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GROWTH AND NODULATION OF PEANUT GROWN IN SANDY SOIL AMENDED WITH OLIVE WASTES

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ABSTRACT

The effect of untreated olive mill wastewater (OMW) and crude olive cake as organic fertilizers, alone or in mixture with poultry manure, on nodulation and growth of peanut plants was investigated in a pot experiment under sandy-soil conditions. The application of low concentrations of either OMW or olive cake significantly increased the number and dry weight of nodules, shoots dry weight and N-content after 50 days of planting. The addition of poultry manure alone or in combination with olive wastes decreased all the abovementioned parameters. Plants fertilized with OMW combined with poultry manure scored the minimum number and dry weight of nodules. Incorporation of olive wastes into soil, with or without poultry manure, decreased nodulation parameters after 70 days of planting. Addition of OMW decreased shoots dry weight and N-content. Generally, soil amended with olive cake exhibited less toxicity compared to soil fertilized with OMW. Amendment with olive wastes and/or poultry manure increased the total flavonoids and soluble phenols of peanut plants compared with those inoculated with *Rhizobium* spp. only. The increase of total soluble phenols was insignificant.

It is concluded that the application of OMW or crude olive waste with or without poultry manure caused inhibitory effect on nodulation and growth of peanut plants in sandy soil.

Key words: *nodulation, olive cake, olive mill wastewater, rhizobium.*

1. INTRODUCTION

Olive oil extraction produces vast amounts of residues, in the form of liquid and solid wastes. Olive mill waste water (OMW) and crude olive cake are produced as waste-products in olive oil factories. Olive mill waste water, commonly called alpechin, is the main waste produced by three-phase extraction of olive oil. The treatment and disposal of this liquid are the main problems of olive-oil industry. The phytotoxic properties of OMW are mainly attributed to the relatively high concentration of polyphenols, which are also known to possess antibacterial properties which resist biological degradation (Perez *et al.*, 1992 and Aktas *et al.*, 2001). Crude olive cake also contains phenolic compounds which are toxic to many plant species and resistant to degradation (Estaun *et al.*, 1985 and Gonzalez *et al.*, 1990).

The advantage of using olive wastes as organic fertilizers is their high contents of plant nutrients such as N, P, K and organic matter, (Paredes *et al.*, 1999). The disadvantage mainly is the high contents of phenols which are toxic to soil and plants (Tomati and Galli, 1992; Sierra *et al.*, 2001; Casa *et al.*, 2003 and Cereti *et al.*, 2004).

Nodulation, nitrogen fixation and growth in legume-*Rhizobium* symbiosis are affected by the application of untreated olive wastes as an organic fertilizer. Al-kahal, *et al.* (2001) found that increasing the levels of olive-oil wastewater or olive-cake suppressed nodulation and growth of faba bean plants cultivated in a clay loam soil after 80 days from planting in the presence of *Bradyrhizobium* spp.. Mekki *et al.* (2006) observed that untreated olive mill wastewater inhibited germination and growth of tomato, chickpea, bean, wheat and barley. Hence, a conditioning treatment of this waste is compulsory to safely produce organic fertilizers. Some authors have pointed out that the toxicity of olive wastes introduced into soil is reduced when mixed with different nitrogen sources such as urea, polymers, anhydrous ammonia or poultry manure. The

mixture of olive wastes and such N sources is characterized by a wider C/N ratio, which stimulates microbial degradation of the phytotoxic compounds of olive wastes (Rodriguez-Kabana *et al.*, 1995).

The aim of the present investigation was to examine the effect of either olive mill wastewater or olive cake alone or in combination with poultry manure on nodulation and growth of peanut plants in sandy soils.

2. MATERIALS AND METHODS

A pot experiment was executed in the greenhouse of the Agricultural Research Center at Giza – Egypt. The soil used was sandy (pH, 7.1; EC, 0.47 dSm^{-1}; organic carbon, 0.25%; total nitrogen, 0.01%). The soil was distributed into earthenware pots at the rate of 10 kg / pot. The used poultry manure has 60% organic matter, 2.50 % total N, 0.21% total P and 0.92% total K. Olive mill wastewater and crude olive cake were obtained from Olive Oil Extraction Unit, ARC - Giza. The major properties of these products are presented in Table (1).

Air dried crude olive cake and the poultry manure were ground and incorporated into soil at a rate of the 20 g/10 kg soil. All pots received super-phosphate at a rate of 2 g/pot (200kg/feddan), and potassium sulfate (48% K_2O) at a rate of 0.5 g/pot, as 24 kg K_2O/fed. Pots were initially kept for 2 weeks to facilitate decomposition of the introduced organic materials. This was followed by allocating the six experimental treatments with six replicates as follows: inoculated plants (peanut seeds inoculated with *Rhizobium* spp. as a control; plants inoculated with *Rhizobium* spp. and amended with OMW at a rate of 20 ml/10kg soil; plants inoculated with *Rhizobium* spp. and amended with poultry manure at a rate of 20 g/10kg soil; plants inoculated with *Rhizobium* spp. and received olive cake and poultry manure; plants inoculated with *Rhizobium* spp. and treated with olive cake along with poultry manure. *Rhizobium* spp. inoculum was prepared by growing the bacteria, in shake flasks, on yeast extract mannitol medium, YEM (Vincent, 1970) at 30°C for 3 days until early log phase (5×10^9 cfu/ml), then transferred to sterile fine peatmoss neutralized with 5% $CaCO_3$. Seeds were inoculated at a rate of 400g inoculum /40 kg seeds/ feddan using Arabic gum solution (16%) at a rate of 400 ml/ feddan.

Table (1): Some physiochemical properties of OMW and crude olive cake.

Character	OMW	Crude olive cake
Moisture content %	80	7
pH	4.9	4.7
EC dSm^{-1}	18.16	3.45
Organic matter %	40.00	55.00
Total Nitrogen %	1.46	0.96
Protein content %	6.20	8.20
Organic carbon%	23.23	32.47
C/N ratio	16	34
Fat %	0.50	6.80
Ash %	3.20	3.52
Total P %	0.20	0.16
Total K %	5.20	1.14
Total phenols %	1.00	1.20

OMW was added at seed sowing. Eight seeds of peanut cv. Giza 6 were sown in each pot. Pots were arranged in a complete randomized block design. Pots were watered when needed. Plants were thinned to 3 plants/ pot after one week. Nitrogen fertilizer was added two weeks after sowing at a rate of 1 g/ pot as ammonium sulfate (20.5 %). Plant samples were collected after 50 and 70 days of planting to determine nodules number and dry weight besides shoots dry weight. N-content of dried peanut shoots was determined by micro-kejldahel method (Page *et al.*, 1983). Data were subjected to analysis of variance using the statistical analysis system computer package M-STAT (Snedecor and Cochran, 1989).

3. RESULTS AND DISCUSSION

3.1. Nodulation and growth of 50-day old peanut

Data shown in Table (2) present the effect of OMW and crude olive cake alone or in combination with poultry manure on the nodulation status of peanut plants after 50 days of planting. The application of either OMW or olive cake as an organic amendment, at low concentrations, caused a significant increase in the number and dry weight of nodules compared with the plants inoculated with *Rhizobium* spp. only. Addition of poultry manure, as such, led to insignificant decrease in the number and dry weight of nodules. Plants supplied with poultry manure together with OMW scored the lowest number and dry weight of nodules.

In spite of untreated olive wastes (either OMW or olive cake) being characterized by the presence of phytotoxic substances, mainly phenolic compounds, which may affect symbiotic nitrogen fixation by *Bradyrhizobium* (Tomati and

Table (2): Nodulation and growth of peanut plants as affected by the application of olive wastes and poultry manure after 50 days of planting.

Treatment	No. of nodules/ plant	Dry weight of nodules (mg/ plant)	Dry weight of shoots (g/plant)	N- content of shoots (mg/plant)
Control (*Rhizobium*)	**62**	**0.33**	**13.48**	**3.08**
OMW	**85**	**0.41**	**15.34**	**3.78**
Olive cake	**102**	**0.42**	**15.58**	**3.57**
Poultry manure (P.M.)	**54**	**0.24**	**13.87**	**3.22**
OMW.+ P.M.	**24**	**0.15**	**13.73**	**2.52**
Olive-Cake.+ P.M.	**53**	**0.24**	**14.83**	**2.17**
L.S.D. at 5%	**12**	**0.6**	**2.16**	**0.80**

Galli, 1992), some investigators reported that some strains of *Bradyrhizobium* spp. are able to utilize a wide range of aromatic compounds such as phenolics as carbon or nitrogen sources. Gajendrian and Mahadevan (1990) reported that *Bradyrhizobium* spp., isolated from *Labab purpureus*, survived for nine months in soil containing the phenolic derivative catechol. Also, Chen *et al.* (1984) mentioned that *Rhizobium leguminosarum* was able to grow on a number of organic compounds and utilized 4-hydroxybenzoate in the presence of glucose and succinate. Abbas (2000) studied the effect of olive cake, at very low concentrations, on Leucaena plants grown in sandy soils. He found that olive cake increased the number of nodules over that due to inoculation with *Bradyrhizobium* spp. only. Al-Kahal (2001) found that faba bean cultivated in clay soil amended with olive oil wastewater at a rate of 1% had higher number and dry weight of nodules after 50 days of planting compared with plants inoculated with *Rhizobium leguminosarum* only. On the other hand, peanut plants fertilized with poultry manure, as a source of organic fertilizer, in the presence of rhizobial inoculation exhibited a reduction in the number and dry weight of nodules. This might be attributed to the high N content of poultry manure; the majority of N is in the form of uric acid that can be rapidly converted to ammonium nitrogen if temperature, moisture and pH are suitable for microbial activity (Sims and Woelf, 1994).

Heavy N dressing inhibited nitrogenase activity and this, in turn, led to a decrease in symbiotic nitrogen fixation. Addition of OMW, even at low concentration, along with poultry manure recorded the lowest numbers and dry weights of nodules.

Concerning the effect of olive wastes, alone or in combination with poultry manure, on the dry weight and N-content of peanut shoots at 50-day growth period, there was an increase in dry weight of shoots in all treatments compared with the plants inoculated with *Rhizobium* spp. only, but this increase was insignificant. Addition of olive wastes or poultry manure increased N-content compared to inoculation with *Rhizobium* spp. only. On the other hand, N-content of the plants treated with a combination of OMW and poultry manure was decreased. This decrease was also observed in the plants amended with olive cake and poultry manure. These results indicate that the addition of either OMW or olive cake, with or without poultry manure, somewhat had a stimulative effect on peanut shoot biomass. This is probably due to the high organic matter content of olive wastes, along with the notable levels of nitrogen in both OMW and olive cake (Paredes *et al.*, 1999).

3.2. Nodulation and growth after 70 days of planting

Results of the influence of olive wastes application alone or in combination with poultry manure on nodulation status and growth of peanut plants after 70 days of planting are given in Table (3). There was an inhibitory effect due to application of olive wastes or poultry manure in all treatments compared to the control. The highest number and dry weight of nodules were recorded in the plants inoculated with *Rhizobium* spp. only, followed by those inoculated with *Rhizobium* spp. simultaneously amended with olive cake. The minimum values of the abovementioned parameters were recorded in the plants fertilized with either OMW plus poultry manure or those amended with olive cake and poultry manure.

Table (3): Nodulation and growth of peanut plants as affected by the application of olive wastes and poultry manure after 70 days of planting.

Treatment	No. of nodules/ plant	Dry weight of nodules (mg/ plant)	Dry weight of shoots (g/plant)	N- content of shoots (mg/plant)
Control (*Rhizobium*)	**200**	**0.53**	**22.49**	**4.72**
OMW	**175**	**0.37**	**19.47**	**4.60**
Olive cake	**178**	**0.49**	**22.24**	**3.85**
Poultry manure (P M)	**123**	**0.37**	**21.37**	**2.94**
OMW+ P M.	**109**	**0.38**	**21.87**	**3.36**
Olive-Cake.+ P M	**141**	**0.45**	**23.54**	**3.99**
L.S.D. at 5%	**10**	**0.10**	**8.19**	**1.30**

Suppressive effect of olive wastes on the nodulation status in peanut plants cultivated in sandy soil was obvious after 70 days from planting, this is probably due to increasing the concentration of phenolic compounds in the soil after degradation of olive wastes, while the application of poultry manure led to significant reduction in the number and dry weight of nodules compared to the plants inoculated with *Rhizobium* spp. only. Addition of poultry manure along with olive wastes did not diminish the toxicity of olive wastes in terms of the number and dry weight of nodules compared with the plants amended with olive wastes only.

Table (3) shows that peanut, treated with *Rhizobium* spp. and amended olive cake, scored the highest shoots dry weight followed by plants inoculated with *Rhizobium* spp. and amended with a mixture of olive cake and poultry manure, however, this increase was insignificant. Plants inoculated with *Rhizobium* spp. only gave the highest N-content followed by those inoculated with *Rhizobium* spp. and fertilized with OMW. In this respect, Ciafardini and Zullo (1998) studied the effect of OMW on the symbiotic activity of *Rhizobium meliloti* and *Rhizobium hedysarii* in soil and in sand, and found that the soil protects the symbionts from the toxic action of OMW better than sand trials.

3.3. Influence of olive wastes and/or poultry manure on total flavonoids and total soluble phenols

Table (4) reveals that there was an increase in total flavonoids and soluble phenols in the peanut plants' dry-weight in all treatments with either olive wastes or poultry manure compared with those inoculated with *Rhizobium* spp. only. The increase of total flavonoids was significant in the first period while the increase was insignificant in the concentrations of soluble phenols in both periods, This indicates that the addition of olive wastes at a low concentration and/or poultry manure as organic amendments is considered a source of phenolic compounds in the soil and in plants as well.

Addition of poultry manure scored the highest value of total flavonoids in peanut plants after 50 days, while addition of O M W combined with poultry manure scored the highest values of flavonoids and total soluble phenols after 70 days. As mentioned before, peanut plants fertilized with poultry manure reduced the number and dry weight of nodules after 50 and 70 days of planting. This was attributed not only to the excess of N provided by poultry manure that inhibit nitrogenase enzyme activity, but also to the high content of phenolic compounds in poultry manure that may affect the nodulation processes.

Therefore, such negative effects of olive wastes and or poultry manure even at low concentrations are associated with the presence of phytotoxic compounds especially high content of phenolics, as reported by (Paredes *et al.*, 1999).

From these results, it could be concluded that the application of olive wastes, either in the form of OMW or crude olive cake, at low concentrations in sandy soil had a suppressive effect on the nodulation and growth of peanut plants in the absence or presence of poultry manure. Such negative effects are associated with its low pH, and mainly to the presence of phytotoxic compounds, especially phenols. Negative effects have also been recorded in soil properties, including the immobilization of available nitrogen (Perez and Gallardo-Lara,

Table (4): Effect of olive wastes and/or poultry manure on total flavonoids and total soluble phenols in the dry weight of peanut plants.

Treatment	Flavonoids%		Soluble phenols%	
	50-days old plants	70-days old plants	50-days old plants	70-days old plants
Control	3.661	3.189	2.311	1.768
O M W	4.000	3.833	2.574	2.61 4
Olive cake	4.267	4.120	2.903	2.68 5
Poultry manure	5.057	4.503	3.406	2.660
O M W + P M	4.420	4.63 7	2.742	2.865
Olive cake+ P M	4.905	3.570	3.472	2.043
L.S.D. at 5%	1.295	1.561	1.226	1.473

1987), the displacement of the exchange complex calcium by potassium in an anfisol, increased salinity (Lopez *et al*., 1996) and decreased plant-available magnesium, perhaps because of the antagonistic effect of potassium (Perez *et al.*, 1986).

These results are in line with those observed by many authors about the phytotoxic effect of OMW on plants when used directly as an organic fertilizer and have therefore opposed its direct application (Jelmini *et al.*, 1976; Paredes *et al.*, 1999). Therefore, before OMW or crude olive cake can be used properly and safely, toxicity must be eliminated or reduced. Different methods have been proposed for treatment of OMW or crude olive cake based on evaporation ponds, thermal concentration and different physicochemical and biological treatments (Paredes *et al.*, 2005). However, most methods are expensive and unable to completely solve the problem because of the need to dispose sludge or other by-products deriving from the process. Recently, composting of olive wastes, especially OMW, is found to be the most acceptable treatment to reduce its toxicity (Cegarra *et al*., 1996; Paredes *et al*., 1999; Paredes *et al*.,1999 and 2005 and Walker and Bernal 2008).

In Egypt, extensive studies are needed on olive wastes composting process and the effect of its application on the yield and quality of different crops.

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النمو والتعقيد في نباتات الفول السوداني المزروعة في تربة رملية معاملة بمخلفات الزيتون

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ملخص

تمت دراسة تأثير إضافة العصير المتخلف بعد استخلاص زيت الزيتون وكذلك كسب الزيتون كأسمدة عضوية سواء كإضافة منفردة أو في صورة مخلوط مع زرق الدواجن علي التعقيد والنمو لنباتات الفول السوداني المزروعة في تربة رملية وذلك في تجربة أصص.

وقد أوضحت النتائج أن إضافة التركيزات المنخفضة من العصير أو كسب الزيتون أدت إلى زيادة معنوية في العدد والوزن الجاف للعقد الجذرية والوزن الجاف و المحتوى النيتروجيني للمجموع الخضري وذلك بعد خمسين يوماً من الزراعة مقارنة بالنباتات التي لقحت بالريزوبيا فقط. وقد وجد أن زرق الدواجن سواء أضيف منفرداً أو مع مخلفات الزيتون أدى إلى تقليل كل القياسات السابق ذكرها.

ومن بين جميع المعاملات وجد أن النباتات التي سمدت بالعصير المتخلف بعد استخلاص زيت الزيتون مع زرق الدواجن أعطت أقل قيم للعدد والوزن الجاف للعقد الجذرية، وقد أوضحت النتائج، بعد سبعين يوماً من الزراعة، أن استخدام مخلفات الزيتون أدى إلى تقليل العدد والوزن الجاف للعقد الجذرية ، وأن إضافة التركيزات المنخفضة من العصير أدت أيضاً إلى تقليل الوزن الجاف والمحتوى النيتروجيني للمجموع الخضري.

وبصفة عامة، كما وجد أن استخدام كسب الزيتون كسماد عضوي يكون اقل سمية مقارنة بالعصير المتخلف بعد استخلاص زيت الزيتون. كما وجد ان استخدام مخلفات الزيتون كسماد عضوي سواء بصورة منفردة أو مع زرق الدواجن ادى الي زيادة تركيزالفلافونيدات الكلية والفينولات الكلية الذائبة لنباتات الفول السوداني مقارنة بالنباتات التي لقحت بالريزوبيا فقط. وكانت الزيادة غير معنوية في حالة تركيزات الفينولات الكلية الذائبة. بناء على هذه النتائج، يمكن استنتاج أن استخدام العصير المتخلف بعد استخلاص زيت الزيتون أو كسب الزيتون سواء بصورة منفردة أو مع زرق الدواجن له تأثير مثبط علي التعقيد والنمو لنباتات الفول السوداني تحت ظروف التربة الرملية.

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