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LIST OF ABBREVIATION

ABBREVIATION	Mean
OMWW	Olive mill wastewater
COD	Chemical Oxygen Demand
GA	Gibberellins
IAA	Indole Acetic Acid
UOMWW	Untreated olive mill wastewater
TOMWW	Treated olive mill wastewater
BOMWW	Bioaugmented olive mill wastewater
TPOMWW	Two-phase olive mill wastewater
TPF	Triphenylformazan
PLI	Phenolic compounds Loss Index
LiP	Lignin peroxidase
MnP	Manganese- dependent peroxidase

SUMMARY

Olive oil industry produced of huge amounts of wastewater which contain large amount of organic matter and phenolic compounds. Olive mill wastewater (OMWW), when discharged into surface water which used for irrigating soils, affects physical and chemical properties of soils and plants due to formations of crust layer on the surface, preventing oxygen to pass the soil. This led to inhibition of microbial activity due to low dissolving oxygen concentrations in the soil, in addition to toxic effect on plants, polluted surface and underground water causes eutrophication process. Chemical oxygen demand (COD) of OMWW can be higher than sewage.

On the other hand, OMWW contains high amount of potassium, calcium, phosphorus and some plant hormones that can be used in agriculture application.

The study was therefore aimed to treat OMWW by degrading the phenolic compounds and reducing COD values using microorganisms and finally evaluate the use of treated OMWW in irrigating pea plant (*Pisum sativum*). Results of the research can be summarized as follows:

1. Physical and chemical properties of OMWW

- Two samples were collected one from waste water of agriculture research center (used for fungal treatment) and the other from Wadi Food Industry (used for algal treatment).
- pH of the two wastes was 5.4 and 4.8, and EC values were 17.2 and 13.4, respectively.

2. Biological treatment of OMWW using fungi

- a. Eight isolates and strains were obtained and cultivated in OMWW at 25% concentration for two weeks. Results revealed that fungal isolate 5 was the most efficient in degradation of phenolic compounds and reducing of chemical oxygen content.
- b. Fungal isolate 5 was compared to the fungus *Pleurotus columbinus*, result showed that *Pleurotus columbinus* had a higher

efficiency in degradation of phenolic compounds than the fungal isolate 5. The highest percentage was 86 and 58% in the lowest concentration of the waste 10% by *P. columbinus* and fungal isolate 5 respectively after the fourth week of the incubation period.

- c. *P. columbinus* recorded the highest rate for decreased COD the percentage was 86.6% and fungal isolate 5 was recorded at 76.6 % after the fourth week of the incubation period at the lowest concentration of 10%.
- d. Different concentrations of the waste ranging from 10 to 40% were used to study the effect of both fungus on their ability to remove the color of the waste. The highest color clearance was 79.4%, with *P. columbinus* in the concentration of 10% for the waste after the fourth week of the incubation period, while fungal isolate 5 achieved a reduction rate reached 49.3% on the same concentration and incubation period, while these percentages decreased at high concentrations of the waste.
- e. **Biomass as a dry weight for both fungi after cultivated on OMWW.**

Highest growth rate was 2.97 g / L in the first week at 10% reach to 7.58 g/L after the fourth week of the incubation period by *P. columbinus* while fungal isolate 5 was less than 2.8 g / L in the first week and 6.0 g /L in the fourth week. The results showed an inverse relationship between dry weight and incubation period at high concentrations 30 and 40%, where the growth of *P. columbinus* decreased from 1.14 to 0.89 g/L, and fungal isolate 5, decreased from 0.95 to 0.86 g/L at 40% of the waste concentration.

- f. **pH and electrical conductivity of OMWW as affected by Fungal isolate 5 and *Pleurotus columbinus*.**

No significant results were found in the change in pH or electrical conductivity of the wastes at concentrations of 10 to 40% for

fungal isolate 5, but pH was reduced from 5.8 to 5.6 and the electrical conductivity was reduced from 2.4 to 1.95 dS/m by *P. columbinus* at 10% concentration of waste.

g. **Carbohydrates and phytohormone as affected by the fungal biotreatment of OMWW.**

Results showed a slight reduction in the total carbohydrate content in the concentration of 30 and 40% while the percentage increased in the concentration of 20 and 10%, being 3.10 and 4.37 g / L in concentration of 20% of the waste, while the quantity increased from 1.46 to 3.90 g / L in concentration of 10% after the fourth week of the period of incubation.

As for the change in level of growth hormones, it was estimated in the concentrations of 20 and 10% of the waste. The results showed a slight decrease in the level of Indole-acetic acid in the two concentrations than control, while increased the level of gibberlic acid in the concentration of 10% from 0.66 to 1.58 mg / l after the fourth week of the incubation period, while at the concentration of 20%, a slight reduction in the level of gibberellins was observed.

3. Biological treatment of OMWW by some algal strains and isolates.

Wollea sp. has the highest rate in degrade of phenolic compounds (68.9%), followed by *Spirulina platensis* (65.7%), at a concentration of 10% OMWW after 30 days. These ratios decreased in the concentration of 20% OMWW to reach 58.7% and 58% for *Wollea* sp and *Spirulina platensis* respectively after 30 days of incubation.

The highest percentage of COD reduction reached 63.98% by *Spirulina platensis* followed by *Wollea* sp., being 58.83% at the concentration of 10% of the waste after 30 days of incubation, and decreased in the concentration of 20% to 53.1% and 47.5% for the *Spirulina platensis* and *Wollea* sp. respectively.

a. Total carbohydrate as affected by treatment of OMWW by algae.

The increase in carbohydrate content after 10 days from 0.5 to 0.6 g /L at 10% of the wastes was decreased and increased from 1.16 to 1.30 g / L after 10 days at 20 % OMWW, the amount of carbohydrate began to decrease after 20 and 30 days of incubation period by *Spirulina platensis*, while the quantity was lower than the control in the other algae.

b. Algae efficiency to reduce the color of OMWW.

At 10% OMWW achieved the highest rate of color reduction, being 47% by *Wolleea* sp. followed by *Spirulina platensis* 44.7% while in the 20% concentration, the percentage dropped to 41.9% and 40.9% by *Wolleea* sp. and *Spirulina platensis* respectively after 30 days of incubation.

c. Algal growth as dry weight on OMWW.

There was a slight change in growth for all species after 10 days of growth in both concentrations but growth increased after 20 days of incubation. At 10% concentration of the waste was more suitable for algal growth than the concentration of 20% where growth increased from 0.51 to 0.841 g / L by *Wolleea* sp followed by *Spirulina platensis*, where growth increased from 0.51 to 0.847 g / L at 10% concentration of the waste. This amount decreased at a concentration of 20% for the waste to 0.820 and 0.814 g / L by *Wolleea* sp. and *Spirulina platensis* respectively after 30 days of incubation.

d. pH value of the waste after treatment by algae.

The pH increased from 4.8 to 7.7 by *Spirulina platensis* after 30 days of incubation followed by *Chlorella vulgaris* where it reached 6.4 at 10% OMWW.

e. Phytohormones as affected by algal treatment of OMWW.

Growth hormones were estimated by *Wollea* sp. and *Spirulina platensis*. The results showed an increase of indole acetic acid from 16.23 to 19 µg/ ml after 30 days of incubation while gibberellins increased from 1.11 to 1.55 mg / ml by *Spirulina platensis* at 10% OMWW. The amount of these hormones is slightly increased by *Wollea* sp. at a concentration of 10% OMWW.

A slight decrease of indole - acetic acid and gibberellins than control at 20% OMWW for both algae.

4. Use of biologically treated OMWW in irrigation of pea plant.

Treated wastewater by *P. columbinus*, *Wollea* sp. and *Spirulina platensis* was applied in irrigated of pea plants after mixing with tap water at rate 50% and compared to untreated OMWW and tap water as control, where use two level of mineral nitrogen fertilization at a rate 100%N and 75%N and impact of these treatments on the plants was studied and the results are summarized as follows:

4.1. Plant lengths and dry weight of shoots and roots

plant lengths and dry weight of shoot and root of the plants were increased in all treatment than plants irrigated with the untreated wastewater, while the highest length and weight achieved by plants irrigated with OMWW treated with *Spirulina platensis* at 100 %N than 75%N followed by *P. columbinus* at 100%N.

4.2. Total Nitrogen, Phosphorus and Potassium in shoot system.

The highest percentage of total nitrogen was found in shoot system with the plants that were irrigated with *Spirulina platensis* at 100% N fertilization rate. The highest percentage of phosphorus achieved in plants irrigated with OMWW treated by *P. columbinus* at 100%N fertilization rate. While the highest yield of potassium was obtained by plants treated with untreated OMWW. The ratio of nitrogen, phosphorus and potassium was increased in all treatments than control.

4.3. Total nitrogen, phosphorus and potassium in the root system.

The highest total nitrogen was given by *Wollea* sp. at fertilization rate of 100%N, while the percentage of phosphorus and potassium increased in plants irrigated with untreated OMWW at rate 100%N.

4.5. Dehydrogenase activity in rhizosphere region.

In contrast to nitrogen, phosphorus and potassium, the enzyme dehydrogenase showed the highest percentage of the plants were fertilizer at the rate of 75% N which irrigated with *P. columbinus*-treated waste.

4.6. Nitrogenase activity in rhizosphere region.

The activity of this enzyme increased in all treatments than control and achieved the highest ratio by plants irrigated with treated OMWW by *Wollea* sp., at 75%N, indicating an inverse relationship between increased fertilization rate and increased activity of dehydrogenase and nitrogenase in soil.

4.7. Chlorophyll content and carotenoids in the leaves

The highest quantity of Chlorophyll content and carotenoids was achieved by plants irrigated with treated wastewater by *Wollea* sp. at rate 100% N followed by plants irrigated with *P. columbinus*-treated OMWW.

4.8. CO₂ evolution in the rhizosphere region.

The rate of CO₂ evolution was increased in all treatments for plants irrigated with treated and untreated OMWW than control. The highest rate was recorded by plants irrigated with OMWW treated by *P. columbinus* at 75%N.

Generally, it could be concluded the following:

- 1- Possibility of treating OMWW resulting from olive oil industry which pollutig the environment can be treated by fungi *Pleurotus columbinus* and some algae *Wollea* sp. and *Spirulina platensis*.

- 2- Transformation of the components of olive mill wastewater from high toxicity components to less toxic components by these microbes was achieved and possibility of using this waste in agriculture application because it contains nutrients and elements which useful for plant and soil.
3. Use of treated olive mill wastewater as bio-fertigation of pea plant resulting in an increased of growth and improve the properties of the plant parameters. Improve of shoots and roots growth and increased the level of activity of dehydrogenase and nitrogenase and rate of carbon dioxide evolution, reflect the increase of microbial activity in soil.