
Effect of Olive Pomace Alone or Combined with Poultry Manure on Biocontrolling of Root-Knot Nematodes *Meloidogyne* spp. Infecting Tomato

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ABSTRACT

This study was conducted to investigate the effectiveness of using olive pomace (OP) alone or in combination with poultry manure (PM) at mixing ratio of 75% OP :25% PM (w/w) as a safe alternative method for biocontrol of root-knot nematodes (*Meloidogyne* spp.) on tomato (cv. Elisa). The application rates of olive pomace (OP) alone or with poultry manure (PM) mixture were 20, 40 and 60 g /kg soil. It was found that, the highest application rate of OP and PM at 60 g/kg soil resulted in the highest reduction percentages of the studied root-knot nematodes (*Meloidogyne* spp.) infection parameters. Final population (Pf) and reproduction factor (RF) percentages of reductions under previous treatment recorded 97.73 and 97.75%, respectively. These results were comparable to those recorded with chemical nematicide (Vydate) treatment (98.50 and 98.45%), respectively. In contrary to root-knot nematodes (*Meloidogyne* spp.) parameters, higher OP application rate over 20 g/kg soil with or without poultry manure resulted in lower growth attributes for all tomato crop growth parameters including length, fresh and dry weights of shoots and roots as well as fruit fresh weights. The highest fruit fresh weight of tomato (471.50 g/plant) was recorded at 20 g/kg soil with PM mixture treatment with Pf and Rf percentages reductions of 94.20 and 94.13%, respectively. In conclusion, olive pomace and poultry manure mixture amendment at rate of 20g/kg soil could be recommended for integrated pest management (IPM) strategies against nematode pests. This approach provided effective nematode suppression, while also reduced the environmental impacts on soil-plant equilibrium, as compared to the use of chemical nematicides.

Key words: Olive pomace, poultry manure, root-knot nematodes, *Meloidogyne* spp., tomato.

INTRODUCTION

Tomato (*Solanum lycopersicum* Mill.) is one of the most important solanaceous crops. The cultivated area in Egypt of tomato was about 413.67 thousand feddans (MALR, 2018, 2019, 2020), representing about 22 % of the total vegetable cultivated area which amounting 1.9 million feddans during 2018-2020 (Faied and Elshater, 2022). The root-knot nematodes (RKNs), *Meloidogyne* spp. are one of the most widespread nematodes with severely injuring on tomato under Egyptian conditions. It causes serious damage to crop productivity (Ibrahim et al., 2010). The deleterious impacts of the chemical nematicides on both human and environment have prompted researchers worldwide to seek safe alternative measures for nematode control. This has become a major challenge for many scientists in the scientific community. Using of organic materials

pesticides is more attractive due to less persistent and little non-target, toxic impacts than agrochemicals (Cayuela et al., 2008).

The biocontrolling of plant-parasitic nematodes using soil amending by organic materials is a long-practiced strategy (Lazarovits et al., 2001). Under Egyptian conditions, the annual olive production is about 671.000 tons from which totally 30 % is used for olive oil production (EOC, 2021). On average 1000 kg produces 200 kg of oil whatever the extraction system used, it also produced the quantities in residues which varies between 400 to 800 kg of olive pomace (Medouni-Haroune et al., 2018). Such wastes of high organic loads as well as their composition are resistant to biological degradation. The olive wastes contain phytotoxic phenolic compounds which inhibit microbial activities (Capasso et al., 1992; Ramos-cormenzana et al., 1996) and adversely affect germination and vegetative growth of plants (Linares et al., 2003). During storage, phenolic compounds are condensed and converted to high-molecular-weight polymers which are difficult to degradation (Ayed et al., 2005). The uncontrolled disposal of such wastes has become a serious problem due to their polluting effects on both soil and water (Sierra et al., 2001; Piotrowska et al., 2006). The phytotoxic and antimicrobial effects of olive wastes are frequently approached as a negative attributes which limits the re-using of such organic materials (Cayuela et al., 2008). Thus, many methods have been developed to degrade the high phenolic content in olive residues (Marttirani et al., 1996; Linares et al., 2003; Greco et al., 2006). The biocidal effect of such wastes can be used to suppress plant pathogens (Cayuela et al., 2008).

Olive residues as a soil amendments, either unprocessed (Brunetti et al., 2005; Lopez-Pineiro et al., 2006) or composted (Cayuela et al., 2004; Hachicha et al., 2006) represents a promising practice under agro-ecosystems of olive cultivation (Cayuela et al., 2008). Olive pomace was demonstrated to be suppressive on soil phytoparasitic nematode populations (D'Addabbo et al., 1997; Radwan et al., 2009 and Kavdir et al., 2019). Sasanelli et al. (2002) found that composted pomace suppressed the gall formation on roots also at the lowest dosage. The population of *M. incognita* in the soil was significantly reduced by olive pomace amendment with no statically significant difference compared to fenamiphos. Additionally, no differences were found among the different amendment rates (D'Addabbo et al., 2009). Cayuela et al. (2008) found that application of olive pomace has suppressed penetration of roots by J2, disrupting the life cycle of the nematode. Chitwood (2002) reported that phenolic compounds such as pyrocatechol caffeic acid, vallinic acid and fatty acid derivatives were among the phytochemical compounds with nematicidal capability. Kavdir et al. (2019) found that olive pomace reduced *M. incognita* populations by 53% compared to control. Radwan et al. (2009) found that *M. incognita* population in the soil and root galling were significantly reduced, when olive pomace was added to the soil.

The highest rate of olive pomace (50 g/kg soil) was the most effective in reduction J2 by 92%. The high pollution potential of olive pomace is due to its high content of phenolic and antimicrobial impacts, which hinder its biodegradation. Co-composting olive pomace with some organic wastes like agricultural residues is one of the approaches which can be used for activation of biological decomposition of such high decomposable resistance wastes. The preferred approach has been co-composting with other residues, cattle manure and poultry farming (Hachicha et al., 2006).

Therefore, the main objectives of the current study were to compare the effect of olive pomace (OP) alone or combined with poultry manure (PM) on controlling root-knot nematodes (*Meloidogyne* spp.) (RKNs) on tomato crop.

MATERIALS AND METHODS

Collection of olive pomace (OP) and poultry manure (PM):

Olive pomace and poultry manure were collected from both Olive Oil Mill and Poultry Farm of Faculty of Agricultural Environmental Sciences, Arish University with geographical coordinates N 55° 07' 31": E 12° 48' 33". Both two organic wastes were left to drying for 3 weeks and then well – ground and sieved through 1 mm aperture stainless screen. Olive pomace and poultry manure were mixed with the ratio of 75% OP: 25% PM W/W, then stored till using.

Pot experiment :

Pot experiment using tomato, *Solanum lycopersicum* (cv. Elisa) was carried out in plastic pots (15 cm diameter) with no water excesses to prevent leaching water. Pots were filled with 2 kg/pot naturally infested loamy sand soil (sand, 86.44%; silt, 11.63% and clay 1.93%) with 12000 infective juveniles (*Meloidogyne* spp.)/kg soil as initial population (Pi). The experiment consisted of six treatments of olive pomace and / or poultry manure in addition to check (control) and comparative Vydate chemical nematicidal treatments. Hence, the studied treatments were as follows: Three different rates of olive pomace (20, 40 and 60 g/Kg soil) were used in combination with or without poultry manure (75%+25%) compared with Vydate chemical nematicide. The treatments were arranged in a randomized complete block design (RCBD) in four replications.

Organic amendments were thoroughly mixed with the soil then irrigated to field capacity (FC) of the soil (Kagai et al., 2012). Each pot was covered with thin layer of transparent plastic sheet (Anita, 2012; Sherbiny et al, 2014). The soil moisture was kept at FC during all decomposition period of 30 days. After that, thin plastic sheets were removed. Vydate chemical nematicide was mixed with the soil according to recommended dose application. Forty days old, healthy and uniform tomato (cv. Elisa) seedlings were transplanted at the rate of one seedling/pot. All tomato potted plants were managed by standard agricultural practices through the growing season. After twelve weeks of transplanting, tomato plants were uprooted. Roots were washed under tap water to remove soil particles. The length of shoots and roots, fresh and dry weights of tomato plant (shoot and root) and fresh fruit weight were recorded. The roots were cut into small pieces and nematode eggs were extracted using NaOCl and counted (Hussey and Daykin,1973). Number of galls, developmental stages, females, egg masses and numbers of eggs/egg mass were assessed (Southey,1986). Soil nematode population was enumerated by extraction root-knot nematode juveniles (J2s) using the tray modification of Baermann funnel (Barker,1985). Final population (Pf) was assessed according to the equation: $Pf = (\text{No of egg masses} \times \text{No. of eggs/ egg mass}) + \text{No. of females} + \text{No. of developmental stages} + \text{No. of juveniles in soil}$, as described by Mousa et al. (2018), $RF = Pf/Pi$, $Pi = \text{initial population}$ (Sasser et al., 1984).

Olive pomace chemical analysis:

Polyphenolic extracts were prepared according to Chtourou et al. (2004) and then analyzed using the Folin-Ciocalteu reagents according to the colorimetric method described by Box (1983). The percentages of total organic carbon (TOC %), total nitrogen (TN %) and C/N ratio were calculated (Paredes et al., 2005) (Table 1).

Table 1: Chemical characteristics of raw olive pomace, poultry manure and olive pomace-poultry manure mixture (dry weight base)

Parameters	Olive pomace (OP)	Poultry manure (PM)	Olive pomace: poultry manure mixture (75%OP:25%PM w/w)
PH	6.83	8.35	8.22
Water content (%)	33.51	37.62	36.30
TOC(%)	47.60	35.21	39.38
TN(%)	0.69	5.71	0.97
C/N	68.99	6.17	40.60
Poly phenols (%)	0.89	0.12	0.70
TOC= Total organic carbon		TN=Total nitrogen	

Statistical analysis:

Analysis of variance (ANOVA) was conducted to compare the studied treatments using a SAS program. Means were compared using Fishers protected LSD values at 5 % level of probability (SAS, 1997).

RESULTS**Effect of olive pomace alone or mixed with poultry manure on parameters of *Meloidogyne* spp. infected tomato plants**

Data in Table (2) and Fig (1) illustrated the effect of olive pomace (OP) alone or combined with poultry manure (PM) on root- knot nematodes, *Meloidogyne* spp. parameters. Concerning number of galls and egg masses per root system, Vydate chemical nematicide showed the highest reductions with percentages of decreases of 96.07 and 91.72 % compared to control treatment. Application of olive pomace alone at all studied treatments resulted in progressive decreases in numbers of galls and egg masses with significant ($P \leq 0.05$) reductions in values of 52.00 (50.50), 35.25 (30.25) and 28.25 (28.25) at 20, 40 and 60 g/kg soil, respectively. These values corresponded to percentages of reductions of 88.39 (65.88), 90.77 (79.22) and 92.60% (84.29%), respectively.

Mixing amendment of poultry manure with olive pomace at 20, 40 and 60 g/kg soil resulted in decreases in gall and egg mass numbers to 44.00 (32.50), 30.75(27.50) and 21.00 (20.50) with percentages of reduction of 88.48 (78.07), 91.95 (81.42) and 94.50% (86.15%), respectively.

The same trend was found with numbers of developmental stages per root system and eggs per egg mass. Since, application of Vydate at the recommended dose gave the best results in suppressing numbers of nematodes within root system as well as number of eggs per egg mass, followed application of OP alone at 20, 40 and 60 g/kg. Moreover, combined addition of olive pomace with poultry manure increased the efficiency of OP in reducing *Meloidogyne* spp. infection on tomato plants. Regarding the effect of the tested treatments on numbers of infective juveniles in soil, final nematode population and reproduction factor, it was clear that Vydate treatment was superior in significantly lowering these criteria with percentages reaching to 89.50 %, 98.50% and 98.45% compared to control treatment. Adding olive pomace alone at 20, 40 and 60g/kg of soil or mixed with poultry manure reduced numbers of second stage juveniles in soil with

Table 2: Effect of olive pomace alone or combined with poultry manure on percent reduction of root-knot nematodes, *Meloidogyne* spp. parameters compared to control treatment.

Treatments	Galls/root system	Egg masses/root system	Eggs/Egg mass	Females /root system	Developmental stages/root system	J2s/250g soil	Final Population	Reproduction factor
Vydate	96.07	91.72	88.72	94.03	92.4	89.5	98.50	98.45
20g OP	88.39	65.88	26.94	77.14	35.92	74.25	74.68	74.61
20g OP +PM	88.48	78.07	76.22	83.64	68.65	82.77	94.20	94.31
40g OP	90.77	79.22	81.08	86.23	73.15	83.82	95.52	95.51
40g OP+PM	91.95	81.42	81.8	88.18	77.5	88.71	96.2	96.2
60g OP	92.6	84.29	84.73	90.39	80.33	88.71	97.2	97.24
60g OP+PM	94.5	86.15	86.12	91.3	86.54	89.24	97.73	97.75

OP= Olive pomace PM= Poultry manure.



Figure 1: Effect of olive pomace and poultry manure on root-knot nematodes (*Meloidogyne* spp.) studied parameters

values and percentages of 74.25 (82.77%), 83.82 (88.10%) and 88.71 (89.24%), respectively (Fig.1).

Similarly, higher olive pomace, with or without poultry manure, the greater percentages of reductions of final population and consequently reproduction factor (Fig.1). Values and percentages of reductions in final population and reproduction factor, when olive pomace was applied alone at 20, 40 and 60 g/kg soil were 74.68 (74.61 %), 95.52 (95.51 %) and 97.20 (97.24%), respectively. When olive pomace added with poultry manure, these values and percentages increased to 94.20 (93.26%), 96.20 (96.20%) and 97.73 (97.75%), respectively. Generally, it could be concluded that the nematicidal effects of olive pomace and poultry manure applied alone or in mixture at 75% OP:25% PM treatments significantly ($P \leq 0.05$) reduced all studied root-knot nematode parameters with different magnitudes. As the application rate of olive pomace increased from 20 to 40 and 60g/kg soil, values and percentages reductions in galling and reproduction parameters of root-knot nematodes also decreased. Moreover, adding poultry manure increased the efficacy of olive pomace in controlling root- knot nematodes infecting tomato.

Effect of olive pomace alone or mixed with poultry manure on growth parameters of tomato plants infected with *Meloidogyne* spp.

Obtained data in Fig. (2) revealed that Vydate treatment significantly ($P \leq 0.05$) increased length, fresh weight and dry weights of shoots and roots as well as fruit fresh weight of tomato plants compared to control treatment. For instances, application of Vydate resulted in significantly ($P \leq 0.05$) higher shoot fresh weight (686.75g/plant), root fresh weight (218.00g/root) and fruit fresh weight (485.25g/plant) compared to control treatment (358.00g/plant), (197.00g/root) and (303.75g/plant), respectively. This trend was also observed in other tomato growth parameters i.e. length and dry weight of shoots and roots as well as shoot/root dry weight. On the other hand, application of olive pomace with poultry manure mixture at 20 g/kg soil significantly ($P \leq 0.05$) increased all studied tomato growth parameters compared other treatment (Fig.2).

Moreover, mixing poultry manure increased efficacy of olive pomace in controlling root-knot nematodes and consequently enhanced all plant growth parameters of tomato plants. For examples, amending soil with 20g/kg soil of olive pomace alone or mixed with poultry manure obviously increased lengths of tomato shoots and roots to 36.13, 12.73cm, and 48.00, 14.30cm compared to control treatment 26.33 and 9.20cm, respectively.

Similarly, olive pomace alone or applied with poultry manure increased shoot fresh and fruit fresh weights to 430.50, 656.25 and 424.00, 471.50g, compared to control treatment 358.00, and 303.75g, respectively. Conversely, application of olive pomace at 40 and 60g/kg soil alone or combined with poultry manure significantly ($P \leq 0.05$) decreased all measured tomato growth parameters compared to control treatment (Fig.2). In most cases, adding poultry manure to olive pomace treatments 40 or 60g /kg soil showed slightly insignificant effect in tomato growth parameters.

DISCUSSION

The use of organic soil amendments represents one of the possible alternatives to chemical nematicides in the control of nematodes. Obtained data showed that application of olive pomace at 20 g/kg soil alone or in combination with poultry manure significantly enhanced plant growth characterizes i.e shoot and root lengths, shoot and



Figure 2: Effect of olive pomace and poultry manure application on tomato crop growth attributes as influenced by *Meloidogyne* spp. Different Letters means significant difference between treatments at P<0.05

root fresh and dry weights and fruit fresh weight of tomato, while the lowest effect was recorded at 60 g/kg soil. According to Radwan et al. (2009), the low application rate of olive pomace significantly increased growth indices of tomato over control treatment, while phytotoxicity was associated with the higher rates (50 g /kg soil). The phytotoxic properties of olive pomace residues are mainly due to phenolic compounds as well as free fatty acids. Ait Baddi et al. (2009) reported that although the addition of olive pomace did not affect the growth of tomato plants, a significant reduction of plant top weight at the highest dosage (5% w/w) revealed a phytotoxic effects.

Results in the present study also indicated that olive pomace alone or mixture with poultry manure were significantly effective ($P \leq 0.05$) in reducing all nematode parameters up to 60g /kg soil. Analysis of the nematode parameters showed that all studied nematode parameters were significantly ($P \leq 0.05$) reduced compared to control plants. Hence, olive pomace at 60g /kg soil alone or mixed with poultry manure recorded the highest percentage of reduction in final population and reproduction of nematode amounted to 97.20, 97.24 and 97.73, 97.75% compared to control treatment, respectively. Such reductions were comparable to those of Vydate chemical nematicide (98.50 and 98.43, respectively). According to D'Addabbo and Sasanelli (1996) amending the soil with olive pomace at 20 g/kg soil +poultry manure suppressed the population of *Meloidogyne* spp. both in tomato soil and on roots with concomitant increases in the growth and yield of tomato. Similar findings were recorded with Nico et al. (2004) who reported that soil amendment with poultry manure, decomposed waste products and dry olive mark reduced *Meloidogyne* spp. by 24.4 to 87.9%. In addition, Radwan et al (2009) reported that application of olive pomace (50g /kg) soil gave higher activity against root-knot nematode both in soil and tomato roots than using low rate (5 g/kg soil). Cayuela et al. (2008) pointed out that antagonistic activity can play the key role in disease suppression, since bioactive compounds released from olive pomace could pass through the nematode egg shell, inhibit J2s motility by 95%, suppress penetration of roots by J2s which finally disrupting the life cycle of nematode.

Rodriguez-Kabanna et al. (1995) demonstrated that olive pomace either unprocessed or composted with poultry manure was more effective method for minimizing hazard phytotoxic effects of such phenolic compounds. The polyphenols turnover and their biodegradation involved lead to non-toxic end-product which can use as organic-soil amendments. Such effects may be due to the high nitrogen content in poultry manure compared to olive pomace. Due to the relatively lower C/N ratio of poultry manure (6.17), mixing with olive pomace resulted in lowing C/N ratio to 40.60 of the mixture which could increase decomposition processes. This result on C/N ratio was on line with the results obtained by El-Nagdi and Youssef (2019). The present experiment indicated that phytotoxicity was avoided, when olive pomace was mixed with poultry manure at 20 g/kg soil which improved tomato crop parameters and caused more suppressive on nematode infection parameters than olive pomace alone. Such effects could be attributed to the synergetic effects of high nitrogen content in poultry manure (5.71%) compared to olive pomace (0.69%). Thus, lower dosages could be used to achieve the same nematicidal effect when used as combined with poultry manure. However, the highest rates of olive pomace alone or combined with poultry manure revealed a phytotoxicity on tomato growing plant that made such rates practically useless.

According to obtained results of tomato crop growth attributes, amending soil with olive pomace at 20g /kg soil combined with poultry manure could be used for RKNs (*Meloidogyne* spp.) management although the significantly ($P \leq 0.05$) highest reductions of all root-knot nematode parameters were found under corresponding treatment of 60 g/kg soil. Such findings may indicate that poultry manure possesses a synergetic effect to mitigate the harmful effects of olive pomace on all tomato crop growth attributes.

In conclusion, soil amendments with low dosage of olive pomace in mixing with poultry manure at the rate of 75 % OP +25 % PM are very beneficial in the biocontrolling of root- knot nematodes, *Meloidogyne* spp. in tomato cultivation and could be recommended to be one component in integrated nematode management in conventional and organic production agriculture system. However, more research activities are needed to determine the actual contribution of olive pomace towards controlling these nematodes which contain toxic compounds in such organic amendments.

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الملخص العربي

تأثير تفل الزيتون منفردا او مع سماد الدواجن فى مكافحة نيماتودا تعقد الجذور التى تصيب الطماطم

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اجريت تجربة اصص تحت ظروف الصوبة لدراسة تأثير استخدام كل من تفل الزيتون منفردا او مخلوط مع سماد الدواجن بنسبة ٧٥% تفل زيتون : ٢٥% سماد دواجن بمعدلات اضافة للتربة ٢٠ و ٤٠ و ٦٠ جرام / كجم تربة فى مكافحة نيماتودا تعقد الجذور *Meloidogyne spp.* فى محصول الطماطم صنف Elisa. ادت الزيادة المتتالية فى معدلات اضافة تفل الزيتون + سماد الدواجن الى انخفاض معنوى فى كل مؤشرات الاصابة بالنيماتودا مما ادى الى انخفاض فى كل من التعداد النهائى للنيماتودا وكذا قيمة معامل تكاثر النيماتودا معنويا مقارنة مع النباتات المصابة فقط (المقارنة) حيث حققت المعاملة بالمبيد الكيماوى (فايديت) اعلى نسبة خفض فى التعداد النهائى بمعدل ٩٨,٥% وقيمة معامل تكاثر النيماتودا الى ٩٨,٤٥% مقارنة مع اكبر معدل انخفاض قدرة ٩٧,٧٣% و ٩٧,٧٥% وذلك فى المعاملة ٦٠ جرام/كيلو جرام تربة +سماد الدواجن. ادت المعاملة بالمبيد فايديت الى اكبر زيادة فى جميع مؤشرات نمو نباتات الطماطم تليها المعاملة ٢٠ جرام / ١٠٠ جرام تربة+سماد الدواجن وكان الوزن الطازج للثمار ٤٨٥,٢٥ جرام/نبات مقارنة بمعاملة الكنترول (٣٠٣,٧٥ جرام/نبات). ادت زيادة معدلات الاضافة من تفل الزيتون ٤٠ و ٦٠ جرام / كيلو جرام سواء منفردا او مخلوط مع سماد الدواجن الى انخفاض جميع مؤشرات نمو النبات وكان اكبر انخفاض فى وزن الثمار الطازج فى المعاملة ٦٠ جرام/كيلو جرام بدون او مع سماد الدواجن حيث سجل (٢٥١,٧٥ و ٢٥٣,٢٥ جرام /نبات) مقارنة بمعاملة الكنترول (الشاهد) (٣٠٣,٧٥ جرام/نبات) من هنا يتضح ان استخدام تفل الزيتون +سماد الدواجن بمعدل ٢٠ جرام / كيلو جرام تربة قبل الزراعة تعتبر طريقة واعدة لاعادة استخدام تفل الزيتون بطريقة امنة بيئيا وغير مكلفة اقتصاديا مقارنة باستخدام المبيدات الكيماوية وذلك فى نظم مكافحة متكاملة وكذا فى حالة الزراعة العضوية حيث سجلت تلك المعاملة انخفاض فى مؤشرات الاصابة بالنيماتودا بمعدل 94.20 و 94.13% فى كل من التعداد النهائى للنيماتودا ومعامل التكاثر النيماتودى على الترتيب مع زيادة الوزن الطازج لثمار الطماطم الى ٤٧١,٥٠ جرام/نبات مقارنة بمعاملة الكنترول (٣٠٣,٧٥ جرام/نبات).