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Effect of Olive Pomace Phenols on the Soil Fungi and Alfalfa Yield

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ABSTRACT

Although the presence of high proportion of organic matter and valuable nutrients creates olive pomace (OP) a valuable natural fertilizer for plant growth, the presence of high phenol concentration turn it into phytotoxic waste. Composting of nine batches of trials using different proportions of olive pomace and agricultural by-product revealed that three factors contributed in the elimination of phytotoxicity of olive pomace. The most effective processing in reducing phenol content was composting followed by addition of agricultural by-product and finally by using of phenol degrading bacteria *A. vinelandii*. The net reduction of phenol content was 94.8% at the end of composting process. The final compost was characterized by high EC, neutral pH, high amount of nutrients, rich with microbial community and free from pathogens. The results showed the efficiency of composting in reducing olive pomace toxicity that the germination percentage increased to 88.1% at the end of the composting process which means that the olive pomace must be composted before using as soil amendments. Fungicidal properties of olive pomace and compost extracts revealed that the growth of tested fungi were positively affected by olive pomace except for *Fusarium oxysporum* which is negatively affected. Olive pomace composts had inhibitory effects on *Aspergillus clavatus*, *Aspergillus flavus*, *Aspergillus terreus* and *F. oxysporum* and stimulatory ones on the growth of both *Rhizoctonia solani* and *Aspergillus niger*. Field experiment was conducted to evaluate the effect of three olive pomace composts selected from the nine batches as fertilizers for alfalfa growing. The significant increase in yields and microbial density in the rhizosphere comparable to control suggesting that most of the nutrients contained in OP composts supported adequately plant growth and microbial community. This study developed a low cost technology that will enable the growers to convert olive pomace into a natural non toxic compost rich with essential nutrients which have positive effects on plants growth.

Key words: olive pomace ,composting, phenol degrading bacteria, antifungal activity, alfalfa cultivar

Introduction

In Egypt, the annual average production of olive is 330.000 tones per year. About 80% of the total production is consumed as table olive and the residual (20%) is used for oil production (Central administration for public mobilization and Statics,2006).The production of olive oil generates three phases and two wastes: olive oil (20 %), solid waste (30%) and aqueous liquor (50%).These olive mill wastes are produced in significantly large quantities during short periods of time. Disposal of olive wastes from olive oil mills is already a major environmental issue in several olive growing countries in the world. Spreading the solid waste on farm lands causes enormous pollution to the land and air.

The large proportion of organic matter, macromolecules such as polysaccharides, lipids, proteins , high amount of valuable nutrients especially potassium, nitrogen, phosphorus as well as micro nutrients which are essential for plant growth make olive mill waste water (OMSW) a valuable resource for beneficial utilization, particularly in degraded agricultural soils. Also, OMSW have no xenobiotics or heavy metal contaminants .In fact, it has been proven that this waste may potentially act as good sources of plant nutrients (Piperidou *et al.*, 2000). But at the same time ,OMSW contain a number of monocyclic and polymeric aromatic molecules generally known as phenolic compounds (Ehaliotis *et al.*, 1999). The levels of phenols in olive husk can vary from 2.9 to 3.7mg g⁻¹(Nair *et al.*,2007).Olive husk is also characterized by its phytotoxicity, hydrophobicity, salinity, low pH and polyphenols (Perucci *et al.*, 2006). The presence of phenols ,short and long chain fatty acids are considered to be responsible for the phytotoxicity and antimicrobial nature of these wastes(Fierentino *et al.*,2003; Isidori *et al.*,2005). So, the toxicity of the waste to microorganisms and plants must be first eliminated or reduced.

Several studies carried out to reduce the phenolic compounds of this waste by using different detoxification methods. Composting seems to be the most logical from both ecological and economical points of view. Different composting processes are used in the olive growing countries in the Mediterranean region as one of

the bioremediation process ,e.g., the technology developed in Italy consists of adding appropriate hygroscopic organic waste to olive husk without stones (seeds), packing in net sacks and storing until required for use in the field (Altieri and Esposito, 2007). Soil amendment using compost has several distinct advantages over the addition of fertilizers to soil. There is significant loss of nutrients from soil after fertilization due to leaching. Compost retains nutrients in the soil and releases them on demand by the plants due to its high cation exchange capacity, maintains the water-holding capacity of soil, thereby helping the plants to be more drought resistant. Well processed composts can be suppressive to a variety of soil-borne pathogens, including those of olives (Raviv *et al.*, 2007), however, little is known about the suppressive capacity of compost made from olive solid waste. Recent work has shown that olive waste compost suppressed *Fusarium oxysporum* sp. *Melonis* (Raviv *et al.*, 2007) and *Verticillium dhaliae* (Alfano *et al.*, 2007).

Alfalfa (*Medicago sativa* L.) is a deep-rooted, perennial legume plant capable to produce high yields with high-quality forage. Its nutritional value makes this crop ideal for hay and silage. It also has the ability to use atmospheric nitrogen (N₂) and deposit significant amount of N in the soil during growth. Also alfalfa crop increases soil organic matter, improve soil structure and builds up nitrogen reserves in top soil (Peterson and Russelle, 1991).

The aim of the study is to convert the olive pomace waste into a value-added product by using a natural remediation process of microbial composting that removes phytotoxic compounds from this waste.

Material and Methods

Isolation of phenol degrading bacteria:

Phenol degrading bacteria was isolated from olive pomace as described by the method of (Goodfeelow, 1994). Briefly:10 g of OP sample was mixed with 100 ml of Ramsay medium (Ramsay *et al.*, 1983), incubated at 30°C with aeration for one week. Then 1 ml of this media was inoculated to 100 ml of new phenol broth media and aerated in 30°C for another one week. These passages were repeated until turbidity was obtained from bacteria growth. After the last passage, it was cultured on phenol agar media as an isolate bacterium .The isolate was identified as *Azotobacter vinelandii* using the Biolog system ,Microlog Version 3-20 (Bochner,1989).

Lab scale composting:

Olive pomace (OP) were collected from an olive mill in Siwa Oases Experimental Station of Desert Research Center (DRC). EC was 0.94 mS.cm⁻¹, pH was 6.1, total C % was 33.6, N % was 0.9 , C/N of 37.3, P % was 0.61, K % was 2.6 and total phenols % was 0.6. Nine batches of composting trials were carried out with different proportions of olive pomace and agricultural by-product. The agricultural by-products used were rice straw, weeds and palm fronds. The compositions of all nine trial batches are given in Table 1. The cellulose degrading bacteria (CDB) used were *Cellulomonas fimi* ATCC 484, *Pseudomonas fluorescens subsp. cellulosa* and *Micrococcus luteus* ATCC 9341 . The CDB and *Azotobacter vinelandii* as phenol degrading bacteria were added to compostable materials at 10% for each .Compost samples were collected at three different stages of composting process. Stage 1: initial non-decomposed mixture ,stage 2:(60 days of composting) and stage 3: (end of composting process).

Chemical assay:

The samples were analyzed for total organic carbon (Jackson,1958), total N (Bremner and Mulvaney,1982) and total phenol content using a modification of the Romero *et al.*,2002). Briefly, five gm of dried compost was weighed into 250mL flask and methanol: water (80:20 v/v, 30mL) was added. The flasks were placed on a shaker for 24 hours. The contents of the flasks were centrifuged at 12000 g for 10 min at 4°C and the supernatants stored at -20°C. The pellets were resuspended in methanol :water (80:20 v/v,30mL) and extracted for 48 hours under the same conditions. The process was repeated again. The volumes of the combined supernatants were evaporated to dryness, re-suspended in a small volume of distilled water (4ml), and placed in a glass vial. One ml of sample (4 replicates) were transferred to glass test tubes. 1ml of 20% sodium bicarbonate was added to each tube followed by 0.5 ml of Folin-Ciocalteu's phenol reagent. The contents of the tubes were mixed by vortexing after each addition. After 60 minutes incubation at room temperature, the tubes were mixed again and the absorbance at 750nm was measured. A standard curve was constructed using Gallic acid as standard. Phenol concentrations were calculated as gallic acid equivalents.

Field scale composting:

This study was conducted at Siwa Experimental Station of the Desert Research Center (DRC). Three ditches of following combinations were conducted: (Bile1:100% OP, Bile 2: 80 % OP + 20% agricultural by-product and Bile 3: 60% OP + 40 % agricultural by-product) were individually spreaded in layers inside each ditch . For each ditch, recommended doses of ammonium nitrate, super phosphate and calcium carbonate were added. Then, all layers were treated with 10% of each highly active cellulolytic inoculum and *Azotobacter vinelandii* as PDB .The material was thoroughly turned for proper aeration and wetted with water and microbial inoculums to maintain the proper moisture level at 45- 55%,. After 120 days, the composted wastes were analyzed. Electrical conductivity (EC) and pH were analyzed in a 1:5 (v/v) water extracts. Organic carbon, total nitrogen were analyzed as described previously, total phosphorus and total potassium as described by (Watanabe and Olsen, 1965; Mason, 1963). For microbiological analysis, total microbial counts on Nutrient medium ,cellulolytic microbial counts on Dubos medium, *Pseudomonas* counts on King B medium, phenol degrading bacteria on Ramsay medium and total coliform , *E.coli* counts on MacConkey medium were determined.

Toxicity assay:

Four extracts (E0, E1 E2and E3) corresponding to raw Olive pomace (OP), bile1, bile2 and bile3 composts, respectively, were prepared according to (Znaïdi, 2002). The tomato (*Lycopersicum esculentum*) seeds were disinfected in absolute ethanol for 5 min., rinsed with sterile distilled water and placed in Petri dishes lined with filter paper containing 5 ml of the four obtained aqueous extracts .Petri dishes were incubated in a growing chamber at 26°C. Seeds were maintained soaked with the corresponding composts extracts and distilled water was used as control. After 7 days, germinated seeds were counted, root and shoot lengths were measured and mean values were recorded. Three Petri dishes were replicated for each treatment and in each Petri dish, 10 seeds were used.

Antifungal assay:

In this experiment ,the effect of (E0, E1 E2and E3) extracts on fungal biomass of some fungi isolated from Siwa Experimental Station soil were tested . Fungal isolates used in the bioassays included: *Aspergillus niger*, *A.clavatus*, *A.flavus* and *A.terreus* *Fusarium oxysporum* and *Rhizoctonia solai*. Briefly, five ml of each OP compost extracts was added to 50 ml Potato dextrose broth which inoculated with 4mm test fungal mat and incubated for 5 days at 28°C. Control flasks were maintained without the extracts. Dry weight of each fungus was taken and compared with control. Five replications were used for each treatment and the experiment was repeated twice. The percentage of suppression was calculated as: dry weight of the test filtrate/ dry weight of the control×100.

Field experiments:

The experiment was conducted in Siwa Experimental Station of Desert Research Center (DRC). The experimental treatments were arranged as split plots on the basis of a Randomized Complete Block Design with three replications. Composted wastes (Bile1, Bile2 and Bile2) were added to the soil at the rate of 5 ton/ feddan, two weeks prior to planting .Soil not amended with compost is served as control. All treatments were amended with recommended doses of super phosphate (15.5% P₂O₅) at a rate of 150 kg/fed before sowing, ammonium nitrate (33.3% N) at a rate of 100 kg/fed. and K-sulphate (48% K₂O) at the rate of 150 kg/fed.

Alfalfa seeds (Siwalcultivar) were coated with *Rhizobium meliloti* using 1% carboxymethylcellulose (CMC) as adhesive, dried in air before sowing (Hameeda, 2008). Alfalfa was harvested three times after 55 ,100 and150 days from planting. The following data were recorded; Plant height ,number of branches/plant, fresh and dry yields. At each harvest, total phosphorus, nitrogen and phenol content were determined as previously mentioned. In addition, total microbial and fungal count in rhizosphere samples were also estimated

Statistical analysis:

Data were statistically analyzed by ANOVA, according to (Snedecor and Cochran, 1990) and treatment means were compared by LSD test at 5% level of probability.

Results And Discussion

A typical pattern of changes in total nitrogen, total carbon and C:N ratio during composting is given in (Table 1).While there was a reduction in total carbon and the C:N ratios in all composts from start to end of the

composting process, the reverse was observed in the levels of total nitrogen. Although the addition of cellulose degrading bacteria to compost significantly decrease both total carbon percent and C:N ratio at the end of the composting, it had non significant effect on N or phenol%. Also, addition of *A. vinelandii* non significantly increase the N % but at the same time cause a significant reduction in phenol content. All batches of composts showed similar pattern of changes in concentrations of phenols during the process. There was a gradual reduction in the level of phenol from start to end of the composting process by the reduction percent of 75% for all compost batches. Addition of 20 and 40% agricultural by-products to the olive pomace cause 24% and 56% reduction in phenol concentration, respectively. Also, addition of *A. vinelandii* reduce the phenols by about 33%. These revealed that three factors contribute in the reduction of phenols of the olive pomace. The net reduction was 94.8% at the finish of composting process. Evolution of polyphenolic compounds by HPLC during the composting of an olive mill waste-wheat straw mixture revealed the strongly reduction of the polyphenol content by 93% and disappearing of nine phenolic compounds at the end of composting (Ait Baddi, 2009).

Table 1: Chemical characteristics of the nine compost trial batches

Compost trial batches	Total carbon (%)			Total nitrogen (%)			C:N			Phenol (%)		
	0 day	60 days	120 days	0 day	60 days	120 days	0 day	60 days	120 days	0 day	60 days	120 days
Batch 1	33.6c*	30.6f	25d	0.9a	1.18a	1.3a	37.3c	25.9g	19.2f	0.65a	0.44a	0.195a
Batch 2	33.6c	30.9e	24.3e	0.9	1.2a	1.28a	37.3	25.7g	18.9f	0.65	0.41ab	0.186a
Batch 3	33.6c	30.2g	23.8f	0.9	1.2a	1.3a	37.3	25.2h	18.3g	0.65	0.37b	0.143b
Batch 4	36.9b	34.2c	25.9c	0.82b	1.09c	1.2ab	45.1b	31.4d	21.6d	0.494b	0.31c	0.12c
Batch 5	36.9b	34.2c	25d	0.82	1.1bc	1.23ab	45.1	27.4f	20.3e	0.494	0.25d	0.1c
Batch 6	36.9b	33.6d	25d	0.82	1.17ab	1.25a	45.1	28.7e	20e	0.494	0.24d	0.078d
Batch 7	38.6a	36.3a	27.4a	0.74c	0.88d	1c	52.2a	41.2a	27.4a	0.286c	0.16e	0.052e
Batch 8	38.6a	35.6b	26.6b	0.74	0.92d	1c	52.2	38.7c	26.6b	0.286	0.15e	0.049e
Batch 9	38.6a	36.1a	26.7b	0.74	0.91d	1.1bc	52.21	39.6b	24.3c	0.286	0.13e	0.033f
LSD5%	0.81	0.27	0.43	0.043	0.07	0.135	0.431	0.412	0.431	0.027	0.045	0.0117

*For every variable, different symbols means there is a significant difference

Batch 1:100%olive pomace (OP) (CDB)

Batch 2:100% OP + Cellulose degrading bacteria

Batch 3:100%olive + (CDB) +*A. vinelandii*

Batch 4: 80% OP +20% agricultural by-products (CDB)

Batch 5: 80% OP +20% agricultural by-products +

Batch 6: 80% OP +20% agricultural by-products + (CDB) + *A. vinelandii*

Batch 7: 60% OP + 40% agricultural by-products (CDB)

Batch 8: 60% OP +40% agricultural by-products +

Batch 9: 60% pomace +40% agricultural by-products + (CDB) + *A. vinelandii*

After composting, all piles recorded higher electrical conductivities (EC) than before composting (0.94 for olive pomace and 2.4 for agricultural by-products) due to the mineralization of the compost materials. Electrical conductivities of composts increased with increasing concentrations of the agricultural by-products. After composting, the pH values tended to diminish to a neutral pH and the recorded values of K and P % increased in all compost piles and they were positively correlated with olive concentrations (Table 2).

All batches are free from pathogenic microbes as: *E.coli*, *Salmonella*, *Shigella* or *Proteus*. Results recorded in (Table 3) revealed that there was a quantitative difference in number of total microbial counts, cellulose decomposers, *Pseudomonas spp.*, phenol degrading bacteria and coliforms between three compost sets. The change in microbial count during composting process was affected by the type of raw material used to prepare compost. Addition of lignocellulolytic wastes to OP cause stimulation in the microbial count except for phenol degrading bacteria and coli form bacteria which exhibited reduction in their numbers.

Table 2: Physical and chemical characteristics of compost trial batches:

Treatments	EC* (mS.cm ⁻¹)	pH*	Total C (%)	Total N (%)	C:N	P (%)	K (%)	Phenol (%)
Bile1	1.54c	6.7b	24.7b	1.37a	18c	0.7a	2.7a	0.15a
Bile2	3.2b	6.9ab	25.2b	1.33ab	18.9b	0.535b	2.55b	0.09b
Bile3	3.35a	7a	27.4a	1.29b	21.2a	0.362c	2.3c	0.053c
LSD 5%	0.14	0.28	0.773	0.062	0.475	0.035	0.136	0.019

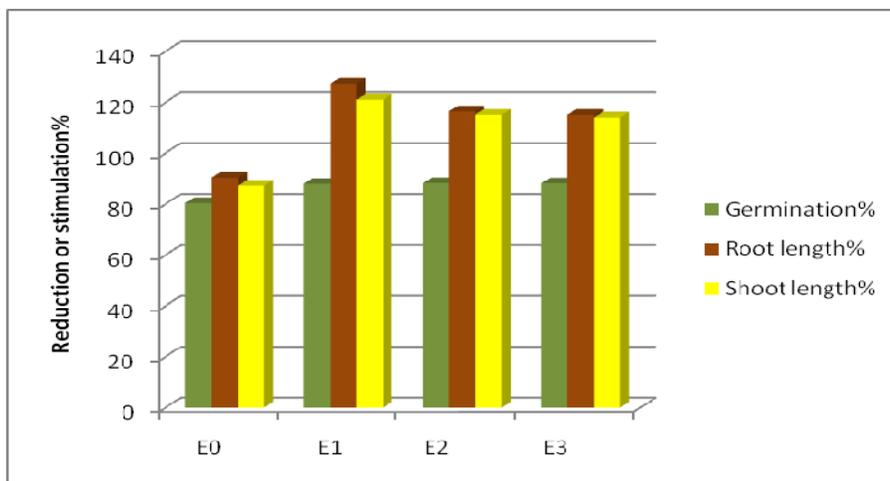
* Solid: water raw material ratio of 1:5

Table 3: Microbiological characteristics of the compost biles :

Treatments	TB# (N/No)**	CDB## (N/No)**	<i>Pseudomonas spp.</i> (N/No)**	PDB### (N/No)**	Azotobacters (N/No)**	Coli form (N/No)**
Bile 1	2.85	1.5	3.5	0.87	2.1	0.5
Bile2	11.42	2.1	6.6	0.65	3.4	0.33
Bile3	17.14	2.6	7.2	0.54	5.9	0.25

TB# : Total bacterial count CDB## :Cellulose degrading bacteria PDB###: Phenol degrading bacteria
 ** N/No: Count of bacteria at the end of composting /Count of bacteria at zero time.

From fig. (1), the uncomposted olive pomace showed the less germination percentage (82.4%) of tomato seeds compared to other treatments and this may be due to the presence of phytotoxic phenolic compounds. It is known that olive waste inhibits germination of seeds of different plant species (Della, 2001; Niaounakis and Hakvadakis, 2004). There was no significant difference between the germination rate in all compost treatments, mean values of seed germination percentage in compost treatments were at least 88.1%. The high rates of tomato germination in all compost treatments suggests that compost extracts are not phytotoxic. While the raw OP negatively affect shoot and root length, all compost extracts stimulate them. This demonstrated the absence of phytotoxicity in the mature compost. As the OP concentration increased, stimulation of shoot and root growth increased. The addition of lignocellulytic wastes to OP decreased its positive effect on tomato growth. The toxicity test of of chicken manure and solid fraction of olive mill residues compost extracts were not toxic to plants but also stimulate seed germination and seedling growth rates compared to control (Raoudha *et al.*, 2009).



- Reduction or stimulation of germination and plant length compared to control (100%)

Fig. 1: Reduction or stimulation effect of olive pomace and different compost biles on seed germination ,root and shoot length:

From (Table 4), neither raw olive pomace nor compost extracts were able to inhibit the growth of the *Rhizoctonia solani*. This points to the fact that the chemical compounds present in OP, or in any stage of compost produced with it are not inhibitory to *R. solani* (Cayuelaa *et al.*, 2008). This also is the same for *A.niger* which is known to be a highly active phenol degrading fungi .This is compatible to the fact that a large number of saprophytic fungi are able to grow on dry olive residue and decompose it (Sampedro *et al.*, 2004). On contrary, all compost extracts stimulated the fungal growth of the two fungi. While the growth of tested fungi *A.clavatus*, *A.flavus* and *A.terreus* were positively affected by raw olive pomace, they were suppressed by compost extracts. These results suggest that the presence of undecomposed dry olive residue may provide energy and nutrients for saprophytic growth of fungi (Giuliano *et al.*, 2006). The higher inhibition capability of mature compost may be explained by the formation of toxic metabolites by some microbial communities that developed in the piles during the composting process (Cayuelaa *et al.*, 2008). Results with *Fusarium oxysporum* showed a trend opposite to that exhibited by the other fungi tested. The extracts of raw olive pomace and composts were undoubtedly inhibitory to it. In plate inhibition trials, OW compost water extracts exerted a significant inhibitory effect on the growth of the pathogens *Fusarium oxysporum f.sp. lycopersici*, *Pythium ultimum*, (Gabriele *et al.*, 2011).

Table 4: Growth percentage of fungi treated with olive pomace and composts with respect to controls:

Treatments	Fungal strains					
	<i>A.niger</i>	<i>Rhizoctonia solani</i>	<i>A.clavatus</i>	<i>A.terreus</i>	<i>A.flavus</i>	<i>Fusarium oxysporum</i>
E0	100	102	100	110	107	77
E1	110	110	98	84	85	80
E2	112	112	96	87	84	84
E3	115	115	93	86	85	87

Data represented in (Table 5) indicated that all compost application were found to enhance the plant height, number of branches/plant ,seed and straw yield of alfalfa plants significantly over the control .While there is no significant differences in plant heights among three compost biles ,the highest remarkable increase in the number of branches was recorded in plant with bile 2 (80% OP and 20 % agricultural by-products). In general, significant increase in the yields of alfalfa plants were recorded in all treatments amended with compost. The highest significant increase in the fresh yield was 33.4% and 33% obtained in the presence of bile 1compost (100%olive + Cellulolytic bacteria +*A. vinelandii*) and bile 2 (80% OP +20% agricultural by-products + Cellulolytic bacteria + *A. vinelandii*), respectively, followed by bile 3 (60% OP +40% agricultural by-products + Cellulolytic bacteria + *A. vinelandii*) which cause 26.3% increase compared to control. The results of dry yield showed the same trend and this may be due to the presence of positive correlation between olive pomace and NPK concentrations in the compost. This result is compatible with the finding that most of the nutrients contained in the OMW composts supported adequately plant growth, even in short-term crops (Roberto and Espositoa ,2009) and that the olive mill waste compost increased markedly the shoot growth of the salt-tolerant sugar beet (David and Bernal, 2008).

Table 5: Effect of of olive pomace and composts on alfalfa growth parameters:

Treatments	Plant height (cm)			Number of branches/pla			Total yield (ton/feddan)							
	Cuttings			Cuttings			Fresh weight of plant				Dry weight of plant			
	1 st	2 nd	3 rd	1 st	2 nd	3 rd	1 st	2 nd	3 rd	Total	1 st	2 nd	3 rd	Total
	Control	24.7a	31a	65b	17.7c	20.3b	22.7c	3.40b	7.9 c	13.9 c	25.2c	1.79c	3.35c	9.0c
Bile1	23.7a	29.7a	85a	28ab	32.7a	34.7ab	3.65ab	10.2a	24a	37.8a	2.83a	4.66a	13.0a	20.49a
Bile2	20.7a	21b	83a	29a	34a	38.7a	3.73a	10 a	23.9 a	37.6a	2.79a	4.31ab	12.9a	20a
Bile3	25.0a	29.3a	77a	24.7b	25.3b	32.0b	3.60ab	9.4b	21.2 b	34.2b	2.45b	4.12b	11.1b	17.67b

In (Table 6), obtained data generally showed that application of olive pomace composts considerably stimulates microbial and fungal counts in the rhizosphere of alfalfa plant compared to control . There is no correlation between microbial count and compost types. Composts caused significant increase in the phenol content of plants relative to the control, higher accumulation of phenol in plants were recorded with plants amended with compost bile 1(100%olive + Cellulolytic bacteria +*A. vinelandii*) followed by the others where there were non significant differences among them. The phenol content of plants increased over the untreated one by about 5, 3.8 and 3.8 %for plants treated with compost bile1, 2 and 3, respectively. While application of all composts caused significant increase in both N and P % in plants, there is no significant differences among compost types. The solid by-product of olive oil extraction and cotton waste compost cause remarkable increase in the ryegrass plant contents of nitrogen, phosphorus and potassium was recorded (*Alburquerque et al.*, 2007).

Table 6: Effect of olive pomace and composts on chemical and microbiological characteristics of alfalfa:

Treatments	Chemical characteristics									Microbiological characteristics					
	N%			P%			Phenols mg/gm FW			Total microbial count×10 ⁵ cfu/g dry soil			Fungal counts×10 ² cfu/g dry soil		
	Cuttings			Cuttings			Cuttings			Cuttings			Cuttings		
	1 st	2 nd	3 rd	1 st	2 nd	3 rd	1 st	2 nd	3 rd	1 st	2 nd	3 rd	1 st	2 nd	3 rd
Control	3.9a	3.74b	3.7b	0.19b	0.17b	0.17b	7.9c	7.9b	7.7b	110	114	115	15	18	18
Bile 1	4a	3.93a	3.92a	0.22a	0.21a	0.2a	8.5a	8.3a	8.1a	160	170	172	27	30	30
Bile 2	3.98a	3.9a	3.9a	0.2ab	0.2a	0.19a	8.4ab	8.1b	8ab	170	178	178	30	34	33
Bile 3	3.9a	3.88a	3.9a	0.21ab	0.2a	0.2a	8.2b	8b	8ab	168	170	169	31	33	33

Initial total microbial counts was 22×10⁵ cfu/g dry soilInitial fungal counts in soil was 12×10² cfu/g drysoil.

Conclusion:

Composting of olive pomace as a soil amendment represents a promising agricultural practice. Olive pomace must be applied to soil only after appropriate decomposition processes such as composting ,addition of lignocellulose residues and phenol degrading bacteria that reduced phytotoxicity scale. The olive pomace composts supported the growth of plants and enhance the microbial community of the soil.

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