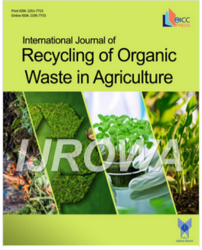

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Isolation of competent actinomycetes strains from soil planted with three species of mint irrigated with olive mill wastewater

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Original Research

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Abstract:

Purpose: This study aimed to investigate the potential of actinomycetes in mitigating the environmental impact of olive mill wastewaters (OMWW) on plant-soil system. The objectives were to investigate the impacts of varying doses of OMWW on soil microflora, and to analyze the enzymatic profile of a selected group of isolated actinomycetes.
Method: A physico-chemical characterization of soil parameters and OMWW was conducted. Microbiological analysis of OMWW was carried out to determine the presence of total aerobic mesophilic flora (FMAT), yeasts, and molds. An interaction study between different doses of OMWW and three varieties of mint (*Mentha aquatica*, *Mentha piperita*, and *Mentha pulegium*) was conducted over a 120-day period.
Results: Analysis revealed that OMWW contained high levels of organic matter and mineral salts, resulting in elevated chemical oxygen demand and salinity. Application of OMWW at a dose of 5 L/m^2 enhanced soil microflora, but this effect diminished beyond this dose, except for actinomycetes, which remained resilient even at 10 L/m^2. Enzymatic profile analysis of isolated actinomycetes indicated that the most competent strains were present in plots irrigated with 5 L/m^2 and 10 L/m^2 of OMWW, in combination with *Mentha piperita* and *Mentha pulegium* cultivation.
Conclusion: This study demonstrates the potential of actinomycetes in addressing to meet the challenges inflicted by OMWW. Optimizing OMWW dosage can positively influence soil microflora, where actinomycetes exhibiting remarkable resilience at higher doses. These results contribute to understanding the use of microorganisms for olive oil industry waste management, in order to offer practical solutions to mitigate OMWW environmental impact.

Keywords: Olive mill wastewater; Microbiology; Actinomycetes; Mentha; Interaction; PCA

1. Introduction

During olive oil extraction process, large quantities of liquid effluents called olive mill wastewaters (OMWW) are generated. They are characterized by low pH, high organic load including polysaccharides and phenolic compounds (Foti et al., 2021). More precisely, OMWW phenolic components would be responsible for phytotoxic and antimicrobial effects (Mechri et al., 2010) leading to seed germination and plant growth inhibition (Ntougias et al., 2013), soil characteristics modification (Kavvadias et al., 2021) and soil microbial diversity upset (Ntougias et al., 2013). For these reasons, increasing attention has been oriented for researching the best methods for spreading OMWW on agricultural land. According to studies done by Yaakoubi and Aghanchich (2021), applying a moderate olive mill wastewater (OMWW) dose ranging from 5 to 20 L/m^2 appears to carry no pollution risk. Conversely, it leads to a soil microflora enhancement, underscoring the potential of utilizing this effluent as an organic soil amendment. This promising approach not only boosts soil fertility (Galliou et al., 2018) but also augments soil microbial biodiversity. Nonetheless, the biodegradation of this liquid waste in the natural environment poses challenges due to its potent antimicrobial impact attributed to various phenolic compounds (Yesilada et al., 1999; Sayadi et al., 2000; Rinaldi et al., 2003). Moreover, introducing such compounds can trigger significant shifts in the structure and function of the microbial community, potentially influencing soil suitability for sustainable agriculture (Mekki et al., 2006). OMWW acts as an antimicrobial agent and contains compounds that are toxic, all of which result in an altered state of soil microbial diversity (Rusan et al., 2016; Mekki et al., 2013). However, some microorganisms interfere with this process, such as actinomycetes.

Actinomycetes are very widespread microorganisms in various biotopes. There are more than 100 genera which are considered soil saprophytic inhabitants. They possess the ability to break down organic matter, such as lignocelluloses and different types of complex carbohydrates, such as starch and chitin (Shanthi, 2021). Recently, actinomycetes have aroused much interest among microbiologists due to their various characteristics, which make them a good source for biomolecules development (Nathan and Kannan, 2021; Shahbaz et al., 2023). Some actinomycetes are also highly valued in various industrial applications. They are being evaluated for their use as probiotics in aquaculture, in compounds production used in plastics development, detergents and other high added value products.

Our previous work conducted in our region (Fez city) (Hassani et al., 2020; Hassani et al., 2022) raised several research questions on the fundamental and applied interest to properly value of OMWW. And this, through the understanding of OMWW irrigated soil microbiology and the changes involved in plant growth and development (Hassani et al., 2010a). Certain metabolic pathways related to the synthesis of essential oils, chlorophyll (a and b) were undertaken by Hassani et al. (2022). In contrast, these researches have reported the change in the metabolic pathways of essential oils synthesis in the specie *Mentha aquatica* (var citrata) used as a plant model for study. The authors reported that OMWW application affects the essential oil composition with appearance of newly synthesized compounds such as menthone and menthol and disappearance of other compounds such as linalool acetate (Hassani et al., 2022).

Works carried out in our region (arid climate) (Hassani, 2020) have cited the microbiology change in soils irrigated by OMWW, but no study has dealt with the potential interaction between the plants that do the phytoremediation and microorganisms that arise after soil treatment with OMWW. In this light, we were interested in an experimental investigation to study the interaction between soil microorganisms

Table 1. Characteristics of OMWW used in plots spreading.

	Parameters	Values
Physico-chemical composition (g/l)	pH	4.5
	DCO	28
	Phenolic compounds	4.8
	SS	3.1
	MS	20.5
	MV	17
	Fatty matter	10
	Total sugars	0.3
Mineral composition (g/l)	Calcium (g/l)	2.1
	Magnesium (g/l)	1.4
	Potassium (g/l)	4.4
	Sodium (g/l)	2.5
	Chlorides (g/l)	3.8
	Orthophosphates (g/l)	0.09
	Total nitrogen (g/l)	0.14
	Ammoniums (g/l)	0.01
Microbial flora (UFC/ml)	TAMF	7.3×10^3
	Fungi	3.8×10^2
	Yeasts	5.6×10^3
	Total Coliforms	0
	Fecal Coliforms	0
	Fecal Streptococci	0

Table 2. Emberger bioclimatic quotient (Q_2) formulas.

Formulas	References	Climatological parameters
$Q_2 = 2000 * \frac{P}{M^2 - m^2}$ $Q_2 = 3.43 * \frac{P}{M - m}$	(Emberger, 1942) (Stewart, 1968)	P: Annual precipitation in mm/m^2/year M: Maximum temperature of the warmest month in °K m: Minimum temperature of the coldest month in °K
$Q_2 = 2000 * \frac{P}{(M+m+546.4)(M-m)}$	(Mokhtari et al., 2013)	P: Annual precipitation in mm/m^2/year M: Maximum temperature of the warmest month in ° C m: Minimum temperature of the coldest month in ° C

and plants.

The choice of plants model was based on the availability of a well-consumed plant resource in Morocco (Hassani, 2020) but the three plants selected are *Mentha aquatica*, *Mentha piperita* and *Mentha peligium*.

Streptomyces, a group well identified among actinomycetes, show important potentialities (Karkouri et al., 2010; Yadav et al., 2021) in biotechnology and bioindustry, which has focused our attention on these microorganisms.

This study target the physicochemical, mineralogical and microbiological characterization of soil and OMWW used for its spreading at different doses (0 L/m^2, 5 L/m^2 and 10 L/m^2). The Tested soil was planted with different species of mint (*Mentha aquatica*, *Mentha piperita* and *Mentha peligium*). The purpose of this study is to investigate the OMWW impact on various soil microbiota and especially Actinomycetes. Therefore, the main aim of this work is to isolate competent Actinomycetes strains, with a high metabolic capacity, from soils irrigated with OMWW.

2. Material and methods

Study of soil intended for OMWW spreading

Before planting the three mint varieties (*Mentha aquatica*, *Mentha piperita* and *Mentha pulegium*), we carried out soil analysis before and after the OMWW spreading. Soil samples contain enough core samples were taken randomly from all the experimental plots, both irrigated and non-irrigated with OMWW.

On each plot, pH values were evaluated with distilled water (H_2O) and with KCl. Measurement was carried out using a pH meter in an aqueous extract obtained according to (NF ISO10390 standard). Total $CaCO_3$ (%) determination was carried out by the volumetric method using Bernard's calcimeter, by breaking down the calcium carbonates with hydrochloric acid, and measuring released volume of CO_2. The active $CaCO_3$ (%) was determined by the Drouineau-Galet method (Mehalaine and Chenchouni, 2020). Mineral nitrogen was determined by colorimetric determination according to the method described by Bremner (Bremner, 1965), and organic carbon (%) was determined using Anne method (Khiari et al., 2021).

Phosphorus extraction and dosage are carried out using the Joret-Hebert method (Leclercq-Dransart et al., 2019). Exchangeable potassium determination was carried out using the Schollenberger method (Chandiona et al., 2021) with flame spectrometry. Soil salt concentration was evaluated by measuring the irrigation water electrical conductivity (ECi) expressed in (mmhos/cm).

OMWW sampling

OMWW samples were collected from local ponds that receive all OMWW from Fez city. They were, then put into 2-liter bottles for physico-chemical assays. Samples intended for microbiological analyzes were collected in sterile bottles, transported to laboratory and stored at 4° C for later use.

OMWW used in this work have the physico-chemical and microbiological characteristics illustrated in Table 1. The methods used for OMWW physico-chemical characterization are those described by Rodier et al. (2009).

Bioclimatic study of Fez city

To investigate the impact of climate conditions on soil microorganisms and interactions with plants, a climatic study utilizing the Emberger bioclimatic quotient was conducted. Bioclimatic parameters such as precipitation (P), minimum (m) and maximum (M) temperature were determined using the Ombrothermal diagram (Emberger, 1942; Debrach, 1953; Bagnouls and Gaussen, 1957; Stewart, 1968; Mokhtari et al., 2013). The bioclimatic stage was identified by creating climatograms, which involved calculating the Emberger bioclimatic quotient (Q_2) using the formulas provided in Table 2.

Microbiological study of used soil before OMWW spreading, after OMWW and after planting Sampling

The study was carried out in an experimental field located within the Faculty of Science and Technology at the Fez botanical garden. Soil sampling was carried out three times with an interval of two months, at nine plots. Sampling was carried out in three periods: The first before the OMWW spreading, the second after the OMWW spreading and the planting of the three varieties of mint (*Mentha aquatica*, *Mentha piperita* and *Mentha pulegium*)

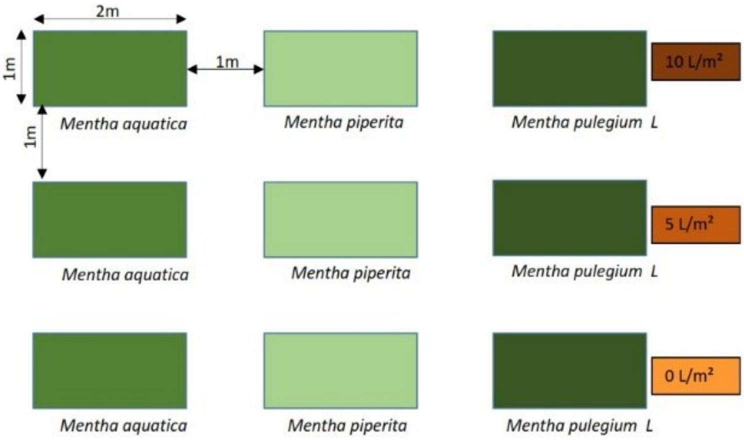


Figure 1. Experimental planting of three varieties of mint (Mentha aquatica, Mentha piperita and Mentha pulegium) applied to plots spread with two concentrations of vegetable water against a control (0, 5 and 10 L/m^2).

and the third period, two months after OMWW spreading just before final harvest. Three OMWW treatments were applied, i.e. a dose of 5 L/m^2 10 L/m^2 against a control of 0 L/m^2 (see Fig. 1). To ensure the representativeness of valid results, sample homogeneity was crucial for evaluating the effect of OMWW spreading on our cultivated plots after two months. To achieve this, three samples from the same plot were mixed and subsequently analyzed. Soil microbiological analysis was focused on bacteria, actinomycetes, yeasts, and molds.

Microbiological enumeration

Total microflora was enumerated from 0.1 mL aliquots of appropriate dilution added to solid media of LB for bacteria, ISP3 medium for actinomycetes, YPG medium for yeasts and malt extract medium for molds.

Isolation and characterization of competent actinomycetes

To select the most competent actinomycete strains according to OMWW doses and mint type, a selection pressure was exerted on the latter to elect those with the capacity to assimilate several carbon sources. For this purpose, several enzymatic activities, such as Amylase, Cellulase, Xylanase, Gelatinase, Pectinase, Lecithinase, Caseinase, Esterase, Chitinase, and Lipase activities, were evaluated. The utilization of carbon and nitrogen as sole carbon sources was determined using ISP9 as the basal medium supplemented with a final concentration of carbon sources (1% w/v) (Shirling and Gottlieb, 1966).

3. Results and discussion

Study of the soil before and after OMWW spreading

To characterize the soils of the three control plots and the six treated with OMWW at different doses (0, 5 and 10 L/m^2) and planted with one of the three species of *Mentha sp*, we studied the acido-base, humus content, major elements and salinity. Table 3 presents the soil analysis results for both the control and OMWW-treated samples.

The acid-base status was not changed much, it seems that it was reduced by soil buffer system (Yaakoubi et al., 2010). On the other hand, total limestone has decreased at a dose of 10 L/m^2 and active limestone has slightly decreased at 5 and 10 L/m^2. Humus content has changed considerably where there is an increase in mineral nitrogen (from 3.97 mg/100g to 7.46 mg/100g). Regarding the major elements, there was also a remarkable increase in assimilable phosphorus and potassium. These elements are very important in soil fertilization. Salinity increase can be explained by OMWW richness in NaCl, which is widely used by farmers in olives conservation at the time of their trituration. These results have shown the beneficial influence of OMWW use on soil fertility. These findings agree with those reported by certain authors (Rouina and Ammar, 1999; Yaakoubi et al., 2010) who have underlined soil fertility improvement through OMWW spreading, particularly in terms of organic matter and potassium.

Bioclimatic study

From the ombrothermal diagram (Fig. 2), we can see that Fez city has hot and dry summers and mild and wet winters. The average maximum temperatures range from 18° C in January to 38° C in July and August, while the average minimum temperatures range from 6° C in January to 22° C in July and August. The difference between the average maximum and minimum temperatures is most pronounced during the summer months and is at its narrowest during the winter months. Precipitation levels remain relatively low throughout the year, with the highest average monthly precipitation occurring in November and the lowest in July. The dry season extends from June to September, characterized by minimal rainfall. Conversely, the wet season spans from October to May, with the heaviest rainfall typically observed in November. Overall, Fez city has a relatively mild climate with low precipitation, and a clear distinction between wet winters and dry summers. The Q_2 values were obtained by applying

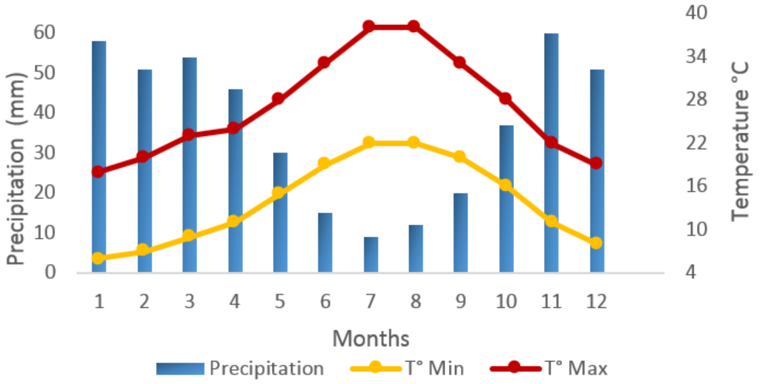


Figure 2. Ombrothermal diagram for Fez city.

the three formulas mentioned earlier, as presented in Table 4.

Based on the climatic parameters, the studied region was positioned on the Emberger diagram (Fig. 3), which indicated that Fez city is situated in a semi-arid climate zone.

The Emberger diagram is used in ecology to visualize the relationship between different environmental factors and vegetation distribution. Mint has a wide range of applications in food, pharmaceutical and cosmetic industries. However, its growth and yield can be severely affected by environmental conditions in semi-arid climates, especially water stress. Yahia et al. (2019) showed that water stress leads to reductions in leaf area, plant height, and essential oil content, all of which are essential for flavor and aroma. Altitude and rainfall are also key factors affecting mint growth and yield. In studies done by Muñoz-Bertomeu et al. (2007) and Shams et al. (2016), the results showed that populations from upper semi-arid regions had higher essential oil production than those from lower humid regions, with precipitation having a more pronounced effect than altitude. In addition, according to Melito et al. (2016), essential oil quality varies with altitude and rainfall, and different populations show different chemotypes. Therefore, selection of populations from upper semi-arid regions for improvement programs can increase essential oil yield and quality (Rao, 1999). Scarcity of water resources in semi-arid areas can seriously affect the growth and yield of mint (Ghamarnia et al., 2021), thus requiring effective management of water resources. However, overwatering can also negatively affect growth, and dry weight of mint plants (Nezami et al., 2016). Therefore, proper management of water and nutrients in semi-arid regions can improve the growth, yield, and quality of mint and other medicinal plants, leading to sustainable agriculture and better human nutritional health (Sborezi et al., 2021). In summary, semi-arid climate conditions significantly influence mint growth and yield, emphasizing the importance of considering climate factors when cultivating and improving mint varieties in such regions. Further research is necessary to optimize the growth of different mint varieties in semi-arid areas. Effective management of water and nutrients is essential to mitigate semi-arid climate effects on plants and enhance soil physicochemical and microbiological properties. Regarding the microbial soil community in Fez city, it can also be greatly affected by the semi-arid climate. In general, soil microorganisms play a vital role in ecosystem processes, including nutrient cycling and soil formation. However, the challenging conditions of the semi-arid climate, characterized by high temperatures, low and erratic rainfall, and nutrient-poor soils, pose difficulties for soil microorganisms. In hot and dry conditions, soil moisture becomes limited (Geirinhas et al., 2022), which can reduce soil microorganisms activity and abundance. This can limit the microbial-mediated processes that occur in soil, such as decomposition and

Table 3. Soil analysis of plots irrigated with different doses of OMWW.

		Doses		
	Characteristics	**0 L/m^2**	**5 L/m^2**	**10 L/m^2**
Acid-base status	pH eau	7.11	7.32	7.08
	pH kcl	6.77	6.87	6.81
	Total limestone%	36.15	37.15	34.81
	Active limestone%	11.22	10.57	10.24
Humus rate	Nitrogen Mineral mg/100g	3.97	7.03	7.46
	Organic matter	3.4	3.88	4.69
Major elements in ppm	P assimilable P_2O_5	25.98	53.33	61.76
	K echangeable K_2O	635.4	687	747.53
Salinity mmhos/cm	Salinity	0.142	0.18	0.197

Table 4. Emberger bioclimatic quotient (Q_2) for the region of Fez.

References	Q_2
(Mokhtari et al., 2013)	46.89
(Stewart, 1968)	47.48
(Emberger, 1942)	46.90
Means	47.09

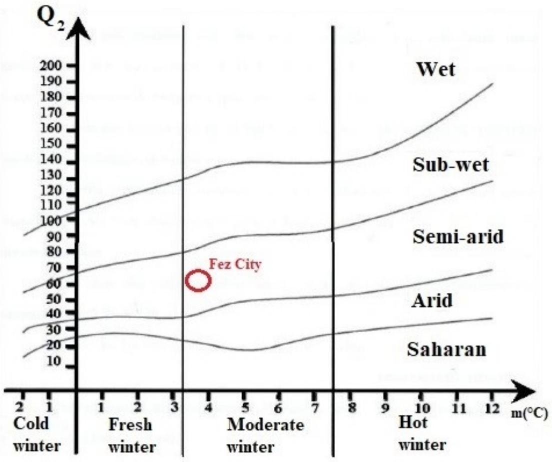
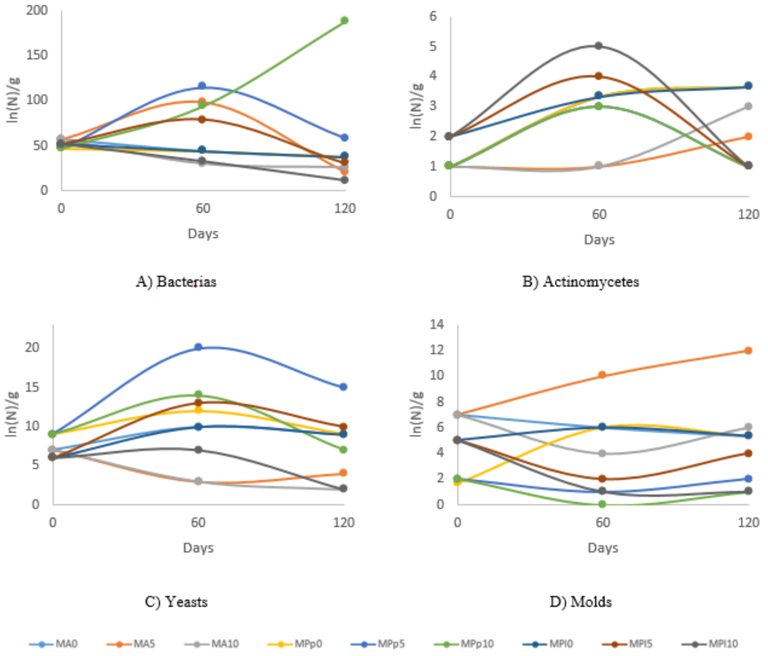


Figure 3. Emberger diagram of the bioclimatic stage of Fez city
Q_2: Emberger bioclimatic quotient; m: Minimum temperature of the coldest month in ° C.

nutrient cycling. Additionally, low rainfall can lead to a buildup of salts in soil, which can be toxic to many microorganisms. On the other hand, during the wetter winter months, the increase in moisture can lead to an increase in microbial activity, with a corresponding increase in nutrient cycling and decomposition rates (Cruz-Paredes et al., 2021). In a study conducted by Gorlach-Lira and Coutinho (2007) in a semi-arid area, bacteria were almost exclusively represented by Gram positive spore-forming isolates and Actinomycetes. These groups of bacteria are characterized by their physiological adaptability and ability to produce spores that are resilient in drastic environmental conditions. As a result, they are able to endure and maintain a stable population even during periods of intense stress, such as high temperatures and dry spells (Wardle, 1998). In semi-arid regions, nutrient-poor soils can limit actinomycetes' growth and activity. Nevertheless, certain species of actinomycetes have developed mechanisms to cope with drastic conditions. These mechanisms include the production of drought-resistant spores and enzymes, enabling them to access nutrients in low-quality soils. This adaptation has been supported by a study conducted by Gorlach-Lira and Coutinho (2007), who found that in a semi-arid area, the production of amylase, chitinase, cellulase and protease enzymes was more common among endospore-forming bacteria and actinomycetes. All substrates tested were 50% of actinomycetes and 8% of spore-forming bacteria.

Microbiological study of the soil before OMWW spreading, after OMWW spreading and after planting

Fig. 4 illustrates the microbial community evolution evaluated per gram of soil (N/g) in the different plots cultivated with the three varieties of mint *Mentha aquatica* (MA), *Mentha piperita* (MPp) and *Mentha pulegium* (MPl) and treated with different doses of OMWW (5 L/m^2 and 10 L/m^2) against three designated controls for each plant.
It is noted that bacteria number per gram of soil always increases in plots treated with OMWW only with a dose of 5 L/m^2 (Fig. 4A). This increase can be explained by soil enrichment in mineral salts (Table 1) following the OMWW application. This bacterial load decreases during treatment with a dose of 10 L/m^2 following the pressure exerted by OMWW phenolics compounds with antimicrobial activity. Thus, we note that mint (*Mentha piperita* MPp$_{10}$) at plot irrigated with 10 L/m^2 shows an increase in bacteria number after 4 months of culture (120 days). This can be explained by the presence of released substrate by plant which presents a source of carbon and energy for bacteria and the absence of an inhibitor for the latter. This means that Mpp plant does not release toxic matter into soil, so we have a synergy between the plant (MPp) and the bacterial load in the plot planted by this specie. Our result is in agreement with AL-Eitan et al. (2021) who reported an increase in bacteria number after the application of OMWW. According to Serio et al. (2008), the presence of bacteria in soils treated with OMWW is attributed to their pivotal role in the organic matter cycle. These microorganisms catalyze activities and possess the capability to decompose complex organic molecules, aligning with our study's findings. In a previous research work done by Abid et al. (2007), during composting of olive mill wastewater sludge, actinomycetes were found to dominate over thermophilic bacteria, indicating their significant role in organic matter breakdown and transformation within the composting system. Also, according to a research conducted by Lanza et al. (2020), the quantities of actinomycetes in soil treated with OMWW with different doses was always higher than the soil control. Monitoring of actinomycetes in different plots showed that the most adequate dose of OMWW for their growth is 5 L/m^2. This applies to the three mint crops (Fig. 4B) where there was an increase in of actinomycetes in the plots treated with a dose of 5 L/m^2, and a decrease in these microorganisms at a dose of 10 L/m^2, except in the cases of *Mentha aquatica* and *Mentha pulegium* where the load of actinomycetes increased. The decline in actinomycetes populations at the 10 L/m^2 dose can be attributed to the high OMWW concentration rich in toxic phenolic compounds, the release of toxic molecules by plants into the soil, or the prevalence of bacteria resistant to actinomycetes' antimicrobial activities, as seen in the case of MPp$_{10}$.
Our results are in agreement with those found by Hassani et al. (2010b) who also noted an abundance of actinomycetes, when applied dose was 8 L/m^2 on *Mentha spicata*



A) Bacterias

B) Actinomycetes

C) Yeasts

D) Molds

Figure 4. Microbial community evolution in plots irrigated with OMWW at different doses and planted with three genus Mentha sp species (A: Bacteria, B: Actinomycetes , C: yeasts, D: molds).
0 days: Before spreading OMWW; 60 days: Spreading OMWW and start of planting; 120 days: End of planting
MA_0: (Mentha aquatica, 0 L/m^2); MA_5: (Mentha aquatica, 5 L/m^2); MA_{10}: (Mentha aquatica, 10 L/m^2),
MPp_0: (Mentha piperita, 0 L/m^2); MPp_5: (Mentha piperita, 5 L/m^2); MPp_{10}: (Mentha piperita, 10 L/m^2),
MPl_0: (Mentha pulegium, 0 L/m^2); MPl_5: (Mentha pulegium, 5 L/m^2); MPl_{10}: (Mentha pulegium, 10 L/m^2).

L culture. Additionally, Serio et al. (2008) reported a rise in actinomycetes, particularly in the superficial soil layers treated with OMWW. Thus conducted study by Regni et al. (2021), shows that among the most abundant genera of bacteria in soil treated with OMWW, we find actinobacteria. Similar results were also found by Pezzolla et al. (2015) who observed that it was the only group of Gram-positive bacteria that accounted for more than 85% of total abundance in a study where OMWW digestate was used as an amendment.

In a study conducted by Mechri et al. (2014), which focused on agronomic OMWW application, it revealed a significant distinction between amended and control soil. This research suggests that the modified soil environment may foster more favorable conditions for the survival and proliferation of actinomycetes, resulting in a higher proportion of these microorganisms. Moreover, the rise in actinomycete population subsequent to the application of organic olive mill wastewater (OMWW) could also be linked to the actinomycetes' capacity to metabolize various phenylpropenoid acids, such as ferulic, vanillic, and p-coumaric acids (Brunati et al., 2004; Mechri et al., 2014). These phenolic acids, commonly found within OMWW, provide insights into the plausible mechanisms contributing to the observed escalation in actinomycete activity.

Yeasts monitoring in plots of this experimental planning showed that regardless of the dose of OMWW, they can develop with small variations (Fig. 4C). This can be explained by the fact that soil yeasts seem to be more adapted to OMWW composition and to the presence of yeasts in OMWW microbial flora (Table 1). Thus peppermint culture of is the most suitable for these microorganisms development.

Indeed, the presence of yeasts during the different treatments is explained by their high capacity to degrade organic matter, in particular that which is not easily degradable such as phenolic compounds (Oskay et al., 2004; Mekki et al., 2006; Sassi et al., 2008; Hassani et al., 2010b).

Molds monitoring showed their presence at the level of the different OMWW (Fig. 4D) since they were already present in the microflora of the latter (Table 1). Thus, 10 L/m^2 dose of vegetable waters and the culture mint enabled them to multiply well.

Fungal populations are renowned for their potent depolymerizing enzymes and resilience in the face of recalcitrant substances. OMWW has been shown to enhance fungi, particularly as crucial agents in lignin and phenolic compound degradation (Mekki et al., 2013). Our result is in agreement with that found by (Serio et al., 2008) which underlined the presence of fungi in areas treated with OMWW.

Soil microbiological study conducted before and after OMWW spreading revealed interesting patterns. In the cases of Mentha aquatica and Mentha piperita, it was consistently observed that the microbial load (including bacteria, yeasts, and molds) in plots treated with a 5 L/m^2 dose of OMWW was higher than in plots treated with a 10 L/m^2 dose, except for actinomycetes. Actinomycetes showed adaptability to different conditions, with no significant difference in their abundance between the two

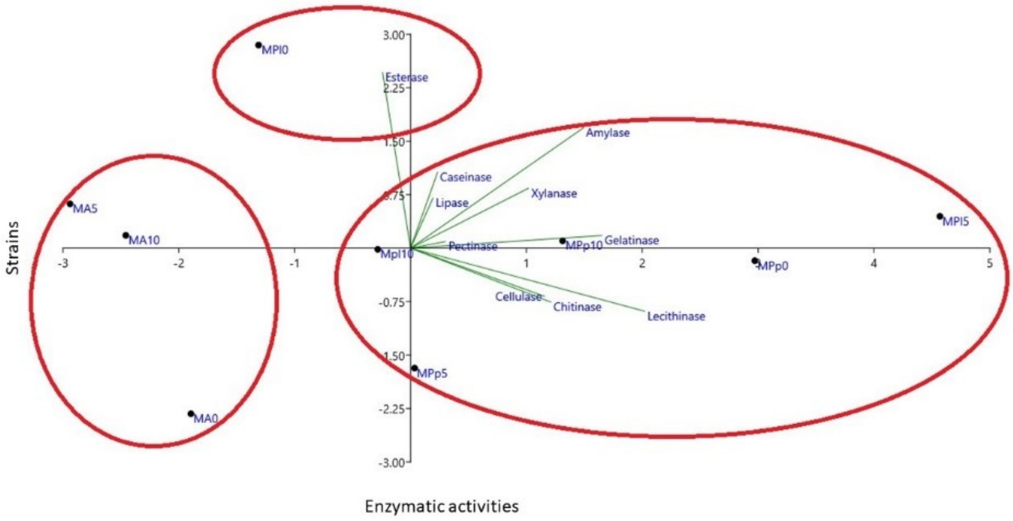


Figure 5. Correlational analysis between enzymatic activities of the strains with OMWW doses and the plantations MA$_0$: (Mentha aquatica, 0 L/m^2); MA$_5$: (Mentha aquatica, 5 L/m^2); MA$_{10}$: (Mentha aquatica, 10 L/m^2), MPp$_0$: (Mentha piperita, 0 L/m^2); MPp$_5$: (Mentha piperita, 5 L/m^2); MPp$_{10}$: (Mentha piperita, 10 L/m^2), MPl$_0$: (Mentha pulegium, 0 L/m^2); MPl$_5$: (Mentha pulegium, 5 L/m^2); MPl$_{10}$: (Mentha pulegium, 10 L/m^2).

doses in the case of Mentha aquatica. However, in the case of Mentha piperita, the quantity of actinomycetes was higher in plots treated with 10 L/m^2, possibly due to their adaptability and efficient assimilation of OMWW phenolic compounds. In the case of Mentha pulegium, the density of microorganisms (bacteria, actinomycetes, yeasts, and molds) was higher in plots treated with a 5 L/m^2 dose of OMWW compared to those treated with a 10 L/m^2 dose.

The results of these experiments shows that OMWW spreading with a dose of 5 L/m^2 generally favors soil microflora, but this increase tends to diminish after exceeding this dose. Our results are similar to those cited by Yaakoubi et al. (2010), who reported that OMWW spreading led to an increase in the abundances of different microbial groups sought. Indeed, Serio et al. (2008) have also shown that OMWW spreading with doses of 8 and 16 L/m^2 on cultivated maize plots generally led to an increase in soil microbial flora.

Microbial community variations have been explained by several authors as possibly resulting from interactions between different factors such as micro-environmental changes (decrease in oxidation conditions, strong competition for mineral nitrogen and phenolic compounds availability) and selective inhibition of other microbial groups by phenolic compounds and altered sources of carbon (Karpouzas et al., 2010). It has been suggested that OMWW spreading has had an impact on microbial communities structure by affecting soil nutritional status (Rousidou et al., 2010). The same authors linked microbial community changes in soil structure modification that

Table 5. Distribution of enzymatic activities of actinomycetes strains isolated from plots treated with OMWW and planted with different mint crops.

	MA$_0$	MA$_5$	MA$_{10}$	MPp$_0$	MPp$_5$	MPp$_{10}$	MPl$_0$	MPl$_5$	Mpl$_{10}$
Amylase	0	1	1	3	0	2	2	4	2
Cellulase	1	0	1	2	3	2	1	3	1
Xylanase	0	1	2	1	1	2	1	4	1
Pectinase	0	0	0	0	0	0	0	1	1
Lecithinase	1	0	0	3	2	3	0	4	2
Esterase	0	2	2	2	1	2	4	1	2
Chitinase	3	2	2	5	3	3	2	4	3
Gelatinase	3	2	2	6	4	4	4	5	3
Caseinase	0	1	0	1	1	1	2	1	0
Lipase	1	1	0	0	1	1	2	2	1

MA$_0$: (*Mentha aquatica*, 0 L/m^2); MA$_5$: (*Mentha aquatica*, 5 L/m^2); MA$_{10}$: (*Mentha aquatica*, 10 L/m^2), MPp$_0$: (*Mentha piperita*, 0 L/m^2); MPp$_5$: (*Mentha piperita*, 5 L/m^2); MPp$_{10}$: (*Mentha piperita*, 10 L/m^2), MPl$_0$: (*Mentha pulegium*, 0 L/m^2); MPl$_5$: (*Mentha pulegium*,5 L/m^2); MPl$_{10}$: (*Mentha pulegium*, 10 L/m^2).

occurs after OMWW application to the substrate. Our results also show that the amount of OMWW used can influence the microbial load in a quantitative and qualitative way. To summarize, the impact of olive mill wastewaters (OMWW) on soil microflora can be understood from three key perspectives: the stimulation of microflora growth through the enrichment of easily degradable carbon, the interactions between plants and OMWW, and the introduction of OMWW components into the soil, which could potentially exert toxic effects on specific microorganisms. Saadi et al. (2007) noted a transient effect lasting three months after OMWW application on vertisol soil. Applying a high dose (72 m^3/ha) resulted in increased microorganism numbers one week after the final application, in comparison to the control. Serio et al. (2008) observed an upsurge in soil microbial biomass, particularly in response to high OMWW concentrations. Furthermore, the concentrations of fungi and actinomycetes notably increased in the upper soil layers treated with OMWW. Hassani et al. (2010b) also observed heightened abundance of yeast, actinomycetes, and cellulolytic bacteria following an OMWW dose of 80 m^3/ha.

Competent actinomycetes strains selection

Table 5 presents screening results of actinomycete strains isolated from the treated plots according to their ability to assimilate various carbon sources.

The effect of OMWW dose and the type of culture on actinomycetes is expressed by evaluating enzymatic activities identified in each plot or by observing the presence of the greatest number of enzymatic activities

Table 6. Assimilation of carbon and azote sources by the Actinomycetes isolates.

Strains/Carbon source	Glucose	Saccharose	Asparagine	Histidine	Fructose	Glycérol	Xylose	Maltose	Mannitol	Galactose	Sorbitol	citrate	mannose	L-proline	L-methionine	Inositol
BOEF1	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+
BOEF2	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
BOEF3	+	+	+	-	+	+	-	-	+	+	+	+	+	+	-	-
BOEF4	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-
BOEF5	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
BOEF6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
BOEF7	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
BOEF8	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	-
BOEF9	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-	+
BOEF10	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
BOEF11	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
BOEF12	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
BOEF13	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
EFBO1	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+
EFBO2	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
EFBO3	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	-
EFBO4	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-	-
EFBO5	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-	-
EFBO6	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
EFBO7	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
EFBO8	+	+	+	-	+	+	-	-	+	+	+	+	+	+	-	-
EFBO9	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	-
EFBO10	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	-
EFBO11	+	+	-	-	+	+	-	-	+	+	+	+	+	+	-	-
EFBO12	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
EFBO13	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
EFBO14	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	-
EFBO15	+	+	-	-	+	+	-	-	+	+	+	+	+	+	-	-
BOEF201	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-
BOEF204	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
BOEF205	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
BOEF202	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-
BOEF203	+	+	-	-	+	+	-	-	+	+	+	+	+	+	-	-
BOEF206	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+

at the level of the MP15, followed by MPp_0 and MPp_{10}. These results corroborate with data in Fig. 4, which show that plant nature can intervene to change the microbial load of the soils treated in untreated by OMWW and this is well shown in control plots, where actinomycetes density in plots cultivated with *Mentha piperita* is higher than those of other mint crops.

Fig. 5 presents the dimensional analysis (PCA) of the enzymatic profile of actinomycete strains in relation to the OMWW doses and the culture type.

Correlational analysis allows us to interpret the correlations between enzymatic activities, OMWW dosage, and the type of mint plantation. It is noted that the most competent actinomycete strains are positively correlated with plots irrigated with 5 L/m^2 and 10 L/m^2 of OMWW, and cultivated with *Mentha piperta* and *Mentha pulegium*. While for *Mentha aquatica*, the performing actinomycetes are noticed in non-irrigated OMWW plot. On the other hand, strains with a low enzymatic potential are present in plots cultivated with *Mentha aquatica* spread with different concentrations of OMWW.

This result indicates that cultivating *Mentha piperita* and *Mentha pulegium* with OMWW doses ranging from 5 to 10 L/m^2 promotes the growth of competent actinomycetes with strong capabilities for degrading various carbon and energy sources. Thus OMWW did increase or decrease the metabolic pathways of actinomycetes depending on the plant type.

All the isolated strains use most of the carbon and nitrogen sources including glucose, sucrose, fructose, galactose, mannose, citrate, glycerol, mannitol and L-proline. However, the other sources were used by only a few strains (Table 6).

The use of different carbon sources by all strains indicated a wide pattern of carbon assimilation (Arasu et al., 2013). Our result is similar to those found by Do et al. (2021); Aallam et al. (2021) and Kuncharoen et al. (2022), having isolated strains that have the capacity to use various carbon and nitrogen sources as a sole source. Also, Meliani et al. (2022) reported that most actinomycetes are known to use a wide range of organic compounds as carbon source for their growth which can explain our results and justify adaptation capacity of our strains to the different conditions present in the plots irrigated by olive mill wastewater and planted by mint.

4. Conclusion

Spreading OMWW on different species of mint has improved the soil nutritional quality and established a new actinomycete-rich microbial population that is conducive to soil fertility. This advantageous action is conditioned by the right dose of OMWW (5 L/m^2). The enzymatic profil of actinomycetes isolated from different soil samples highlight the presence of highly efficient strains, particularly in plots irrigated with 5 L/m^2 and 10 L/m^2 of OMWW and cultivated with *Mentha piperita* and *Mentha pulegium*. The results of this work emphasize that using OMWW in soils treatment for plants of different varieties of mint allows for the selection of competent actinomycetes strains with a good ability to degrade different carbon and energy sources. The pursuit of novel molecules with promising properties remains an active research endeavor. In this context, investigations into actinomycetes offer valuable insights across multiple fields, especially in agronomy.

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Ethical approval
This manuscript does not report on or involve the use of any animal or human data or tissue. So the ethical approval is not applicable.

Author contribution
The authors confirm the study conception and design: O. Beroigui, F. Errachidi ; data collection: O. Beroigui, F. Errachidi, L. Elghadraoui ; analysis and interpretation of results: O. Beroigui, F. Errachidi, L. Elghadraoui ; draft manuscript preparation: O. Beroigui. The results were evaluated by all authors, and the final version of the manuscript was approved. The contribution of the authors to the research effort must adhere to the authorship standards outlined in the IJROWA Authorship Guidelines and as advised by the Committee on Publication Ethics (COPE).

Availability of data and materials
Data presented in the manuscript are available via request.

Conflict of interest statement
The authors declare that there are no conflicts of interest associated with this study.

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