British Journal of Global Ecology and Sustainable Development
Volume-15, April, 2023
ISSN (E): 2754-9291

EFFECT OF OIL WASTE TREATMENT ON PLANT GROWTH AND PRODUCTIVITY

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Abstract:

Simple Summary: Olive oil is the most common vegetable oil used for human nutrition, and its production represents a major economic sector in Mediterranean countries. The milling industry generates large amounts of liquid and solid residues, whose disposal is complicated and costly due to their polluting properties. However, olive mill waste (OMW) may also be seen as a source of valuable biomolecules including plant nutrients, anthocyanins, flavonoids, polysaccharides, and phenolic compounds. This review describes recent advances and multidisciplinary approaches in the identification and isolation of valuable natural OMW-derived bioactive molecules. Such natural compounds may be potentially used in numerous sustainable applications in agriculture such as fertilizers, biostimulants, and biopesticides in alternative to synthetic substances that have a negative impact on the environment and are harmful to human health.

Olive oil production generates high amounts of liquid and solid wastes. For a long time, such complex matrices were considered only as an environmental issue, due to their polluting properties. On the other hand, olive mill wastes (OMWs) exert a positive effect on plant growth when applied to soil due to the high content of organic matter and mineral nutrients. Moreover, OMWs also exhibit antimicrobial activity and protective properties against plant pathogens possibly due to the presence of bioactive molecules including phenols and polysaccharides. This review covers the recent advances made in the identification, isolation, and characterization of OMW-derived bioactive molecules able to influence important plant processes such as plant growth and defend against pathogens. in plant biology may benefit from the isolation and characterization of new biomolecules to be potentially applied in crop growth and protection against diseases. Moreover, the valorization of waste materials is necessary for the development of a circular economy, which is foreseen to drive the future development of a more sustainable agriculture.

Keywords: Olea europaea L.; olive mill wastes; plant growth; plant nutrition; plant protection; phenols; oligosaccharides; bioactive molecules

1. Introduction:

Olive tree (Olea europaea L.) cultivation for olive oil production is one of the most ancient agricultural practices known by mankind. Olive oil is an important component of the Mediterranean diet known for its high nutritional properties and beneficial health effects. On average, 3 million tons of olive oil are produced around the world every year and 2 million tons of this production takes place in the European Union (EU) representing the first producer, exporter, and consumer of olive oil in the world. The Mediterranean area alone including Spain, Italy, Greece, and Portugal covers around 99% of the olive oil EU production .

Olive oil extraction includes washing of olive fruits, fruit crushing, malaxation to brake off the emulsion and facilitate coalescence, and finally oil separation and extraction.

Olive oil extraction processes have improved over time due to the intensification of oil production and to the modernization of the technology aimed to upgrade the quality of the final product.

The milling industry generates in a short period, usually 4 months (October–January), large amounts of waste (olive mill wastes, OMWs). For instance, an estimated average volume of olive mill wastewater (OMWW) ranging from about 0.3 to 1.2 m3/tons of processed olives and an average quantity of solid residue ranging from 500 to 735 kg/tons of processed olives have been reported depending on the adopted extraction systems .OMWs due to their acidity, high levels of biological oxygen demand (BOD) and chemical oxygen demand (COD) are characterized by a high polluting and phytotoxic degree . On the other hand, OMWs are a source of valuable molecules including plant nutrients, anthocyanins, flavonoids, polysaccharides, and several phenolic compounds with potential industrial applications as fertilizers, antioxidants, antifungal and antibacterial drugs, cytoprotective agents, gelling and stabilizing agents in food preservation .

Consequently, significant efforts have been devoted to the transition from OMW detoxification to its valorization by optimizing the recovery of high added-value bioactive compounds to be commercially reused.

The increasing consumption of vegetables and the ongoing climate changes, with negative effects on crop production and plant diseases diffusion, requires the large utilization of stimulants, fertilizers, and pesticides to improve plant growth, crop yield, and phytopathogens control .

The future challenge for modern agriculture is to operate in a sustainable way reducing the over-application of synthetic fertilizers and pesticides that have a negative impact on the environment and on human health and high persistence in the ecosystems. As an alternative to synthetic chemicals, biostimulants and biopesticides are the best candidates for sustainable integrated crop productivity and pest management Bioactive molecules with growth promotion and antimicrobial effects, identified and characterized in OMW by-products, have stimulated many researchers to employ these compounds as biostimulants, biopesticides, and plant protectants for crop improvement. However, more extensive field research is required to evaluate their effects to solve serious plant diseases affecting commercially important crops with a sustainable, large-scale, agro-economical perspective.

2. Olive oil Extractive Methods and Olive Mill Wastes

From the classic traditional discontinuous system, modern mills have moved to the use of continuous cycle extraction processes including two-, three-phase decanter systems and, more recently, multi-phase decanter (MPD) technology in which all the oil extraction steps take place automatically and in succession with higher efficiency and capacity of centrifuge-based extraction

In the three-phase decanter system, warm water up to 50 L for 100 kg olive paste is added during malaxation to enhance oil extraction. At the end of the process, a large quantity of OMWWs (0.3–1.2 m3/tons of processed olives) and dried olive pomace (DOP) (about 580 kg/tons of processed olives; 55% moisture) are produced .

The two-phase decanter system requires lower water addition, thus smaller amounts of OMWWs (about 0.2–0.3 m3/tons of processed olives) are generated and a semi-solid waste, defined wet olive pomace (WOP) (about 740 kg/tons of processed olive; 62% moisture) is produced.

The WOP treatment is difficult due to the high moisture and high concentration in solids, lipids, carbohydrates, and polyphenols,

The MPD technology is a modern two-phase system performed without adding water during the process.

The introduction of a pulp-kernel separation system produces a dehydrated kernel-enriched fraction and a novel by-product, named Patè olive cake (POC) recovered during and not after the milling process by an exclusively mechanical treatment. POC consists of a wet fraction composed by olive pulp, olive skin, and vegetative water. POC is rich in several bioactive molecules, more concentrated with respect to OP derived from three- or two-phase systems.

OMWW is an expensive waste to dispose of and a major environmental concern in olive producing countries due to its high pollutant charge. OMWW is a dark-brown liquid (pH 3–6), constituted by a stable emulsion of vegetative water, process water, olive oil residues, and fragments of olive pulp. The OMWW composition depends on the extraction system, processed fruits, and processing conditions. OMWW consists of water (83–94% w/w) and organic compounds (4–18% w/w) including sugars, polysaccharides, tannins, organic acids, phenolic compounds, and lipids .

Its great complexity and variability in chemical composition represents a limit to its direct use as a raw material for industrial purposes.

3. Active Molecules in OMW and Their Analytical Characterization

In order to valorize wastes and introduce valuable by-products into the production cycle, it is of paramount importance to define their chemical-physical properties, as well as their chemical composition. For a long time, the most important parameters to define wastes were the chemical-physical ones, such as total solid, volatile solid, fixed solid, oil and grease content, polyphenol content, volatile phenol content, organic nitrogen, COD, and reducing sugar content.

More recently, analytical platforms were employed to better characterize the OMW parameters and a greater emphasis was placed on the evaluation of the antioxidant properties of these matrices ,The most used methods in order to detect the antioxidant capacity of single or multiple molecules, are oxygen radical absorbance capacity ,, total radical trapping antioxidant parameter ,Ferric reducing antioxidant power ,antioxidant reaction with an organic cation radical ,(diphenyl-1-picrylhydrazyl) copper (II) reduction capacity ,and crocin bleaching assay .The natural evolution of such studies is the investigation of the composition of OMW in terms of phenols, polyphenols, and sugars with more powerful techniques such as Fourier Transform IR, Mass Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, or a combination of these .

The content of bioactive molecules in OMW is heavily influenced by agronomic factors, such as pedoclimatic conditions of olive groves, as well as olive variety, harvest period, production year, and extraction process, as well as microbial treatments ,This observation implies that the chemical characterization of OMW is a step which must be repeated every time a new batch is to be employed for any kind of application.

Molecule	**Analytical Platform**	**Amount in OMWW (mg/g Dry Matter)**	**OMWW References**	**Amount in POC (mg/g Dry Matter)**	**POC References**
Phenols Tyrosol Hydroxytyrosol Hydroxybenzoic acid Coumaric acid Gallic acid Vanillic acid Caffeic acid Hydroxycinnamic acid	**HPLC, MS, GC, NMR HPLC, MS, GC, NMR HPLC, MS, GC, NMR HPLC, MS, GC, NMR HPLC, MS HPLC, MS HPLC, MS HPLC, MS**	**1.0–2.8 [6,32,39–42] 0.9–24 [6,32,39–42] [13–16] // 2–9 1–2 2–6 0.1–0.6 0.3–1.9 Detected**	**6,32,39–42]**	**0.4–1 0.6–2**	**13–16] 1.0–2.8 [6,32,39–42] 0.9–24 [6,32,39–42] [13–16]**

Phenols and Polyphenols:

Phenolics in plants are mainly synthesized through the phenylpropanoid pathway. Several phenols and polyphenols have been detected in OMWs (e.g., 32.8–103.4 mg/g dry matter in OMWW and 3.0–10.6 mg/g dry matter in POC, the most important ones being tyrosol, hydroxytyrosol, and their secoiridoid derivatives oleuropein and ligstroside. These compounds play an important role against inflammatory, aging, cancer, bacterial, etc. From a technological point of view, hydroxytyrosol could be very useful, but its synthesis is very expensive and it is not commercially available in large amounts For such reasons, the possibility to recover these compounds from natural matrices such as OMW could be an important goal.

Seco Iridoids:

The most diffused secoiridoids in OMW (e.g., 18–91 mg/g dry matter in OMWW and 0.2–9.6 mg/g dry matter in POC,) are derived from elenolic acid, which is often esterified with hydroxytyrosol or tyrosol to form the aglycons of, respectively, oleuropein and ligstroside .

Other common derivatives are based on dicarboxymethyl dialdehyde elenolic acid (oleacein) instead of elenolic acid. These molecules possess several properties in addition to their antioxidant potential,with oleuropein, ligstroside, and their aglycons showing significant antifungal and antimicrobial activity, as well as potential health benefits .

METHODOLOGY

A total of fifty one buckets each filled with 4000 g of loam soil obtained from the Biological Garden of the University of Lagos were used for this study. Three of the buckets were used for control studies and were not polluted with spent oil while the others were divided into four groups. Each group was subdivided into four subgroups with each subgroup containing three buckets. Each group represented a period of application of spent oil while each subgroup represented a volume of spent oil added to the soil. The spent oil was applied at week 1, 3, 5 and 7 after germination of the seeds of the test plant while the quantities of spent oil applied are 5, 10, 15 and 20 ml.

The plants samples from each bucket were obtained two weeks after the 7th week application of the spent oil by carefully uprooting one plant from each bucket. The shoot length, dry matter content, leaf area and chlorophyll content of the uprooted plant samples were determined. The shoot length was determined by measuring.

the plants from the base of each plant to the tip while the dry matter content was determined as was described by Merkl et al. (2004). Plant samples were oven dried at 60°C to constant weight for 24 h after which the weights of the dry samples were determined using a sensitive weighing balance (Acculab-USA VIC 300 Model). The leaf area was determined as was described by Pearcy et al. (1989) after measuring the length of the longest part of the leaf and the width of the widest part of leaf by using the formula 0.5 x L x B (L = length and B = breadth). The chlorophyll content of the plant was determined using the method of Heidcamp (2003). It involved the extraction of the chlorophyll of 1 g of each leaf with 10 ml of 80% acetone. The optical density (OD) of each extract was read off at 652 nm using spectrophotometer. The chlorophyll content (mg/l) of each leaf was determined by dividing the OD reading with 34.5 (Heidcamp, 2003).

The data obtained for the different parameters were statistically analyzed using Graphpad prism 4.0 software. This was done to determine the impact of the different quantities of spent oil applied to the plants and also the impact of the different times of application of the spent oil on the plant. These were done at 5, 1 and 0.1% levels of significance.

RESULTS:

The shoot lengths of maize seedlings exposed to different amount of spent oil at different points of growth are shown in Table 1. The shoot length of the plant treated with 10 mls of spent oil at the first and seventh weeks was significantly shorter than the shoot length of the plant from the control treatment ($P<0.01$) at same period. Treatment of the seedlings with 15 ml spent oil at first and seventh weeks of growth led significantly shorter shoot than the control treatment at the same period ($P<0.001$; $P<0.01$). At all weeks of application, 20 ml treatment led to significant reduction of the shoot length of maize ($P<0.05$; $P<0.01$; $P<0.001$). Plants treated with 15 and 20 ml spent oil were also significantly shorter than those with 5 ml spent oil at one week after germination ($P<0.05$). The dry matter content the plant was significantly affected by the quantity of spent oil added to the soil ($P< 0.05$, 0.01, 0.001) as shown in Table 2. Application of 15 and 20 ml of spent oil led to significant reduction of the dry matter content of maize ($P<0.05$, $P<0.01$; $P<0.001$) at all weeks of treatment. 10 ml treatment significantly reduced the dry matter content of maize ($P<0.01$) only when it was applied seven weeks after the germination of the seeds. Significant differences were also observed in the dry matter content of maize treated with different quantities of spent oil at the different weeks of application. For the dry matter of the maize.

Discussion:

The reduction of the plant growth observed in this study could be due to reduction of mineral element with increasing oil concentration in the soil reported by Odjegba and Atebe.. This could have occurred as a result of reduced availability of mineral elements because according to Clarkson and Hanson .plant nutrition is based not only on the presence of mineral elements in the soil but their availability. Another possible cause of the effects of spent oil on the maize plant observed in this study could be due to either the increased acidity in the soil or reduction in the catalase activity reported by Achuba and Peretiemo-Clark). Such increase soil acidity can affect the microbial distribution in the soil reducing their activities in the rhizosphere. The reduction of the catalase activity can affect the optimal soil conditions required for plant growth hence the reduction of plant growth observed in this study.

According to Meinz ,spent oil contains heavy metals and polycyclic aromatic hydrocarbons and chemical additives including amines, phenols, benzenes, calcium, zinc, lead, barium, manganese, phosphorus and sulphur which are dangerous to living organisms. The high level of toxic heavy metals and polycyclic aromatic hydrocarbon which has been reported to be present in spent oil can also account for the growth inhibition observed in this study.

Conclusions and Future Perspectives:

While OMWs have been for a long time an environmental issue, nowadays they are a source of bioactive molecules to be used in agriculture as natural pesticides, biostimulants, or plant protectants in alternative to harmful agrochemicals. New types of wastes, in particular POC, must be thoroughly studied to identify all potentially useful components, such as oligosaccharides to be employed as plant protectanta, as well as phenols and secoiridoids with potential antimicrobial properties. The definition of molecular mechanisms of action, coupling and complementing the protection activity of phenol and carbohydrate fractions could represent an attractive scientific challenge. In particular, basic research in plant biology may benefit from the isolation and characterization of new biomolecules to be potentially applied in crop growth and protection against diseases.

Researches on OMW as fertilizers have demonstrated two different potential uses As biostimulants or as soil amendments.

The phenolic contents in OMW composition, at adequate concentrations, is able to determine positive metabolic and physiological responses in plants. In addition to phenols, attention should be devoted to olive oligosaccharides for their potential role as elicitors of defense responses. Their characterization and effects as plant elicitors of defense responses have not yet been investigated.

There are few studies aimed to investigate the specific and/or synergistic actions of phenols and oligosaccharides in the complexity of the soil-plant system.

Further and more focused researches in these topics are needed as a challenge in the valorization of Olive Mill by-products in formulations complying to the new EU biostimulants regulations and towards a sustainable agriculture.

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