH	0.646	90.67	9.87	7.78

OP : Olive pomace , GWH: Green Walnut husk

Phenolic compounds of OP and GWH extracts are presented in Table 4. Juglone content of GWH were significantly higher than that of OP. A chromatogram of OP and GWH) showed the presence of catechin, epicathecin, caftaric acid, cafeic acid, ferulic acid and quercetin. On the other hand GWH did not contain epicathecin, caftaric acid and cafeic acid. Quercetin which is known to increase stress resistance in the nematode *Caenorhabditis elegans* was higher in GWH than OP. Quercetin concentration of GWH extract was 2.27 while it was 1.20 mg L^{-1} in OP.

Table 4. Phenolic compounds in green walnut husk (GWH) and olive pomace (OP)

Plant material	Juglone	Catechin	Epicatechin	Caftaric acid	Cafeic acid	Ferulic Acid	Quercetin
	(mg g^{-1})	(mg L^{-1})					
OP	7.01	1.24	1.92	1.46	0.12	0.27	1.20
GWH	62.37	2.10	N.V.	N.V.	N.V.	0.54	2.27

N.V. : No value , OP : Olive pomace , GWH: Green Walnut husk.

Juglone (5-hydroxy-1,4- naphthoquinone) cause oxidative stress and generates free radicals in *Caenorhabditis elegans* (8) and is naturally present in walnut trees. Juglone is toxic due to formation of naphthosemiquinone and reactive oxygen species (9). In our research hot water extractable juglone concentration were higher in GWH (62.37 mg g^{-1}) than OP (7.01 mg g^{-1}).

***In-vitro M. incognita* mortality**

Green walnut husk and olive pomace extracts were tested after dilution with ultra-purewater at 25, 50 and 75%. Percentage second stage juvenile (J2) mortality of *M. incognita* was recorded after 12, 24, 48 and 72 h of exposure (Table 5). 100% and 75% GWH possessed higher J2 mortality (100%) followed by 50% GWH (79%) after 72 h of exposure. Mortality rates of *M. incognita* J2 increased with increasing both concentration and exposure time of GWH extract (Table 5). The efficacy of the GWH extract started after12 hours and the mortality rates of J2 increased up to 100% after 72 hrs.

Table 5. Effects of green walnut husk (GWH) and olive pomace (OP) extracts on mortality rates of *Meloidogyne incognita* juveniles

Exposure time (h)	Extracts concentrations (%)				
	0	25	50	75	100
	Mortality (%) (mean± SE)				
	GWH				
12	0.0	11.0 ± 3.1	12.0 ± 0.9	22.0 ± 2.7	30.0 ± 1.3
24	0.0	24.0 ± 3.2	26.0 ± 1.6	44.0 ± 2.0	63.0 ± 1.8
48	0.0	45.0 ± 5.4	53.0 ± 1.2	81.0 ± 2.8	100.0 ± 0.0
72	0.0	69.0 ± 5.5	79.0 ± 2.7	**100.0 ± 0.0**	**100.0 ± 0.0**
	OP				
12	0.0	3.0 ± 1.8	5.0 ± 0.9	5.0 ± 0.8	8.0 ± 3.9
24	0.0	10.0 ± 2.0	16.0 ± 1.5	19.0 ± 9.2	20.0 ± 0.8
48	0.0	27.0 ± 7.0	29.0 ± 5.8	31.0± 1.6	34.0 ± 2.2
72	0.0	37.0 ± 0 .0	49.0 ± 0.0	64.0 ± 0.0	67.0 ± 0.0

Values are means ± SE of three replicates.

As the concentration of the OP extract and the time elapsed after application increased, the mortality rates J2 also increased (Table 5). The effectiveness of the 25 and 50% OP concentrations were generally lower than other concentrations. The J2 mortality caused by the 100% OP application reached 8%, 20%, 34% and 67% at 12, 24, 48 and 72 h, respectively. Application of 75% OP caused higher mortality rates at 12, 24, 48 and 72 h than 25% and 50% OP application rates (Table 5). Mortality rates of J2 were between 0-67% for OP (Table 5). The highest death rate was achieved (67%) after 72 hours of 100% OP treatment. It was determined that GWH extract application was more effective in all doses compared to OP extract applications ($p<0.0001$, Table 6).

Length of exposure of GWH and OP extracts also had significant effects on nematode mortality. Results of the showed that J2 mortality rates were significantly affected by type of material, concentrations and the exposure time ($p < 0.0001$). Additionally, interaction between material and concentrations, material and time, concentrations, time and material were found to be significant at $p < 0.0001$. Application of GWH increased nematode mortality regardless of exposure time and concentration ($p < 0.0001$, Table 6).

In this research, exposure of second stage juveniles of *M. incognita* to OP and GWH extracts resulted in dose and time dependent increased mortality. In juvenile mortality test (*in vitro*) with the extracts, 100% mortality occurred within 72 h at 75% and 100% concentration, hence, GWH have strong nematicidal properties. Dama (7) reported that mortality was 97.9% in juglone at dose of 200 µg/10 ml/flask for 24 h exposure of *M. javanica.* Under *in vitro* conditions juglone is effective against the root-lesion nematode (*Pratylenchus thornei*) within 72 h of exposure (12). Recent results reported that juglone concentration of < 20 mg/kg of soil seemed to be safe for environment (4) however, more studies are needed.

Table 6. Tukey test for mortality rates of *M. incognita* juveniles at different exposure times and various concentrations of green walnut husk (GWH) and olive pomace (OP) extracts.

Plant material	Mortality rates of *M.incognita* juveniles (%)
GWH	43.01A
OP	21.23B

Different uppercase letters indicate statistical significance between materials (p < 0.0001).
OP : Olive pomace , GWH: Green Walnut husk

Gall Index (GI) and Egg Mass Index

As the concentration of GWH and OP increases, the egg mass indexes in the roots decrease because of the increased juvenile mortality (Table 5 and Fig. 1). 2% GWH application was the most effective treatment for nematode death and a similar result was also observed for GI. Egg mass index and GI showed great variation among treatments. Egg mass index ranged from 0.375 to 2.75 for GWH and from 0.875 to 1.875 for OP applications. GWH applications to soils were more effective on the reducing egg masses of the tomato root than OP applications (Fig. 1).

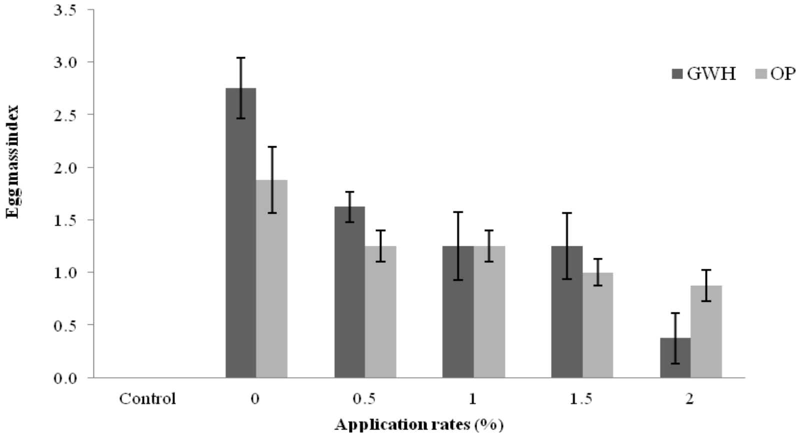


Figure 1. Mean egg mass index in tomato roots grown in green walnut husk (GWH) and olive pomace (OP) amended soil. Bars represent the standard error of the mean, n=4.

All concentrations tested caused a significant reduction in the egg mass index compared to the inoculated control (0% application rate; p < 0.001). Treating soil with 2%

GWH and 2% OP significantly reduced the egg mass index (Fig. 1). The efficacy on nematodes increased as the application rate increased in both GWH and OP treatments. Same reduction rates of *M. incognita's* egg mass indexes were observed with 1% applications of GWH and OP (Figure 1). GWH was more effective than OP in all doses applied, and this difference was statistically significant (p <0.01) when comparing the efficacy on egg mass index.

The effectiveness of the GWH and OP applications on the GI of tomato plants are presented in Fig. 2. Tomato plants grown in inoculated control (0%) had more galled roots, while plants grown on GWH and OP treated soils had moderate galling or absence of galls depending on the concentration used. Increasing concentrations of OP and GWH powder reduced the GI of tomato seedling roots. GWH (2%) applications reduced the GI of tomato seedlings by 85% while OP (2%) reduced GI of tomato seedlings by 53% compared to unamanded control treatment (Fig. 2). Significantly less root galling in 2% GWH rather than 2% OP (Fig. 2) indicates that GWH possesses one or more mechanisms to resist root-knot nematodes.

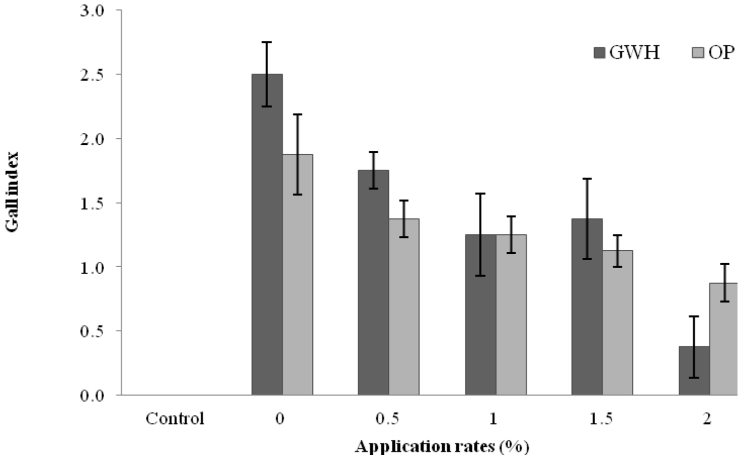


Figure 2. Gall index in tomato roots grown in GWH and OP amended soil. Root rating scale (0-5): 0=0 galls, 1=1-2, 2=3-10, 3=11-30, 4=31-100, 5>100 galls or egg masses of the root-knot nematode, *Meloidogyne incognita*. Bars represent the standard error of the mean, n=4.

The highest efficiency occurred at a 2% GWH and was significantly reduced knot formation of the root compared to other rates. Similarly 2% in OP applications significantly reduced galling on tomato roots compared to other OP rates. Both material and rate effects on galling severity were found to be statistically significant (p <0.0001).

GWH and OP amendments affected the GI and egg mass index, and these results are in agreement with D'Addabbo and Sasanelli (5) who showed that olive pomace reduced the nematode reproduction. Fekrat *et al*. (14) reported that the aqueous extracts of walnut leaf are nematicidal to *M. javanica*. The NPK fertilization had adverse effects on galling of root-knot nematodes (1). High nitrogen content in GWH and OP reduces in nematodes numbers in soil. Materials with low C/N or high content of ammonia will either result in plasmolysis of nematodes (38). In addition, a high electrical conductivity (EC), i.e., 7.78 mS cm^{-1} of GWH and high pH play a vital role to control those nematodes.

Tomato growth

The effects of ground GWH and OP application rates on tomato plant lengths are presented in Fig. 3. Effect of OP applications on root lenghts were statistically significant (Fig. 3). 1.5% OP dose, that increases the root length the most, followed by doses of 0.5, 1 and 2% (Fig. 4) (F=3.4 p<0.04). GWH application rates did not change root lengths significantly.

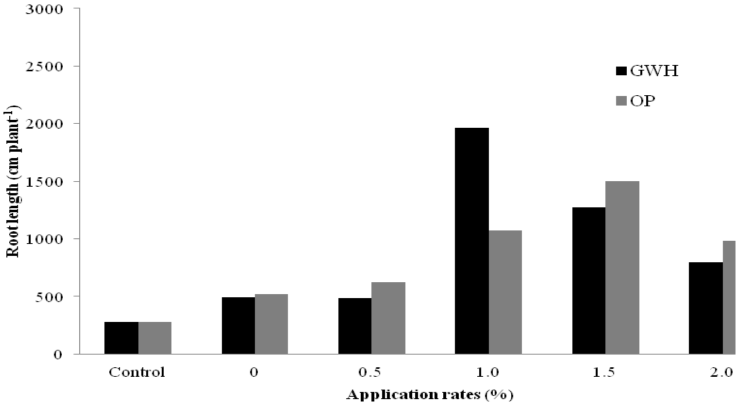


Figure 3. Effects of different rates of GWH and OP applications on tomato root root lengths (n = 4). Different letters above the bar indicate the significance of differences according to application rates (p<0.01, Tukey's test).

The effect of GWH application rates on the surface area of tomato root was not significant (p <0.67). Roots of control plant had no nematode infection, therefore, thickness of roots was smaller as compared to other treatments. Because of the thickening with nematode infection, root surface areas increased in infected plants compared to the control plants, although root development was not healthy in 0% and 0.5% GWH applications (Fig. 4). The largest root surface area and length was obtained in the 1% and 1.5% GWH applications, however, this difference was not statistically significant (Figures 3 and 4) because of large differences among the replications. The effect of OP application rates on the tomato root surface area was statistically significant (F=14,88, p <0.0001). The maximum root surface area was obtained at 1.5% OP application rate, followed by 1% and 2 % OP (Fig. 4). Application of both GWH and OP increased root development as compared to nematode infected "0%" doses.

After about 8 weeks of transplanting of the tomatoes, plant fresh and dry weight increased in all treatments compared to control (non-infected) and nematode infected "0%" doses (Table 7). The highest plant fresh weight was found in 1% rate (14.14 g) for OP and 2% rate (12.86 g) for GWH treatments however differences among rates were not statistically significant (p>0.05). The highest root fresh and dry weights were found in 1% GWH (5.17 g and 0.34 g respectively) and 1% OP treatments (3.72 g and 0.28 g respectively). GWH and OP applications resulted in greater plant and root weights in the soil as compared to control treatment. Both 2% GWH and OP applied plant roots had lower galls and better root development as compared to control plants.

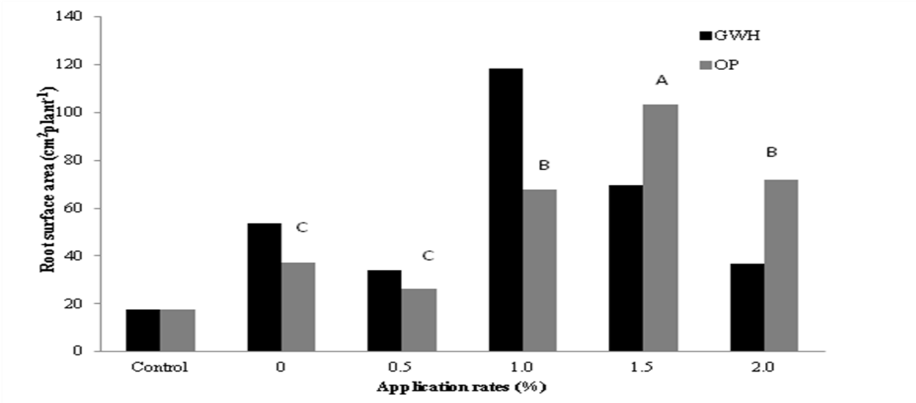


Figure 4. Effects of different rates of GWH and OP applications on root surface area of tomato plants (n = 4). Different letters above the bar indicate the significance of differences according to application rates (p<0.01, Tukey's test).

Table 7. Effects of different rates of green walnut husk (GWH) and olive pomace (OP) applications on root and stem weights and lengths of tomato plants. Values after ± represent the standard error of the mean (n = 4).

Treatments	Plant length (cm)	Root F.W. (g)	Root D.W. (g)	Plant F.W. (g)	Plant D.W. (g)
Control	43.63 ± 3.53	2.13 ± 0.55	0.16 ± 0.04	8.82 ± 2.29	1.00 ± 0.30
GWH-0%	44.00 ± 4.10	3.83 ± 0.48	0.30 ± 0.04	10.10 ± 2.45	0.87 ± 0.32
GWH -0.5%	45.63 ± 1.52	3.00 ± 0.62	0.23 ± 0.04	11.04 ± 1.71	1.22 ± 0.16
GWH -1%	44.00 ± 4.67	5.17 ±2.13	0.34 ±0.17	11.93 ± 3.23	1.33 ± 0.40
GWH -1.5%	37.50 ± 3.75	3.46 ± 0.89	0.25 ± 0.05	9.19 ± 2.56	1.06 ± 0.30
GWH -2%	43.63 ± 1.86	2.81 ± 0.82	0.21 ± 0.04	12.86 ± 1.85	1.42 ± 0.22
OP-0%	45.63 ± 3.51	2.85 ± 0.44	0.23 ± 0.03	10.54 ± 1.61	1.23 ± 0.19
OP-0.5%	49.75 ± 3.09	2.52 ± 0.66	0.20 ± 0.05	12.98 ± 2.68	1.39 ± 0.30
OP-1%	50.00 ± 3.11	3.72 ± 0.71	0.28 ± 0.05	14.14 ± 1.71	1.66 ± 0.18
OP-1.5%	44.50 ± 2.35	3.68 ± 0.69	0.26 ± 0.04	13.49 ± 2.35	1.47 ± 0.33
OP-2%	41.38 ± 1.25	3.28 ± 0.58	0.26 ± 0.03	12.69 ± 0.81	1.31 ± 0.13

OP : Olive pomace , GWH: Green Walnut husk

Another mechanism of GWH and OP incorporation on root-knot nematode might be related to the better plant growth and development. GWH and OP applications increased the tomato development, which resulted in higher root and shoot biomass (Table 7). An increase in root development provides an advantage in combating nematodes. Because as root length and mass increase, the amount of roots affected by nematode will remain low. If the plant roots are well developed the intake of the plant nutrients will continue and the nematode damage will be overcome. For this reason, organic materials are mixed with soil to reduce nematode damage. Roots showed the weakest development in non-amended soil but root development increased in GWH and OP amended soils (Fig. 3). Incorporating higher rates of GWH and OP into the soil, lead to healthy and much longer root systems.

Weak plants are more vulnerable to pest attack (2). Researches showed that the ability of a plant to resist insect, pests and diseases is related to optimal soil conditions (24). D'Addabbo *et al.* (6) reported that OP was phytotoxic at high doses, as application of 1, 2, 4 and 8% OP caused significant reduction in tomato growth compared to control plants. However in this research OP application up to 2% rate increased the tomato fresh and dry weight compared to control plants. Application of GWH and OP can effectively improve tomato production and suppress the root-knot nematode, *M. incognita*. Use of GWH and OP suppressed the nematodes without causing phytotoxicity to tomato plant. Results also confirmed the suppressivity of gound GWH and OP on *M. incognita* were related with application rates and similar results have also been reported previously for other concentrations of OP (5,6). The improved tomato growth and yields in plots or pots, where OP was applied were expected in responses to additions of organic matter to soil and agreed with previous studies (22,25). Additionally even at the highest concentration neither GWH nor OP did not produce signs of toxicity on tomato plants in contrast to results of Echeverrigaray *et al*. (11). They reported that use of borneol, carveol, citral, geraniol and α-terpineol showed the highest nematicidal activity at 100 and 250 mg kg^{-1} concentrations, however, at highest concentration they were toxic to tomato plants.

Decomposing green walnut husks and olive pomaces added different phenols including juglone to the soil and caused allelopathic effects. Increasing application rate of OP and GWH could reduce reproduction of nematodes but it may have negative allelopathic effect on tomato growth. On the other hand GWH and OP application rates up to 2% increased the tomato root and shoot dry and fresh weights than control (no-amandement) treatments. Both GWH and OP were able to reduced the *M. incognita* survival after short exposure periods. Since *M. incognita* can live within plant roots or in soil, the application of GWH and OP are suggested pre-planting to reduce the population densities of nematodes. It was concluded that certain agricultural wastes such as GWH and OP may be used as source of cheap and effective nematicides of root knot nematodes.

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