Effect of thermophilic pre-composting of olive mill wastewaters on reproduction and growth of earthworms Eisenia andrei

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Abstract

The aim of the present work was to test the effect of thermophilic pre-composting of olive by-products on reproduction and growth of earthworms *Eisenia andrei*. Three mixtures were composed of olive cake (600 kg), horse manure (300 kg) and wheat straw (100 kg). Olive Mill Wastewater (OMWW), was added different ratio (0%, 25%, and 50%), to prepare three treatment T1, T2 and T3 respectively, then placed in trapezoidal piles with dimensions (1m height and 3×2m base) and pre-composted for two months. Three vermibed were made using 200 kg of mixtures previously pre-composted and inoculated with 2 kg of earthworms. Another three vermibed were made from same non pre-composted mixture to test the effect of thermophilic pre-composting of olive by-product on earthworm's growth and reproduction rate. Earthworm's growth and chemical composition changes of each treatment were measured during vermicomposting process. The growth rate mg (worm^{-1}day^{-1}) was better in pre-composted treatment. Indeed pre-composted T_1 (control mixture) present maximum value of growth rate (11.79 mg worm^{-1} day^{-1}), against non-pre-composted T_4 (10.04 mg worm^{-1} day^{-1}), followed by T_2 (10.93 mg worm^{-1} day^{-1}), against non-pre-composted T_5 (7.54 mg worm^{-1} day^{-}1), and T_3 (8.57 mg worm^{-1}day^{-1}). In fact these results could be attributed to the OMWW's toxicity reduction.

Keywords: Olive pomace; Olive mille wastewaters; Pre-composting; Vermicomposting; *Eisenia andrei.*

1. Introduction

In Morocco, olive oil production is estimated at about 60.000 tones year^{-1} and generates an equivalent of 11 million working days (DPV/MAEE). However this sector also causes serious environmental problems due to the production of large quantities of olive by-products; OP and OMW, during short periods (Roig *et al*. 2006). To date, recycling of solid olive residues is generally limited to their use as a cheap, local source of fuel for ovens and water heating (Ait Baddi *et al*., 2004). However, is simply dumped directly into the sewage system, kept in evaporation lagoons or simply spread on the land, all too frequently resulting in an important environmental pollution (El Hajjouji *et al*., 2007). Given this situation, several chemical physical and biological techniques have been developed to treat and recycle OMW but despite the efficiency of these processes, their high cost is the main drawback to their industrial application (Suthar 2008). Vermicomposting is an alternative cost-effective and rapid biotechnological process to convert organic substances into a stabilized humus-like product (Garg *et al*., 2006a). Furthermore, some species of earthworms are able to consume a wide range of organic wastes from sewage sludge, animal wastes, agricultural residues, domestic wastes and industrial wastes (Yadav *et al*., 2011), *Eisenia andrei* has been shown to have broad international potential for bioconversion of organic wastes into high-value useful plant growth media and earthworm biomass, in windrows and a large-scale continuous-flow reactor (Edwards and Bohlen 1996). The aim of the present study was to test the effect of thermophilic pre-composting process on earthworms growth and reproduction as a means of recycling and treating olive by-product (OMWs and olive pomace), in order to reduce their toxicity, and process them into organic soil.

2. Material and methods

2.1 Earthworms and collection of olive by-products

Earthworms *Eisenia andrei* (Bouche 1972) was chosen thanks to its high activity, short time of growth, high rate of reproduction and can be susceptible to temperature variations and be handled easily (Dominguez and Edwards 2010). Olive by-products; olive cake (OC) and olive mill wastewater (OMWW) were collected from three-phase centrifugation system. Horse manure was used as nitrogen source and wheat straw was used as high-absorbency material.

2.2 Experimental set up

Three mixtures composed of olive cake (600 kg), horse manure (300 kg) and wheat straw (100 kg) were made. Olive Mill Wastewater (OMWW) was added to each one, in different ratio (0%, 25%, and 50%), to prepare three mixtures M1, M2, and M3 respectively, then placed in trapezoidal piles (1m height and 3×2m base), by the Rutgers system with occasional turnings (Cayuela *et al*., 2006). Water was regularly added to maintain appropriate moisture (50%). Throughout the process, the piles were aerated from the bottom with forced air (through a blower) in order to induce movement of air into the material and deliver oxygen to microorganisms. In addition to the forced ventilation, the piles were turned more frequent at the beginning, since the organic matter biodegradation was more active and less frequent when the thermophilic temperature started to decrease, in order to homogenize the mass, and to avoid compaction of the substrate and subsequent low porosity and poor air distribution. To test the thermophilic pre-composting effect of olive by-product on earthworm's growth and reproduction rate, three vermibed pre-composted T_1, T_2 and T_3 (1 m height and 3×1m base) were made using 200kg of mixtures previously pre-composted, and inoculated with 2 kg of earthworms *Eisenia andrei* (Bouche 1972) chosen randomly in a culture of our laboratory. Three other mixtures were prepared without thermophilic pre-composting test non composted T_4, T_5 and T_6. The moisture level of all vermibed was maintained about 75-80% throughout the study period by periodic sprinkling of adequate quantity of water.

The biomass evolution and reproduction ratio of earthworms and the changes in chemical composition of each mixture during the vermicomposting process have been controlled each ten days for two months. The growth of earthworm was measured in each vermibed; ten earthworms were separated from the substrate material by hand sorting after which worms were washed in tap water to remove the adhering material from their bodies and subsequently weighed on live weight basis. Then all measured earthworms were returned to the concerned container. Each measure was run in five replicate. On the basis of the obtained data about the biomass and cocoon numbers, other parameters of earthworm such as biomass increase rate (mg day^{-1}), maximum weight achieved was produced with the help of the recorded data for different vermibed. The homogenized sub-samples of the substrate material (1kg dry weight basis) were collected every teen day during two months from each mixture, and they were analyzed for pH, electric conductivity (Garg *et al*., 2006a).

3. Resultants and discussion

3.1Earthworm growth and reproduction performance in different treatments

The mean earthworm biomass increased significantly during vermicomposting period in all treatment, except T_6 where slightly decrease of biomass was observed after ten days of vermicomposting process. Indeed, the maximum biomass was recorded in T_1 (41.04 ± 0.56 g), followed by T_2 (38.29± 0.37 g), followed by T_3 (32.37± 0.54 g), followed by T_4 (36.67± 0.15 g), followed by T_5 (31.91±0.02 g) after 40 days (T_1, T_2 and T_4), 50 days (T_5) and 60 days (T_3) (table 1). However in T_6 mean earthworm biomass increase after 10 days (because of litter refuge) to reach a value of 15.35 ± 0.07g, followed by weight loss by the time of termination of the vermicomposting process.

The weight of earthworms was used to assess changes in biomass of earthworms in different treatments (table 1). Earthworms showed significant difference in growth, i.e. mean individual live weight acquired, growth rate (mg $worm^{-1}day^{-1}$) ($F = 1047.43$, $P < 0.00001$), among different treatments. *E. andrei* exhibited significantly higher mean biomass achieved in three pre-composted treatment T_1 (38.90 ±0.64 mg) followed by T_2 (37.01±0.58 mg) followed by T_3 (32.37 ± 0.53 mg), in contrary treatment without thermophilic pre-composted test showed lowest mean individual live weight achieved; T_4 (35.6 ±0.6 mg) followed by T_5 (30.6 ±0.1 mg), however mean biomass achieved decrease after one week in treatment T_6 to reached 9.3±0.4 mg (fig). Gunadi *et al*., (2001) reported that thermophilic composting of organic waste (precomposting) prior to vermicomposting has been shown to reduce pathogenic organisms and potentially toxic components (such as OMW). *E. andrei* exhibited the maximum individual live weight after 40 (T_1, T2 and T_4), 50 (T_5) and 60 (T_3) days, however in T_6 earthworms biomass decrease after 10 days of vermicomposting. It was reported that raw materials with high phenols fraction and lignin concentration (such as OP and OMW) are not well adequate for growth and development of most earthworm's species (Ganesh *et al*., 2009). Moreover, the chemical composition of the feed substrate has an important role in feed rate of earthworms (Alburquerque *et al*., 2006) consequently the difference biomass gain between earthworm can be explained by the difference of phenol concentration of each mixture.

The highest growth rate achieved was11.79±0.03 mg $worm^{-1}day^{-1}$, and this occurred in T_1, however the lowest growth rate was -0.79±0.02 mg $worm^{-1}day^{-1}$, this value was reported in T_6 because of earthworm's mortality (20%). Growth rate was important in treatment with thermophilic pre-composted test, thane non pre-composted treatment; in fact growth rate was in the order $T_1>T_2>T_3>T_4>T_5>T_6$ (table 2). The difference in growth rate between mixtures can be attributed to antimicrobial and toxic effect of OMW (Suthar 2008b), which inhibits microbial activity that is essential for organic matter degradation by earthworms (Suthar 2008a). There was a consistent pattern in worm growth rate tented to be decreased with increasing of OMWW concentration in

treatments. Thermophilic pre-composting efficiency in treatments T_1-T_3 was positively affected earthworm's mean individual live weight acquired and growth rate. Since organic matter content plays an important role in decomposition process due to its direct relation with microbial population and their mineralization activities.

3.2 pH

Edwards (1998) have reported that pH was an important factor in vermicomposting process and earthworm's development; in fact earthworms can survive in a pH range of 5 to 9. In deed evolution of pH may be a good indicator of bio-oxidation evolution and microbial development. OMWW hade acid pH (Mekki *et al*., 2008) that caused serious environmental problems and earthworms can't support OMWW acidity, for this reason we worked with dilutions of 0%, 25% and 50% of OMWW. Initial pH of different treatments varies between 8.7 and 6.4 these values have been measured in T_1 and T_6 respectively. A progressive increase of pH during vermicomposting process was observed in all treatment, reaching a maximum of 9.51 (T_6) (fig.1). In the end of experiences no significant difference was detected between different mixtures for this parameter (ANOVA, Newman-Keuls test, $P>0.05$). This increase was also reported by (Fernández-Gómez *et al*., 2010a), in fact several factors affecting pH; initial decarboxylation of organic acids, the formation of

Table 1: Earthworms biomass during vermicomposting process (mean ± SD, n = 3).

Mixture	Mean initial earthworm biomass (g)	Mean maximum biomass achieved (g)	Mean biomass achieved at the end (g)	Net biomass acquired earthworm^{-1} at the end (mg)	growth rate mg worm^{-1}day^{-1}
T1	10.59±0.3 [a]	41.01±0.6[a]	38.90±0.6 [a]	707.75 ±0.3[a]	11.79±0.03[a]
T2	10.76±0.2 [a]	38.29±0.4[b]	37.01±0.5[b]	656.25±0.4[b]	10.93±0.03[b]
T3	10.75±0.4 [a]	32.4±0.5[c]	32.4±0.5[c]	541.25 ±0.2[c]	8.57±0.01[c]
T4	11.5 ±0.1[a]	37.1 ±0.4[d]	35.6 ±0.6 [d]	602.5 ±0.3[d]	10.04±0.03[d]
T5	11.1 ±0.1 [a]	30.9 ± 0.5[e]	29.2 ±0.1[e]	452.5 ±0.1[e]	7.54±0.01 [e]
T6	11.2±0.4[a]	20.7 ±0.4[f]	9.3±0.4[f]	-47.5 ± 0.2[f]	-0.79±0.02[f]

*Mean value followed by different letters is statistically different (ANOVA; Newman-Keuls t-test, P < 0.05).

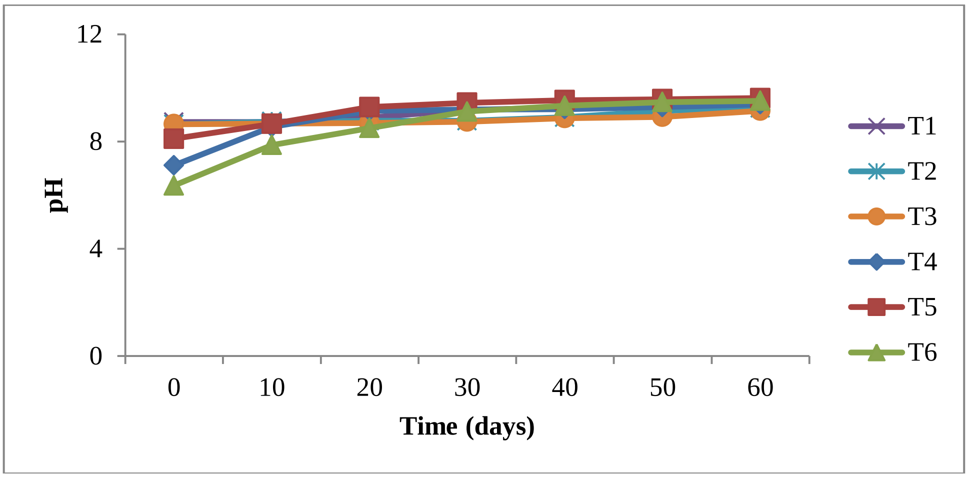

Figure 1: Comparison of pH change in different treatments

ammonium from protein degradation, the mineralization of nitrogen followed by nitrification (NH_4^+ is transformed into NO_3^-) and the production of humic acids (Dias *et al.*, 2010). During vermicomposting process the mineralization of proteins, amino acids and peptides leads to the release of ammonium or volatile ammonia and also contribute to the increase in pH. This trend could be due to the degradation of short-chain fatty acids and intensive nitrogen mineralization by microorganisms (Tognetti *et al.*, 2007a).

3.3Electrical conductivity (EC)

Gunadi and Edwards (2003) have reported that EC of feed could be the limiting factor for the survival and earthworm's growth. Mitchell (1997) reported that earthworms were unable to survive in the cattle solids with electrical conductivity of 5.0 dS m^{-1}.Soluble salts, as estimated by electrical conductivity determination (fig. 2), were generally fairly high throughout the vermicomposting process, with initial values in the range of 1073.22–1756 mS cm^{-1}, this values were reported in $T_1$and T_6 respectively. The electrical conductivity (EC) was decreased in the range of 60–21% (T_1 and T_6 respectively) two months of vermicomposting; the variation was significant (ANOVA, Newman-Keuls test F= 1508.08, P<0.0001) among all the treatment. The decrease in EC during the vermicomposting process has been related to the drop in soluble ion concentrations, the reason for this being that the soluble ions are leached due to the irrigation of beds during the vermicomposting process; that they are immobilized by the prolific microbes or worms; or that they are precipitated in the form of non-soluble salts (Alburquerque *et al.*, 2006).

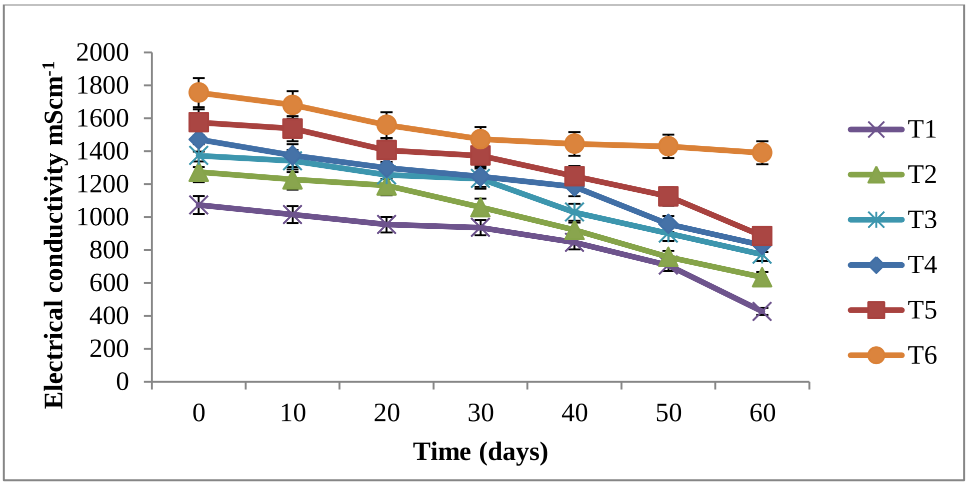

Figure 2: Comparison of electrical conductivity change in different treatments

4.Conclusion

Although OMW is a recalcitrant organic by-product for biodegradation, thermophilic pre-composting prior to vermicomposting was positively affected earthworms growth and reproduction. Thermophilic pre-composting was helpful in olive by-product, pH and EC stabilization. The study provides that vermicomposting can be a potential technology with low cost for the olive by-product bioconversion, i.e. vermicompost and earthworm biomass.

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