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Research article

Long term effect of Olive Mill Waste Water spreading on microbial population and the natural floristic composition of a sandy soil

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Abstract

The high organic content and the toxic nature of olive mill waste water compounds prevent its direct discharge into domestic wastewater treatment systems. Controlled land spreading of untreated olive mill waste water has been proposed as an alternative mean of disposal. A field study was conducted between 1995 and 2005 to assess the long term effect of OMW on microbial activity and the natural floristic species of a sandy soil. Three doses of olive mill waster water (50 m3/ha, 100 m3/ha, and 200 m3/ha) were applied in a sandy soil located at the region of Chammakh-zarzis (southern Tunisia 33° 36'N, 11° 02'E) and compared to control soils without any treatment. The spreading of OMW induced a significant increase of organic matter content and therefore results in abundance of bacterial and fungal population. OMW spreading increased the density of two beneficial plants Chenopodia and Mesembryanthemum cristallinum, and reduced the density of Diplotaxis harra known as a plant without any economic value. The obtained result revealed that OMW is an interesting soil fertilizer that can be used in sandy soils to improve not only its physical and chemical characteristics but also its biological activity.

Keywords: Olive mill waste water, microbial population, natural floristic species, sandy soil

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1. Introduction

Oil extraction generates a sub product such as olive mill wastewater, which constitutes a serious environmental problem. In Tunisia, olive production generates more than 800000 t/year of OMW mainly after the extension of the three-phase system of olive oil extraction. OMW is considered nowadays as a pollutant waste owing to its high content of organic substances (BOD5 up to 90 103 mg/l, COD up to 200 103 mg/l) with a preponderance of phenolic compounds [2] ; [12]. The current management of this waste is based on its collect in evaporation ponds near the manufactories. This practice can produce harmful effects to the environment, such as volatilization of some substances contained in OMW (phenols and sulphur dioxide) and their emissions into the atmosphere [14], strong odours and infiltration risks. Further more this practice is also expensive since the storage aeries are located far from the extraction factories. Thus, many authors have been searching for potentially better solutions. Several studies focusing on OMW valorisation have been carried out. Among these investigations, many experiments have been undertaken to use this effluent as a fertilizer. A positive effect of the small doses of OMW on olive plant growth has been reported [1].

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Despite the valorisation of OMW as a fertiliser, little is known on the long term effect of OMW on microbial activity of soils. This is particularly important for soil microbial community structure since OMW has been reported to exhibit antimicrobial activity [9]. It has been found that the addition of OMW to soils increased the total microbial population as measured by microbial biomass C and N, soil respiration, and dehydrogenase activity [10]; [11] ; [13] ; [6] and [5]. [12] found a significant increase of denitrifying and nitrifying bacteria after OMW soil application, whereas [7] reported a stimulatory effect of OMW on nitrogen-fixing bacteria. [11] found a significant reduction in the number of soil nitrifying bacteria at the highest OMW dose (400 m^3 /ha) applied. Moreover, [5] showed that high amounts of OMW increased the soil-denitrifying community and decreased slightly the population of nitrifying bacteria. Therefore, OMW appears to have an effect on different bacterial groups involved in N cycling. However, all of the above studies were based on culture-dependent methods (plate counting) which do not provide a full picture of the soil bacterial community. It is now well documented that only a small proportion (1–5%) of the total microbial community can be cultured in the currently known growth media [17]. The introduction of culture-independent techniques like denaturating gradient gel electrophoresis (DGGE) or terminal restriction fragment length polymorphism (TRFLP) analyses have provided a more thorough insight into changes occurring at the structure of the whole microbial community.

To the best of our knowledge, there is no information on the effect of OMW on the natural floristic composition of soils. In this investigation, we have evaluated the effect of the incorporation of three doses OMW (50 m^3/ha, 100 m^3/ha and 200 m^3/ha) to a sandy soil located at the southern Tunisia (Chammakh-zarzis) on the microbial community (total flora) 10 years after olive mill waster water spreading.

1. Materials and Methods

1.1.Olive mill waster water origin (OMW) and its characteristics

The olive mill waste water was taken from a three-phase continuous extraction factory located at the region of Chemmakh-Zarzis (Southern Tunisia, 33° 36’N, 11° 02’E) (Fig1). It was taken since 1995 and used for incorporation in a sandy soil. Its composition is reported in table 1.

Figure 1: Studied area

Table 1: Chemical characteristics of OMW (average values for five years)

Characteristics	Value
Humidity (%)	87.9
pH	5.5

CE (mS/cm)	18.6
DCO (g/l)	105.0
DBO_5 (g/l)	55.0
Organic matter (g/l)	107.0
Reducing sugar (g/l)	11.4
Glucose (g/l)	3.9
Phenols (g/l)	5.8
Greasiness matter (g/l)	4.5
Mineral matter (g/l)	13.7
Nitrogen (g/l)	1.4
Phosphates (g/l)	0.32
Potassium (g/l)	7.5
Magnesium (g/l)	0.65
Sodium (g/l)	1.31
Calcium (g/l)	0.71
Chlorures (g/l)	0.56
Actif chlore	Absent
Sulfures	Absent

1.2.Characteristics of the studied region and the treated soil

The treated soil was located in the region of Chammakh-Zarzis located in southern Tunisia in an environment with an arid Mediterranean climate with a mean annual rainfall of 180 mm, as long term average for the period of 1923-2004. The soil is moderately deep with a sandy texture and poor in organic matter. The soil of this area is moderately deep with a slight texture, very filterable and relatively poor in organic matter (0.17%). Its physical and chemical characteristics is reported in table 2.

Table 2: Physical and chemical characteristics of the treated soil

Profondeur (cm)	Granulometry (%)				Calcaire (%)		Gypsum (%)	EC(mS/cm)	pH
	Clay	Silt	Fin Sand	Coarse sand	total	Actif			
0 – 15	7.7	0.7	85.9	5.1	30.5	2.5	0.5	1.9	8.1

1.3. Soil treatment

The OMW is pumped from a pit cistern in a tank and brought by a tractor to the field. Then it was sprayed homogeneously on the sandy soil surface, previously tilled to a 10-15 cm depth. OMW was sprayed yearly since 1995 during the winter period (December-January) using three doses: 50 m^3/ha, 100 m^3/ha, 200 m^3/ha. A control orchard without OMW amendment was considered. Each orchard contains 16 olive trees aged of 70 years planted at intervals of 25 meters. The orchards were separated by 2 non-treated olive tree rows (50 m distance) (Fig 2).

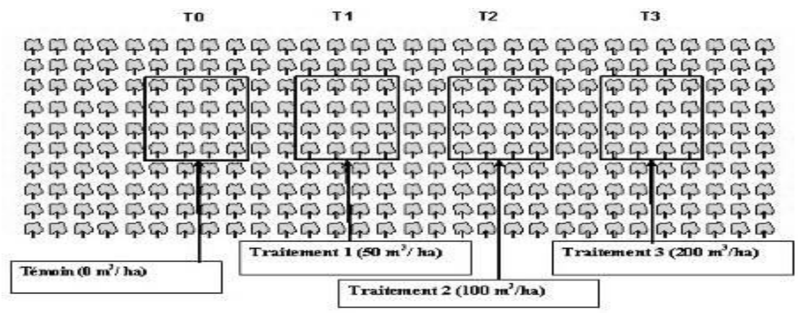

Figure 2: Schematic plan of Chammakh-Zarzis experimental orchards

1.4.Organic matter content

The organic matter rate was determined by Walkley and Black methods which consisted on cold oxidization with bichromate of potassium ($K_2Cr_2O_7$) in acid environment and titration with ferrous sulfate ($FeSO_4$ $7H_2O$). The organic matter rate was calculated by the following equation: MO% = C% x 1.725 Soil samples for organic matter content determination were taken in 2006 on each orchard and followed during 10 years of OMW application.

1.5.Microbiological analysis

Soil samples (10 g) were suspended in 90 ml of sterile phosphate buffer solution (1 mM, pH = 7, 1- 7, 4). The total bacterial flora was enumerated on PCA (Plate Count Agar) medium after incubation for 48 hours at 25 °C, with light and dark alternation. The total flora of yeast and fungi were enumerated on a malt extract agar medium for 72 hours at 28 °C. Results were expressed as colony forming units per gram of wet soil (CFU/g wet soil).

1.6.Natural floristic analysis

The parameters used for the quantitative assessment of vegetation are:
- The density: The number of plants was counted yearly existing in a delimited surface.
- The global recovery of the vegetation: it is the projection of the aerial organ of the plant to the soil. It represents the report between the number of points where there are presences of species and the number of score sampled. This recovery is expressed as the percentage of the soil surface.
- Expense ratio: it is determined by the method of the points quadrats (Goodall, 1953). It is a prompt measure, materialized by a fine needle slipping in a structure. The distance between the needles is function of the closing and the opening of the plant formation (the more grouping is closed the more density is weak). In our case, at the level of every treatment, we installed two lines of 20 m of length each. Reading takes place to intervals of 10 cm, either 200 points by line. The contact of the needle with vegetation or all other nature of soil (naked soil, litter.) has been recorded. In addition to the expense ratio, this method provides the following information: the recovery by species (centesimal frequency) and the contribution of the species to the recovery (CSP).

Before analyzing the effect of the olive mill waster water on the specific composition of the yearly plant table setting, we try to present the features, the ecological requirements and the economic interest of the main species met.

1.7.Data analysis

Data were subjected to analysis of variance (ANOVA) with the SPSS software (version 18). Significance of mean differences was determined using the Duncan's test, and responses were judged significant at the 5% level (P=0.05).

2. Results and discussion

2.1.Effect of OMW spreading on organic matter content

Rich in organic substances (107 kg/m^3), the OMW spray improved the content of soil in these substance. Indeed, the organic matter rate, initially very low (0.06), raised to 0,41 %, 0,71% and 1,27% for the doses of 50 m^3 / ha, 100 m^3 / ha and 200 m^3/ha respectively after ten successive years of OMW spray. Therefore, the organic matter rates increase is proportional to the applied doses (Fig 3).

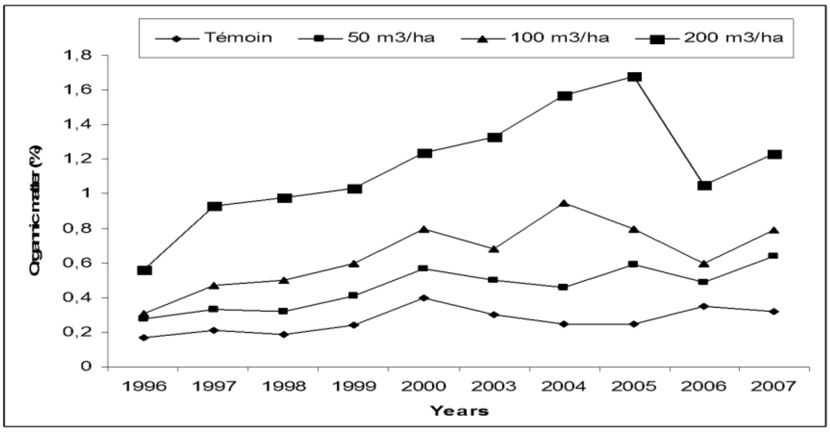

Figure 3: Evolution of organic matter rate 10 years after OMW spreading.

The differences observed could be explained by the kinetics of the organic matter mineralization process which depended on the abounding, in number and quality, of micro-organisms in the treated soil. Similar results were obtained by Cabrera and al. (1996) who indicate that the yearly supply, during three years, of 37 l/m² or 61 l/m² of OMW, on sandy soil with an initial organic matter rate of 0,45 %, could increase the organic matter content to1,62% and 1,98% respectively.

2.2.Effect of OMW spreading of bacterial flora

The spreading of olive mill waste water results in a significant increase of bacterial population. This increase is proportional to the dose of olive mill waster water (Fig 4). Enrichment in bacteria might be explained by the increase of mineral nitrogen content of the soil following the activation of the ammonifying bacteria and the increase in soluble C resulting from OMW spreading. This result demonstrated that OMW spreading may serve as a good substrate for bacteria strains in the soil

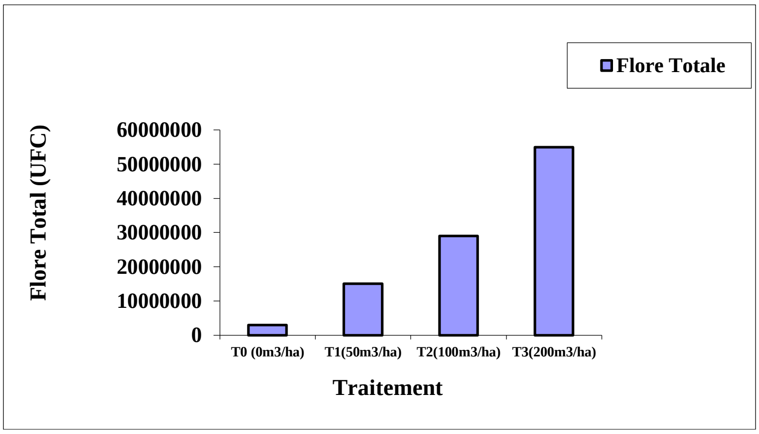

Figure 4: Effect of olive mill waste water doses on bacterial population

The research conducted by [2] on the experimental site of Chaal (Sfax), demonstrated that OMW release nitric soil nitrogen when conditions of temperature and humidity are favorable to the microbial activity in a soil receiving successive annual spreading since 1994 under the same conditions of the present study.

2.3.Effect of OMW spreading of fungal flora

The population of fungi was also increased by the application of OMW (Fig 5). As found for bacterial flora, the increase of total fungi is proportional to the doses of OMW. This finding may be explained by the effect of organic matter on the multiplication of cell fungi.

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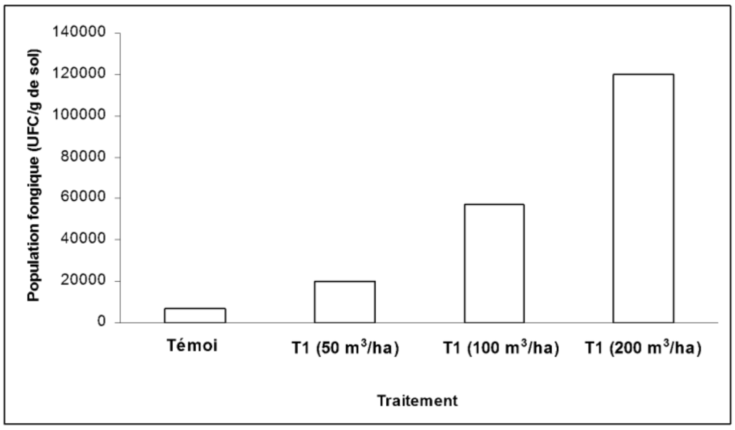

Figure 5: Effect of OMW of fungal flora population

2.4.Effect of OMW spreading on density of natural floristic species

The density of the yearly plant species in the different orchards is reported in (Fig 6). According to these results, one notices that the density of *Chenopodia* and *Mesembryanthemum cristallinum* raised on the orchards that received the highest dose of OMW (200 m^3/ha): 111 and 84 individuals / m2 and with a contribution specific presence (CSP 53% and 37%.) respectively. However, the density of these two species is reduced in the control orchards and those that received the lowest dose of OMW (50 m^3/ha). This is due to the biologic and ecological features of these two species by the presence of an important quantity of nitrates and salts (elevated electric conductivity in the orchard that received 200 m^3 / ha of olive mill waster water) (Fig 6).

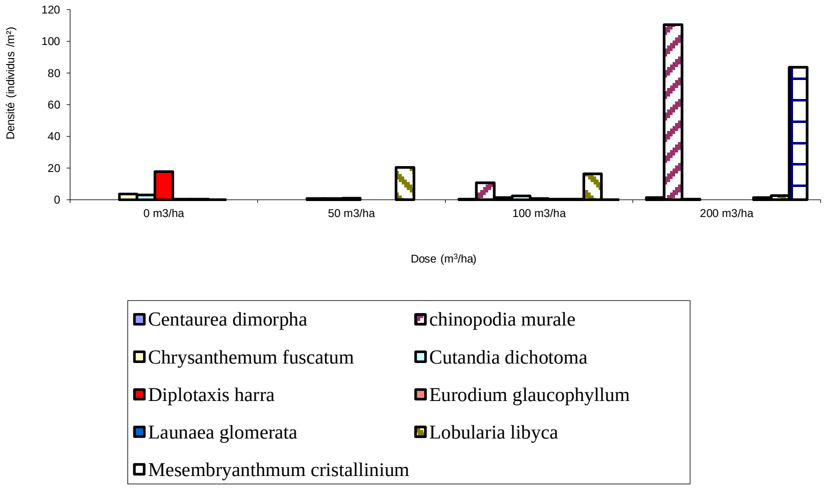

Figure 6: Effect of the spreading olive mill waster water on the density of some natural floristic species.

The species *Diplotaxis harra* is found in relatively elevated density (18 specimens/m2) in the non treated soil. Its density is reduced in the orchard that received 200 m^3/ha. It can be due to the inhibition of the germination of its seeds by olive mill waster water because of the salts and the presence of phnenolic compounds very toxic towards some sensitive species.

2.5.Effect of OMW spreading on the global recovery of the soil

A light increase of expense ratio has been observed in the orchard that received the highest dose of 200 m^3/ha (38%). It could be explained by the high level of density *chenopodia* and *Mesembryanthemum cristallinum*, tolerant species to the saltiness and to the conditions of stress, in addition to their botanical features permitting an important expense ratio (Fig 7).

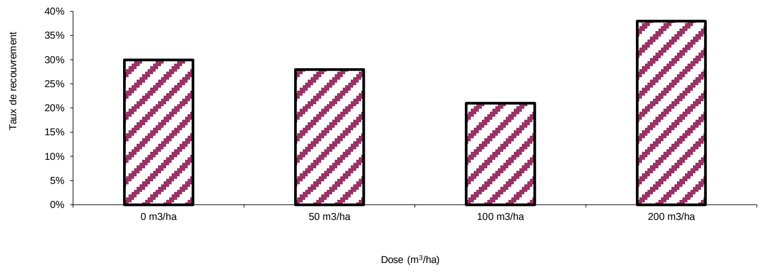

Figure 7: Effect of olive mill wastewater spreading on the global recovery of the soil

According to these results, we can conclude that the mulching of OMW in the abandon and pastoral area could be beneficial to control the messicoles annual species development. In most cases, the fallow lands are dominated by *Diplotaxis harra* which is a very weak economic value species (pastoral, medicinal or other). Therefore, it seems that OMW could decrease the competition of this species by others species more beneficial as well as pastoral and industrial plan like the *Mesumbryanthemum cristallinium*, species used for soap manufacture.
The survey of the effect of olive mill wastewater on the spontaneous vegetation showed that the specific diversity is more important in the orchard having received 100 m^3/ha as compared to the other orchards (Fig 7). It demonstrated the state of balance between phytotoxic and fertilizing effect of olive mill wasterwater. The dose 100 m^3/ha seems to be the adequate dose that minimizes the ominous effects and maximize the beneficial effects

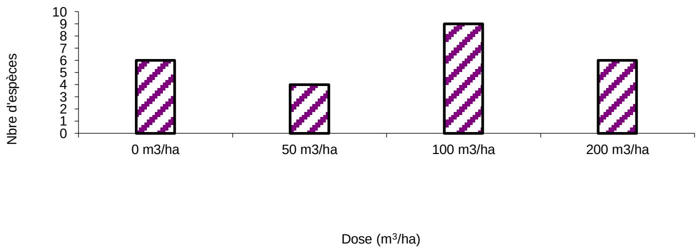

Figure 8: Effect of OMW doses on the number yearly species

Results revealed a low number of plant species has been observed in the orchard that received the small dose of OMW dose (50 m^3 /ha) because of the absence of *Chenopodia* and *Mesembryanthemum cristallinum*. Being based on this result, the use of the olive mill wasterwater in particular in the abundant and the former fallow lands post - cultures, could be beneficial to control the development of the species yearly messicoles. In most cases, the fallow lands of the arid zones are dominated by *Diplotaxis harra*, species of very weak economic value (pastoral, medicinal or other). It seems that the use of the olive mill wasterwater decreases the competition of this species and enhance others species that can be more useful on the pastoral or industrial plan and this according to the used dose. If the dose is weak to average (50 to 100 m^3 / ha), pastoral species that dominate as Lobularia libycapour our test. To highest dose, it is especially *Mesumbryanthemum cristallinium*, for species used for the manufacture of soap that dominates following the increase of the quantity of nitrate and salt.

Mesembryanthemum L. cristallinum
Familly : Aizoaceae
Vernaculaire Name : Ghassoul (Fig 9)

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Figure 9: Orchard T4 (dose 200 $^{m3 / ha}$)(Mesembryanthemum L. cristallinum)

Ecology and geographical localization: this plant grows on the maritime sands; on the salty depressions. Their debris are rich in nitrates. It is therefore a halophyte and a nitrophyte plant. It is common in the North (Tunis, Zambra), the Center (Sousse, Kairouan) but rarer in the South (Sfax, Zarzis, Djerba, El Hamma of Djerid). Outside of the oasis, it is mainly absent on the desert regions.
Interest: the plant was used once like soap to wash the linen. The arabian name of this plant is " ghassoul " (cleaner). (Chaieb.M., Boukhris M. 1998)

***Chenopodia murale L. .* (Fig 10**).

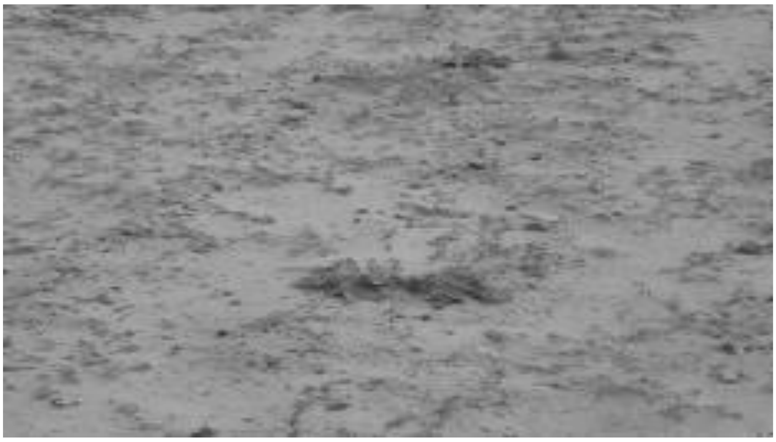

Figure 10 : Orchard T3 (dose 100 m^3/ha**)** (chenopodia murale)

Ecology and geographical localization: sides of the paths and on the walls. Nitrophile. In the whole Tunisia.
Interest: fairly palatable by goat and cheep [4].

Launaea glomerata Boiss -L. agglomérée

Ecology and geographical localization: grazing area, beds of river. All sites. Geographic Area: Algeria, Libya and Tunisia.
Interest : species a lot of palatable by goat and cheep[4].

Diplotaxis harra (Fig 11)
Familly : Cruciferae
Vernaculaire Name: Harra

Figure 11: Control (dose 0 m^3/ha) (*Diplotaxis harra*)

Ecology and geographical localization: Although it appears on the steppe, it can be founded, with strong density, in cultivated region. Species of slimy soils, it can also exist on the sandy, gypseous soils or even clayey. It exists everywhere in arid Tunisia until the Ouara and the Jeffara.
Intérêt : it is especially consumed like vegetable, especially when it is confounded to the *Diplotaxis simplex* that is barefaced. It should be consumed when it is fresh. It can cause many problems like the paralysis among the animals [4].

Lobularia libyca (Fig 12)
Familly : Cruciferae

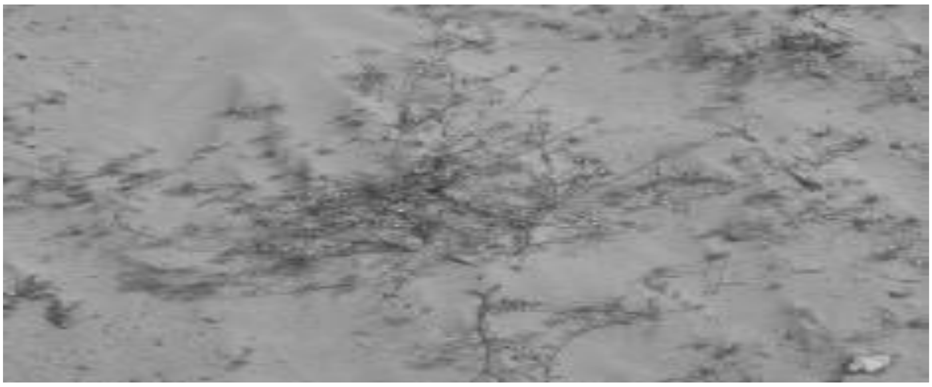

Figure 12: Orchard T2 (dose s 50 m^3 / ha) (*Lobularia lubica*)

Ecology and geographical localization: species very commun in central and southern Tunisia, it is especially found on the sandy steppes and in the cultivated fields, in particular the olivettes. it exists in Sousse, Sfax, Gabès, Médenine, Tataouine and in the Djerid and Nefzaoua.
Interest : it is rather a pastoral species [4].

Concerning the effect of the olive mill wastewater on the floristic composition of the spontaneous vegetation, the obtained results showed a beneficial effect for some species and ominous for others. The parameters studied as the density, the contribution specific presence and expense ratio deserves to be deepened by analyses during all vegetative stages of the plants. According to the preliminary results we can deduce that the use of the olive mill waster water in particular in the abundant and the former fallow lands post - cultural, could be beneficial to control the development of the yearly messicoles species. In most cases, the fallow lands of the arid zones are dominated by *Diplotaxis harra*, species of very weak economic value (pastorale, medicinal or other). It seems that the use of the olive mill wasterwater decreases the competition of this species to the profit of others that can be more useful on the pastoral or industrial plan and this according to the used dose.

Conclusion

The spreading of OMW induced a significant increase of organic matter content and therefore results in abundance of bacterial and fungal population. OMW spreading increased the density of naturals plants The obtained result revealed that OMW is an interesting soil fertilizer that can be used in sandy soils to improve not only its physical and chemical characteristics but also its biological activity.

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