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Evaluation of Biologically Treated Olive Mill Wastewater for Irrigation of Pea Plant

Rokia YA Awadallah[1], Azza A Mohamed[1], Ahmed AM Abdelhafez[2*]
1- Microbiology Dept, Institute of Water, Soil and Environment, ARC, Giza, Egypt
2- Agricultural Microbiology Dept, Fac of Agric, Ain Shams Univ, P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt

*Corresponding author: aabdelwahab@agr.asu.edu.eg

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Abstract: This study evaluated the use of biologically treated olive mill wastewater (OMWW) for irrigation of pea plants, rather than discharging this nutrient-rich liquid and polluting the environment. Pea seeds were planted in pots containing soil irrigated with tap water (control), untreated (crude) OMWW, or OMWW treated with the fungus, *Pleurotus columbinus*, or algae *Spirulina platensis* or *Wollea* sp., with two NPK rates. Plant length, shoot and root dry weight, nitrogen, phosphorus, potassium, chlorophyll, and carotene contents were measured, along with to nitrogenase and dehydrogenase activity. The highest shoots N and P content were recorded in plants irrigated with *Sp. platensis*-treated OMWW + 100% NPK; while the highest K content was in plants irrigated with crude OMWW + 100% NPK. The highest dehydrogenase activity, 59.01 µg TPF/100 g soils, was recorded in plants irrigated with *P. columbinus*-treated OMWW supported with 75% NPK, while maximum nitrogenase activity (261.82 µmol/100g soil/day) occurred in plants irrigated with *Wollea* sp.-treated OMWW with 75% NPK. The highest content of chlorophylls a & b and carotene (0.838, 0.276, 0.252 mg/g dry weight, respectively) were found in plants irrigated with OMWW treated with *Wollea sp*. and 100% NPK. Thus, biologically-treated OMWW showed promising impacts on plant growth parameters.

1 Introduction

Extraction of olive oil utilizes mechanical procedures, producing large amounts of liquid and solid waste with high organic content. The nature of the waste varies depending on the technology used for extraction and the system employed (Aly et al 2014). Egypt produced about 16% of the worldwide table olive yield, and in 2015, its production of table olives and olive oil reached 47,000 and 25,000 tons, respectively (Yacout et al 2016).

Extraction of olive oil generates 20% oil, 30% solid waste (pomace or olive pulp), and 50% aqueous liquor (wastewater). The two wastes are produced in significant quantities and considered to be of major environmental concern in several olive-producing countries. A study evaluated the impact of spreading OMWW, without treatment, on barley farmland, from 15 to 45 m³/ha for three consecutive years, although improved soil fertility, soil salinity increased to exceed 6 dS /m and all the components of barley yield, except 1000 grain weight, were negatively affected (Dakhli et al 2018). Raw (untreated) olive mill wastewater (OMWW) is polluting and phytotoxic to varying degrees, due to high acidity and elevated levels of biological oxygen (BOD) and chemical oxygen demand (COD) (Sciubba et al 2020). Further, management of olive oil production residues is an economic burden on producers (Esteve et al 2015).

On the other hand, organic matter in OMWW contains large quantities of useful compounds; namely polysaccharides, lipids and proteins, in addition to substantial potassium, nitrogen, phosphorus, and other elements. Therefore, detoxification of this wastewater before it can be used in agriculture has been of research interest (Martinezs-Gallardo et al 2020, Al-Qodah et al 2014).

Moreover, OMWW also contains bioactive constituents, which could be applied as natural pesticides as an alternative to harmful agrochemicals. Others show antimicrobial and antagonistic properties against plant pathogens, possibly due to the presence of phenols, in addition to high content of organic matter which serves as fertilizer (Sciubba et al 2020).

Biologically remediated and detoxified OMWW can be used directly as fertilizer and for crop irrigation. These applications enhance soil microbial activity and improve soil water capacity (Mekki et al 2013). OMWW can be applied as a biofertilizer by combining it with solar drying and composting. This process produces stable humic substances and minerals, improving soil fertility and plant production (Galliou et al 2018).

Application of OMWW treated using various protocols for irrigation improved plant growth, lowered soil pH, and variably impacted soil properties depending on treatment method (Rusan et al 2016).

A review article showed that treated OMWW (TOMWW) improved soil water holding capacity, salinity, organic carbon content, humus, total nitrogen, phosphate, and potassium along with adding organic and mineral matter. The review also reported that plants irrigated with TOMMW showed higher biomass, spike number, plant growth and similar or improved productivity than plants irrigated with tap water (Mekki et al 2013).

Conversely, applying untreated (raw) OMWW to a barley field at 15 to 45 m^3/ha for three consecutive years negatively affected barley growth, especially for soil receiving greater application. Even so, application considerably improved soil fertility, indicating a need for pretreatment before application (Dakhli et al 2018).

Applying untreated OMWW in fertigation of tomato cultivation increased total soil organic C, extractable N and C, available P, and extractable Mn and Fe. Additionally, increased soil respiration, dehydrogenase, urease activities, and microbial biomass of OMWW-amended soils were reported. In contrast, activities of phosphatase, b-glucosidase, nitrate reductase, and diphenol oxidase decreased. Moreover, soil became highly phytotoxic after crude OMWW application (Piotrowska et al 2006).

The above observations show that OMWW could be used for irrigation or fertilization after it is properly treated, anticipating possessing significant positive impact on soil properties and crop yields when treated OMWW is applied. Therefore, the present study was designed to evaluate the utilization of efficient microorganisms to biologically-treat OMWW for crop irrigation of peas.

2 Materials and Methods

2.1 Olive mill wastewater

Crude OMWW (diluted to 30, 20 and 10%), originally collected from the outlet of olive presser at Agriculture Research center (ARC), Giza, Egypt, and 30, 20 and 10% OMWW, treated with *Pleurotus. columbinus*, *Spirulina platensis*, or *Wollea sp.*, were obtained from the work of Awadallah (2017). The diluted OMWW were used in irrigation of pea plants. **Table 1** illustrates the chemical composition of crude, undiluted, OMWW, obtained from olive presser at ARC, Giza, Egypt.

2.2 Plant seeds

Seeds of pea (*Pisum sativum*), obtained from Vegetable Research Department, Agriculture Research Center, Giza, Egypt, were used for germination tests. Only pea seeds were used for pot experiments.

2.3 Germination test

The effects of untreated (crude) and treated OMWWs on germination of pea seeds were assessed according to the method as described by Enaime et al (2020) as follows: 10 pea seeds were placed in sterilized Petri dishes filled with washed sandy soil and irrigated with either tap water, 10, 20 and 30% crude OMWW, or 10, 20 and 30% OMWW treated with *Pleurotus columbinus* (fungus), *Spirulina platensis*, or *Wollea* sp. (algae). Plates were incubated at room temperature for 5-7 days and germination percentage was recorded.

Table 1. Chemical composition of OMWW from Agricultural Research Center

pH	E.C d.s/m	Cations (meq/l)			Anions (meq/l)		
		Ca^{++}	Mg^{++}	Na^{++}	HCO_3^-	Cl^-	SO_4^-
5.4	17.2	38.7	35.5	68.9	4.2	115.5	11.3

Macro nutrients (ppm)			Micro Elements (ppm)				Organic materials (g/L)		
N	P	K	Mn	Zn	Fe	Cu	COD	Phenolic compound	Total Carb.
0.257	0.126	0.06	0.266	0.660	0.577	0.052	99	7	14

2.4 Pot experiments

Pots of 20 cm diameter containing 6 kg of sandy soil were planted with 7 pea seeds/pot and maintained under greenhouse conditions at the Department of Agriculture Microbiology Research, Soil, Water and Environment Research Institute, Giza, Egypt at the end of October 2015. The pot experiment was conducted to assess the effect of OMWW on plant growth parameters and soil biological properties with replicates/treatment. Physical and chemical properties of soil **(Table 2)** were determined according to Jakson (1958).

According to the recommendations of Egyptian Ministry of Agriculture, plants were fertilized as follows:

- Super phosphate (15% P_2O_2) at 100% and 75% of the recommended application rate (200 kg/feddan, 480kg/hectare), one week before planting.
- ammonium sulfate $(NH_4)_2SO_4$ at 100% and 75% of recommended application rates, and potassium sulfate (K_2SO_4) at 100% of the recommended rate (200kg/fed, 480kg/hectare) both added 15 days after planting.

Plants were irrigated with either untreated OMWW or *P. columbinus*-treated OMWW, at the concentration that supported the highest germination rate, every other day. Soil samples were collected from rhizospheres 45 days after planting and analyzed for nitrogenase and dehydrogenase activities and CO_2 evolution. Samples of whole plant were collected 45 days after planting. The plant height, fresh and dry weight of shoots and roots, chlorophyll content, and NPK content in shoot and root systems were assessed.

2.5 Determination of microelements

K and Na were determined using flame photometer model 400, while P was determined using spectophotemeter and Mn, Zn, Cu, Fe, Mg and Ca were determined using atomic absorption spectrophotometer according to the methods described by (Cottenie et al 1982).

2.6 Determination of total nitrogen

Total nitrogen was determined by Kjeldahl according to the method described by (Cottenie et al 1982).

2.7 Determination of chlorophyll pigmentation.

Chlorophyll a, b, and total carotenoids were determined colorimetrically in leaf samples (mg/100g of fresh matter) according to the method described by (Senthilkumar et al 2021a).

2.8. Dehydrogenase activity

Dehydrogenase activity (DHA) of soil samples was determined according to the method described by Solaiman (2007). Enzyme activity was calculated as µg of triphenylformazan (TPF)/g dry soil/day.

2.9 Nitrogenase activity

Nitrogenase activity of soil samples was determined by acetylene reduction assay according to Senthilkumar et al (2021b).

2.10 CO_2 evolution

CO_2 evolution of soil samples was determined as mg CO_2 /100g soil according to the method described by Giacomo et al (2014).

3 Results and Discussion

3.1 Effect of Treated OMWW on seed germination

Crude OMWW cannot be used in irrigation in its native form, i.e. without dilution and treatment. Enaime et al (2020) reported that 100% and 75% OMWW completely inhibited maize seed germination, and phytotoxicity decreased with dilution to 25% and 50%. They also found that pre-treated OMWW (combined filtration on olive stone and coagulation-flocculation) possess significantly high phytotoxicity to tomato seeds germination in high waste concentrations, and decreased with dilution, and best germination rates were in 5, 10 and 15% OMWW. Biological treatment doesn't neither work well in the high concentrations due to the toxic compounds present in it toward the microorganisms involved in the treatment.

Therefore, phytotoxicity was conducted by irrigating pea seeds with OMWW (diluted to 30, 20 and 10%) untreated or treated with the fungus *P. columbinus,* or the algae *Sp. platensis,* or *Wollea* sp., or tap water as control treatment, all in sterilized Petri dishes filled with washed sandy soil.

Pea seeds irrigated with tap water had 100% germination, while those irrigated with 30, 20 and 10% crude OMWW showed 4%, 20% and 52% germination, respectively. These findings indicate significant phytotoxicity of the waste, considering the correlation between the OMWW concentration and germination rate.

Pea seeds irrigated with 30, 20 and 10% *Pl. columbinus*-treated OMWW had 23%, 48% and 96% germination, respectively. Seeds irrigated with 30, 20 and 10% *Sp. platensis*-treated OMWW had 21%, 50%, and 95% germination, while in case of *Wollea* sp.-treated OMWW, germination rates were 18%, 44%, and 93%, respectively.

These findings indicate that biological treatments of OMWW significantly reduced OMWW toxicity, particularly due to the removal of phenolic compounds, and the efficiency of any of the microorganisms to detoxify the OMWW decreases with the higher concentrations of the wastewater used.

A comparable study showed that 100, 75, and 50% OMWW were very phytotoxic and eliminated barley seed germination, and OMWW must be treated and diluted to at least a 1:3 ratio before use in crop irrigation (Rusan et al 2015).

Another study showed that chemical structures of nine phenolic constituents of OMWW are responsible for inhibition of Fenugreek seed germination and that hydrophobicity is a key factor in phytotoxicity, partly linked to the presence of catechol function (Bouknana et al 2019).

Therefore, 10% of treated and untreated OMWW were selected for the pot experiment.

3.2 Characteristics of treated OMWW

Table 3 illustrates the characteristics of the obtained 10% crude and treated OMWW, which were later applied in the pot experiment. Data revealed that *P. columbinus* is more efficient in reducing phenol content (0.07g/L) and COD (0.86g/L) of the crude OMWW than the two algae species used. IAA was slightly reduced in OMMW treated with *P. columbinus* and *Wollea sp.* and slightly increased by the treatment with *Sp. platensis*. Gibberellic acid increased two folds by *P. columbinus* and slightly by *Sp. platensis* and did not change by *Wollea sp.* treatments.

In a study conducted on biodegradation of OMWW by two fungal genera, *Pleurotus* spp. possessed significant (60–65%) decolorization, 74–81%reduction of phenolics, COD decrease by 12–29%, and increase in Cress-seeds germination by 30–40% when irrigated with OMW treated by *Pleurotus* strains (Ntougias et al 2012).

3.2.1 Plant height and shoot and root dry weight

Plants irrigated with 10% crude OMWW, regardless of NPK application rate, gave the least height plants, followed by plants irrigated with tap water-then all other treatments **(Table 4 and Fig 1)**.

For shoot and root dry weights, crude OMWW was slightly, but significantly, higher than tap water, which must be due to organic content in wastewater **(Table 4 and Fig 2)**. No significant differences were found between 75% and 100% NPK applications regardless of the OMWW treatment, which may interpreted by OMWW compensating for the 25% reduction of NPK fertilization doses.

Table 2. Physical and chemical properties of experimental soil

Physical properties					
Sand %	**Silt %**	**Clay%**	**Texture grade**	**$CaCO_3$ %**	**Saturation percent (S.P%)**
90.00	03.50	06.50	Sandy	01.66	22.30

Chemical properties					
pH	**E.C. (dS m-1)**	**Organic matter (%)**	**Total soluble N (mg kg-1)**	**Available P (mg kg-1)**	**Available K (mg kg-1)**
07.38	00.30	00.21	17.62	08.00	82.60

Soluble cations and anions (meq l^{-1})							
Cations				**Anions**			
Ca^{++}	**Mg^{++}**	**Na^{+}**	**K^{+}**	**$CO_3^{=}$**	**HCO^{-3}**	**Cl^{-}**	**$SO4^{=}$**
0.48	0.30	1.68	0.53	0.0	0.82	0.57	01.60

DTPA-extractable (mg kg-1)			
Fe	**Mn**	**Zn**	**Cu**
1.10	00.34	00.40	00.22

Table 3. Characteristics of OMWW crude, and treated with fungi and algae (at 10%)

Treatment	pH	E.C. d.s/m	Phenols g/L	COD g/L	Carb g/L	IAA µg/ml	GA µg/ml
crude OMWW 10%	5.2	2.4	0.52	6.4	1.46	15.93	0.667
treated 10% OMWW *P. columbinus*	5.8	1.95	0.07	0.86	3.9	13.15	1.58
treated 10% OMWW *Sp. platensis*	7.7	2.1	0.18	2.14	0.54	16.01	0.94
treated 10% OMWW *Wollea* sp.	5.4	2.2	0.16	2.45	0.31	14.75	0.654

OMWW: Olive Mill wastewater; COD: Chemical oxygen demand; Carb: Carbohydrate; IAA: Indole Acetic Acid; GA: Gibberellins

Table 4. Growth parameters of pea plants irrigated with crude and treated OMWW (10%) after 45 days of planting

Treatment		NPK fertilization	Plant height (cm)	Shoot DW (g/plant)	Roots DW (g/plant)
Tap water (control)	1	100%	55	0.93	0.46
	2	75%	53	0.82	0.40
crude OMWW 10%	3	100%	52	1.1	0.54
	4	75%	49	1.05	0.43
treated 10% OMWW *P. columbinus*	5	100%	58	1.71	0.71
	6	75%	56	1.60	0.60
treated 10% OMWW *Sp. platensis*	7	100%	59	1.79	0.75
	8	75%	57	1.65	0.67
treated 10% OMWW *Wollea* sp.	9	100%	57	1.61	0.66
	10	75%	56	1.53	0.59
LSD			**3.2**	**0.13**	**0.04**

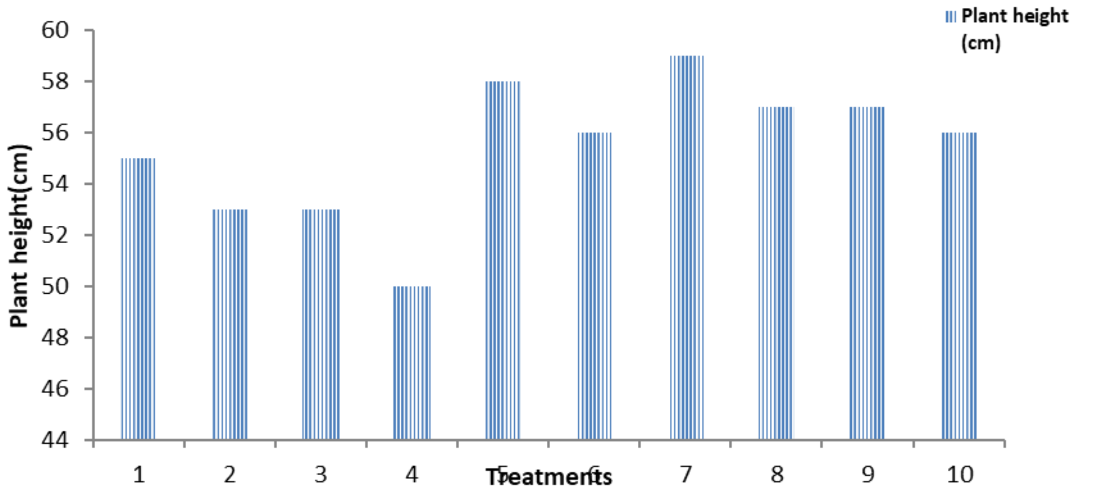

Fig 1. Plant height of pea, irrigated with 10% crude and treated OMWW, after 45 days of planting
1: Tap water (control) + 100% NPK, 2: Tap water (control) + 75% NPK, 3: Untreated $(OMWW)_1$ + 100% NPK, 4: Untreated OMWW + 75% NPK, 5: Treated OMWW (*P. columbinus*) +100%NPK, 6: Treated OMWW (*P. columbinus*) + 75% NPK, 7: Treated (*Sp. platensis*) + 100% NPK, 8: Treated (*Sp. platensis*) + 75%NPK, 9: Treated (*Wollea* sp) + 100% NPK, and 10: Treated (*Wollea* sp) + 75% NPK.

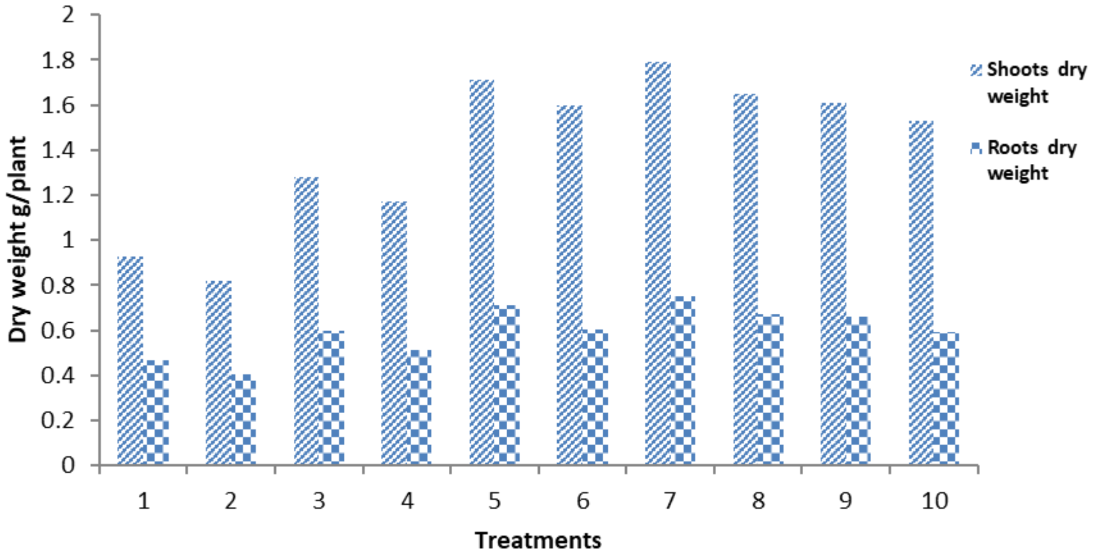

Fig 2. Shoot and root dry weight of Pea plants irrigated with 10% crude and treated OMWW, after 45 days of planting

1: Tap water (control) + 100% NPK, 2: Tap water (control) + 75% NPK, 3: Untreated $(OMWW)_1$ + 100% NPK, 4: Untreated OMWW + 75% NPK, 5: Treated OMWW (*P. columbinus*) +100%NPK, 6: Treated OMWW (*P. columbinus*) + 75% NPK, 7: Treated (*Sp. platensis*) + 100% NPK, 8: Treated (*Sp. platensis*) + 75%NPK, 9: Treated (*Wollea* sp) + 100% NPK, and 10: Treated (*Wollea* sp) + 75% NPK.

Maximum plant heights were recorded for plants of treated OMWWs, with no significant differences, ranging from 56 to 59cm **(Table 4 and Fig 1).**

A study conducted on maize plants irrigated with OMWW showed that untreated OMWW had increased soil salinity and lowered plant weights, while treated OMWW improved plant growth and lowered soil pH. This can be attributed to the removal phenols and other phytotoxic compounds from raw OMWW (Rusan et al 2016). In a review analyses, treated OMWW improved plant growth and induced better productivity than plants irrigated with tap water (Mekki et al 2013).

Shoot and root dry weight were significantly and positively affected by irrigation with treated OMWW. *Sp. platensis* + 100 NPK treatment recorded maximum shoot and root dry weights (1.79 & 0.75g/L respectively), followed by *P. columbinus* + 100% NPK treatment (1.71 and 0.71g/L, respectively), then *Wollea* sp. +100% NPK treatment (1.61 and 0.66 g/L, respectively). This can be due the IAA produced by *Sp. platensis* **(Table 3)**.

3.2.2 Determination of shoot and root NPK content

Shoot and root NPK contents were determined, as a function of irrigation treatment. Results, illustrated in **Table 5,** showed no significant differences between 75% and 100% NPK rate in N and P contents of both shoot and root in all treatments, which could be due to presence of some amounts of these elements in the soil prior plantation, and more profoundly due the presence of these element in the OMWW **(Table 1)**. For K content, there are significant differences in K contents in the root system between 75 and 100% NPK fertilization treatments **(Table 5).**

In the shoot system, the maximum nitrogen content for shoots was recorded in plants irrigated with OMWW treated with *Sp. platensis* (4.46-4.90%) and with *Wollea* sp. (4.38-4.73%), with no significant difference between the two treatments.
Plants irrigated with crude OMWW contained the highest % of both P and K, followed by all treated OMWW with no significant differences between the treatments.

In the roots, the highest nitrogen content (2.97%) was recorded plants irrigated with OMWW treated by *Wollea* sp. at 100% NPK.

Phosphorus content did not show a consistent trend. The highest content was in plants irrigated with untreated OMWW and 100% NPK, 0.72% for shoots; the lowest value was found for tap water and 75% NPK, 0.5%. Phosphorus content of roots recorded 0.39% for plants irrigated with OMWW treated with *Sp. platensis* with 100% NPK. Notably, irrigation with untreated OMWW and 100% NPK produced a similar result, 0.4%. Untreated OMW significantly increased potassium content in roots and shoots.

The above-mentioned results can be explained by the action of the treated OMWW when it is added into the soil, in that its acidic nature lowers the soil pH, thus dissolving and releasing the bound forms of phosphorus and potassium species present in the soil and make it available to the plants. Rusan et al (2016) concluded from their work that treated OMW by different technologies improved plant growth and resulted in lower soil pH. and lowering the soil pH would lead to free immobile phosphorus to mobile and dissolved form, making it available to be up taken by plant roots.

A comparable study in maize showed that quality and quantity parameters, including yield and kernel quality, were similar when plants were irrigated with mineral N fertilizer application or treated OMWW. Thus, treated OMWW might be useful to substitute, even partially, mineral fertilizer (Kokkora et al 2015).

3.2.3 Determination of chlorophyll and carotene

Chlorophyll and carotene contents were positively affected by NPK application **(Table 6).** The highest content of chlorophylls a & b and carotene were found in plants irrigated with OMWW treated with *Wollea sp*. and 100% NPK, being 0.838, 0.276, 0.252 mg/g dry weight, respectively, followed by plants irrigated by OMWW treated with *P. columbinus* combined with 100% NPK, being 0.782, 0.267, 0.249 mg/g dry weight, respectively **(Table 6).** This finding confirms a positive correlation between NPK application and chlorophyll content.

3.2.4 Dehydrogenase and nitrogenase activity and CO_2 evolution in pea plant rhizosphere

Dehydrogenases are oxidoreductase enzymes which is essential in respiration of microbial cells, catalyzing many reactions and thus have potential as an indicator of microbial activity in soils. Therefore, measuring dehydrogenases activity (DHA) was used in this study to determine the effect of OMWW application on soil microbial activity.

Table 5. Shoot and root NPK content of pea plants irrigated with crude and treated OMWW (10%) after 45 days of planting

Treatment		NPK fertili-zation	Shoot NPK content (%)			Root NPK content (%)		
			N	P	K	N	P	K
Tap water (control)	1	100%	3.94	0.54	1.19	1.92	0.27	0.28
	2	75%	3.68	0.51	1.09	1.83	0.26	0.25
crude OMWW 10%	3	100%	4.51	0.69	2.10	2.54	0.38	0.42
	4	75%	4.21	0.66	1.87	2.32	0.34	0.38
treated 10% OMWW	5	100%	4.29	0.62	1.66	2.27	0.32	0.36
P. columbinus	6	75%	3.76	0.61	1.5	2.10	0.31	0.35
treated 10% OMWW	7	100%	4.90	0.68	1.51	2.62	0.39	0.31
Sp. platensis	8	75%	4.46	0.63	1.47	2.62	0.31	0.29
treated 10% OMWW	9	100%	4.73	0.64	1.44	2.97	0.33	0.32
Wollea sp.	10	75%	4.38	0.63	1.38	2.62	0.32	0.30
LSD			0.52	0.038	0.51	0.38	0.06	0.01

Table 6. Chlorophyll a and b and carotene contents of pea plants irrigated with crude and treated OMWW (10%) after 45 days of planting

Treatments		NPK fertilization	Chl a (mg/g DW)	Chl b (mg/g DW)	Carotene (mg/g DW)
Tap water	1	100%	0.482	0.164	0.128
(control)	2	75%	0.396	0.134	0.152
crude OMWW 10%	3	100%	0.650	0.230	0.203
	4	75%	0.525	0.199	0.159
treated 10% OMWW	5	100%	0.782	0.267	0.249
P. columbinus	6	75%	0.649	0.246	0.172
treated 10% OMWW	7	100%	0.553	0.248	0.165
Sp. platensis	8	75%	0.533	0.167	0.209
treated 10% OMWW	9	100%	0.838	0.276	0.252
Wollea sp.	10	75%	0.552	0.219	0.190
LSD			0.01	0.03	0.014

Table 7. Dehydrogenase and nitrogenase activities and CO_2 evolution in pea plant rhizosphere, irrigated with crude and treated OMWW (at 10%) after 45 days of planting

Treatments		NPK fertili-zation	Dehydrogenase µg TPF/g soil	Nitrogenase µmol/100g soil/day	mg CO_2 /100g soil
Tap water (control)	1	100%	31.05	75.40	303.4
	2	75%	29.57	76.162	278.1
crude OMWW 10%	3	100%	36.23	172	360.3
	4	75%	34.9	235	398.25
treated 10% OMWW	5	100%	52.26	233.65	556.3
P. columbinus	6	75%	59.01	255.25	594.2
treated 10% OMWW	7	100%	36.15	230.18	455.1
Sp. platensis	8	75%	53.41	224.39	480.4
treated 10% OMWW	9	100%	42.72	228.30	543.6
Wollea sp.	10	75%	43.88	261.82	568.9
LSD			3.56	5.48	181.3

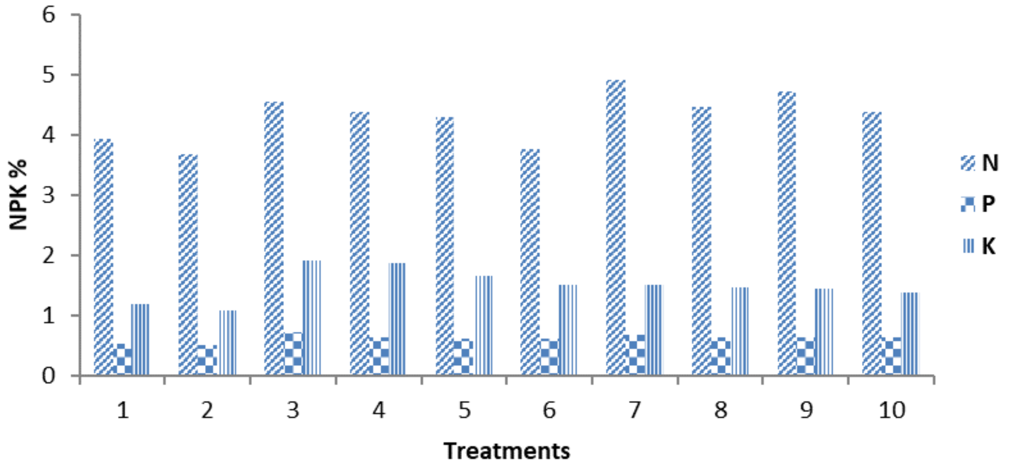

Fig 3a. Shoot NPK of Pea plants irrigated with 10% crude and treated OMWW, after 45 days of planting

1: Tap water (control) + 100% NPK, 2: Tap water (control) + 75% NPK, 3: Untreated $(OMWW)_1$ + 100% NPK, 4: Untreated OMWW + 75% NPK, 5: Treated OMWW (*P. columbinus*) +100%NPK, 6: Treated OMWW (*P. columbinus*) + 75% NPK, 7: Treated (*Sp. platensis*) + 100% NPK, 8: Treated (*Sp. platensis*) + 75%NPK, 9: Treated (*Wollea* sp) + 100% NPK, and 10: Treated (*Wollea* sp) + 75% NPK.

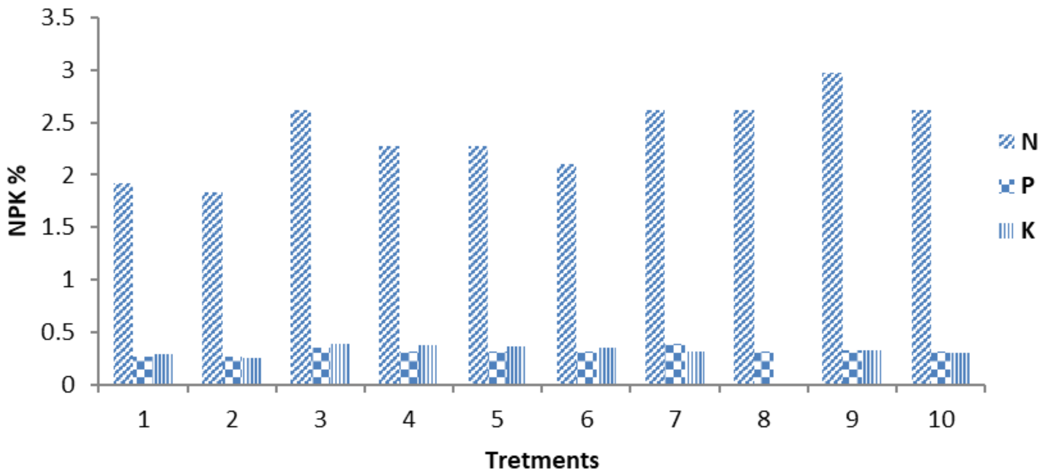

Fig 3b. Root NPK of Pea plants irrigated with 10% crude and treated OMWW, after 45 days of planting

1: Tap water (control) + 100% NPK, 2: Tap water (control) + 75% NPK, 3: Untreated $(OMWW)_1$ + 100% NPK, 4: Untreated OMWW + 75% NPK, 5: Treated OMWW (*P. columbinus*) +100%NPK, 6: Treated OMWW (*P. columbinus*) + 75% NPK, 7: Treated (*Sp. platensis*) + 100% NPK, 8: Treated (*Sp. platensis*) + 75%NPK, 9: Treated (*Wollea* sp) + 100% NPK, and 10: Treated (*Wollea* sp) + 75% NPK

Results, **Table 7**, showed that DHA in the 75% NPK applications was either higher than 100% NPK (in *P. columbinus* and *Sp. plantesis* treatments) or not significantly different from 100% NPK (in all other treatments). This may indicate that 100% NPK application did not benefit soil microbial community, and thus wasted.

The lowest DHA was recorded in tap water treatment, while the highest (59 µg TPF/g soil) was recorded in *P. columbinus* treated OMWW with 75% NPK fertilization, and significantly higher than the same treatment supplemented with 100% NPK (52.26 µg TPF/g soil**).** These findings could be attributed to the presence of higher amount of carbohydrates and less phenolic compounds than what is present in the crude and other treated OMWW, which would benefit the soil microbial community possessing DHA.

Data of nitrogenase activity **(Table 7)** showed that the application of 75% NPK along with crude, *P. columbinus* and *Wollea sp.* treatments recorded values significantly higher than 100% NPK with same treatments. The presence of available mineral nitrogen in the soil is thought to inhibit nitrogenase activity of the N_2-fixing microorganisms (Reinprecht et al 2020). The highest nitrogenase activity (261.82 µmol/100g soil/day) occurred in the *Wollea sp*. treatment supplemented with 75% NPK**,** followed by *P. columbinus* treatment with 75% NPK rate (255.25 µmol/100g soil/day).

Carbon dioxide (CO_2) is released from the soil as a result of respiration processes related to degradation of organic matter in soils (Giacomo et al 2014), and thus usually used as indicator to biological activity of the soil.

Except tap treatment, 75% NPK application combined with crude of treated OMWW caused more CO_2 emission than 100% NPK application with the same irrigation treatment. This may be explained by the higher dose of mineral fertilizers inhibits the microbial activity in the rhizosphere, since it narrows the C/N ratio, which means less carbon per cellular population number.

The highest CO_2 emission, 594.2 mg CO_2/100g soil, was detected in the rhizosphere of plants irrigated OMWW treated with *P. columbinus* supplemented with 75% NPK, followed by *Wollea sp.*-OMWW + 75% NPK treatment, producing 568.9 mg CO_2/100 g soil. Generally, the values of CO_2 generation with treated and crude OMWW treatments were higher than in controls. Piotrowska et al (2011) reported that application of OMWW, after removing its phenolic component, resulted in higher values of respiratory quotient CO2.

4 Conclusions

Disposal of olive mill wastes into the environment is a major concern due to its high content of phenolic and other toxic constituents. However, organic matter and valuable nutrient content, especially NPK, as well as micronutrients and plant growth hormones, makes the waste a viable candidate for plant fertilization. Therefore, the use of OMWW for crop irrigation, after detoxification, is assumed to produce positive effects on plant growth and productivity. The present work illustrates that application of biologically-treated OMWW has a positive impact on pea plants, resulting in increased growth and improved plant properties as measured by shoot and root dry weight and dehydrogenase and nitrogenase activities. These findings most likely reflect the significant nutrient content of OMWW. Further, the increase in CO_2 evolution from the soil reflects increased microbial activity, which is an added positive value to the treated OMWW. However, OMWW concentration higher than 10% exhibits more toxic materials that inhibit microbial growth and plant seed germination. Therefore, treated OMWW may be considered as a good strategy for restoring soils, especially in soils with poor organic matter, however smore work is still needed to further detoxify OMWW for use without high dilution.

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