Wool Wax, a Natural Product that Promote Olive growth by Auxin Production Enhancement

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Research Article

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Abstract

Effluents from textile industry using wool pose serious environmental nuisances that are mainly due to their pollutant load and the release of unpleasant odors. In order to minimize these hazards and to take advantage of these wastes for the sake of our environment, the present work consists on valuing wool wax from washing effluent on olive (*Olea europea*), one of the main agricultural products in Tunisia, germination and growth. The results showed that this waste is characterized by its richness in dry matter and total lipid content when we used water and hexan for washing with 60.7 and 95.6 % respectively. GCMS analysis of wax showed its richenes on fatty acids. Six saturated fatty acids ranking from 15 to 27 carbon atoms were characterized. Furthermore, diluted wax in water improves germination and growth of this plant. The best result was noted at 1.25 mg/g. GCMS analysis showed that slight enhancement in auxin production was noted in plants treated with 1.25 mg/g of wax with 0.9±0.1 ng/mg comparing to control (0.7± 0.2 ng/mg). This leads us to valuate wool wax as environmental frendly natural product in agricultural and fertigation practice.

Introduction

Wool is an animal fiber used by humans since 4.000 BC. It is the fiber that protects and covers the body of some animals such as sheep, camels, etc. For human use, the mainly fiber used has been, over the time, the sheep wool. Once obtained and before the processing, animal wool washing is necessary in order to ensure the quality of the final product. The original fiber is usually dirty with three different components, the animal grease, secreted by the sebaceous glands and usually called wool wax, the suintin, secreted by the sweat glands and the dirty related to the daily life of the animal. To remove the grease and the dirty materials the most commonly used method consumes large amounts of water and surfactants. At the end of the washing process, the waters with the remaining material constitute the liquid phase. In Tunisia, apart from being unsightly and emitting odours, wool washing wastes are usually located in environmentally sensitive areas thereby causing environmental problems which must be addressed [1]. Thus alternative treatment systems which are both more efficient and more environmentally friendly are required. Liquid effluents of the washings are usually dried to reduce waste volume and due to its high content in potassium and organic material this sludge could be used as fertilizer in agricultural [2]. The aims of the present study were : 1) to extract wax or grease from wool washing effluent, 2) characterize fatty acids presents in wax by GCMS, 3) use of wax for *Olea europaea* germination and fertigation and 4) understand the mode of action of emprovement of plant groth by characterization of phytohormones such as auxin.

Material And Methods

Wool Samples collection

Wool was collected after mowing season in Mai 2018 and stored in G-TEX SARL center of collection located in Ksour Essef-Mahdia, Tunisia. Almost of sheep races in Tunisia is composed by Barbarine and

Black of Thibar.

Wool washing and wax extraction

100 g of wool were washed by 500 ml of hot water at 70°C or hexan for 1 houre using a sonicator. The liquide effluent was then filtred througouth a funnel every 10 minutes and concentrated using a soxhlet apparatus and a yellowish cream was recovered. After that, a kinetics of the extraction yield was made. To avoid degradation, samples were stored at 4°C at the Laboratory of Functional Physiology and Valorization of Bioresources, High Institute of Biotechnology of Beja-Tunisia, until use.

Wax characterization

The pH was measured using a pH meter (INOLAB) according to the potentiometric method. Electrical conductivity and salinity were measured using a MEAS / Cond 8 conductivity meter. Determination of the dry matter was carried out by adding 5 g of cream to 20 g of dry sand. The whole is dried for 2 h in the oven at 105°C. Total nitrogen was determined by the Kjeldahl method [3]. Total phosphorus was measured calorimetrically.

Total lipid determination

The total lipid determinantion in cream was carried on accarding to CM Lee et al [4]. Brievely, 25 ml of chloroform methanol were added to 3g of cream. After adition of 10 ml 0.5 NaCl, the solvent was evaporated on a hot plate. The beaker was weighted and the obtained weight gain represent the weight of lipid extracted.

Lipid content (%) = [Lipid extracted (g) / Sample weight (g)] x [(chloroform layer + amounts lost) (ml)* / 3 ml] x 100

Gas chromatography analysis

GC/MS analysis of the wax was performed on a DB-1 HT fused-silica capillary column (15 m x 0.25 mm, film thickness of 0.10 µm; 6890 N, Agilent Technologies, USA). The injector and detector temperatures were set at 390°C, and the column temperature was programed from 120°C to 240°C at 15°C/min and then from 240°C to 390°C at 8°C/ min and finally maintained at 390°C for 6 min.

For auxin characterization, the same methode was used except the ion source held at 220°C, the injector 250°C, and the transfer line 290°C.

Samples (1 µl) were injected through a split-injector (1/5). MS spectra were detected in EI mode. Samples and standards were dissolved in hexane (0.1–1.0 mg/mL). Each sample solution was injected in duplicate, and reproducibility of the results was confirmed.

Fatty acid methyl esters of wax were identified by comparison with the standard fatty acid esters (Sigma, USA) and were quantified as percentages of the total peak areas.

In vitro **germination test**

Germination test was assessed using Zucconi test by measuring seed germination [5]. Olive seeds were placed, after moving external pit, on a screen in a glass petri dishes with dimensions of 110 mm × 20 mm. Seeds were irrigated with 0.5 ml of wax diluted to 10% (10-0.62 mg/g) in water then was capped and kept in a dark incubator at 25°C temperature for 15 days. A germination index (GI) was calculated by counting the grown seeds and determining the average sum of seeds root elongation in each tested sample. The results were finally expressed as a percentage. The germination index was determined by the following formula:

$$GI\ (\%) = NE / NT\ X\ LE / LT\ X\ 100$$

NE: number of germinated grains irrigated by diluted wax NT: number of germinated grains in the control irrigated by water, LE: average length of the radicle of germinated grains for the sample, LT: average length of the radicle of germinated grains for the control.

All the experiments are carried on triplicates.

Fertigation practice

The main objective of this experiment is to test the effects cream on plant growth, number of leaves and branches and to optimize its beneficial concentrations for the speace. This essay was conducted in accordance with the natural climatic conditions favorable to the growth of olive. Indeed, all the pots were placed in a greenhouse designed as a growth chamber programmed for a photoperiod of 12 h day 712 night, with a photosynthetic photon flux density of 300 µmol m-2 s-1; temperature, 24 ± 1/18 ± 1°C day / night; and relative humidity, 60/70 ± 3%. The test was carried out in a polystyrene honeycomb plate filled with soil. Pants aged of two weeks are carefully irrigated with 10 ml of water or diluted wax with a dose of 10-0.62 mg/g soil. The monitoring of plant growth for 90 days (total plant length, leaves and ramifications number) allows us to study the possibility of upgrading the wax.

Auxin characterization in treated plants

Leaves of plants previousley treated with wax were moved, dried, ground and extracted with water at 4°C as previosley reported by Jager et al [6].

Subsequently, the extract was dried on a rotary evaporator. Extracts for analysis of auxin were taken up in 30 ml of $KHSO_4$ (0.3 N) and distilled water, respectively, and partitioned three times with 10 ml chloroform. The organic phase was then dried under rotary evaporation, transferred to a tapered-bottom vial, and taken to complete dryness in a sample concentrator. Trimethylsilylation was then performed by adding 40 ml N,O-bis (trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) to the dry sample with 10 mL pyridine to aid dissolution, and heating to 80°C for 30 min. Subsequently, the extract was dried under nitrogen and 15 mL BSTFA (1% TMCS) was added. The sample was then placed in an oven at 80°C for a further 30 min and then injected in GCMS.

Statistical analysis

All experiments were carried out on triplicates. Analysis of variance (ANOVA) was done with the software STATISTICA, using Tukey's test at $P < 0.05$. GC-MS data sets were imported into SIMCA 13 (Umetrics AB, Umea,Sweden) for processing using principal component analysis.

Results And Discussion

To obtain the best wool wax recovery using an optimal extraction time, a kinetic study was carried out using two different solvants of water and hexan (Fig. 1). The results highlight the importance of this parameter. When we used water at 70°C, a low proportion of wool wax was extracted during the first 20 min. However, when hexan was used, the extraction rate of lanolin increased. The solvant did not have any influence on the extraction rate after 50 min (20 and 10 µg of extract/g of wool using hexane and water respectively). The resultant extraction profiles suggest that the extraction rate is limited by the solubility of some wool wax components. This is logic because hexan is an apolar solavnt. This result is similar with those found by Dominguez et al. in 2003 [7].

Physiochemical characterization showed that wax presents a slightly acidic pH which extends from 6 to 6.5 and a high dry matter content of 60.2 to 62.3% (Table 1). This effluent is characterized by the absence of alcohol which could not therefore be at the origin of a possible toxicity.

Table 1
Wool wax physiochemical and total lipid content analysis

	Water extract	Hexan extract
pH	6.5	6
Conductivity (ms/cm)	17.9	18.4
Salinity (g/l)	18.6	18.9
Protein (%)	0.13	0.08
Nitrogen (%)	0.1	0.07
Phosphate (%)	4.2	4.7
Alcohol test (%)	0	0
Water (%)	6	3
Dry matter (%)	48.2	62.3
Total lipid content (%)	60.7	95.6

The mineral composition of this effluent shows a low composition of water moisture (3 to 6%) coupled with a large amount of phosphate (4.2 to 4.7%) and conversely low doses of protein (0.08%) and nitrogen (0.07%). This disagree with previous work dealing with the the physiochimical characterization of baker yeast separation effluent [8].

By means of gaz chromatography-mass spectrometry, more than fifty compounds present in wax sample were identified in form of their methyl derivatives. 6 compounds were determinated comparing to NIST library. Cholesterol is strongly dominating followed by 2-MeO methyl ester of fatty acid with 18 carbon atoms (18:0) followed by methyl ester of 21:0, methyl ester of 16:0 and metyl ester of 15:0 (Table 2). 7-ketocholesterol, which is known to be present in lanolin especially as a product of aging, was not detected in our study. This is in conflict with conventional data of lanolin, where it was found in form of its degradation product 7-keto-3,5-cholestadiene [9]. Our results are in accordance with those found by the same authors in another publication [10].

Table 2
Fatty acids compsosition of wool wax

Peak number	Peak identification
10	FAME 15:0
13	FAME 16:0
16	FAME 17:0
18	MeO-FAME 18:0
24	FAME 21:0
47	Cholesteryl methyl ester
FAME : fatty acid methyl ester, MeO-FAME : Methoxy fatty acid methyl ester	

Lanolin consists of a complex mixture of esters and polyesters of high molecular weight alcohols and fatty acids [11]. Isolation of lanolin esters is extremely difficult and no conclusions were found that identify the individual esters which exist in lanolin. It has been reported from gas chromatographic investigations that the aliphatic alcoholic compounds in lanoline comprise 17.1% aliphatic nonalcohols, 8.7% aliphatic alkane-diols, 68.3% sterol and triterpene alcohols, and 5.9% unidentified and polyols [12].

The determination of the germination index of olive seeds during 15 days of treatment with different concentrations showed that this parameter is more important using diluted cream comparing by water control. This germination index reaches a maximum at a dose of 1.25 mg/g and then gradually decreases (Fig. 2). These results are in agreement with those found by Lan et al and Abida et al [13–14]. This can be explained, on the one hand by the richness of wax in nutritional elements as phosphate that stimulate germination and on the other hand by fatty acid content. In the same fashion, diethyl aminoethyl hexanoate (DA-6), a plant growth regulator, increases germination and seedling establishment from soybean seeds by increasing fatty acid metabolism and glycometabolism [15].

Irrigation with diluated wax showed an improvement of increasing average lengths of stems compared to the control, this growth in length reaches its maximum in plants irrigated by the dose set at 1.25 mg/g soil, the latter is of the order of 45.7 ± 2.52 cm (Fig. 3). Nevertheless, beyond this volumes irrigation, it

causes an antagonistic effect resulting in a decrease of this length and confirming, the toxicity of the wax at high doses. To hypothesise the mode of action of wool wax and its fatty acids on phytohormones, auxin in treated plants was charactirized by gaz chromatographic analysis after derivatization step. Slight emprovement in auxin was noted in plantes treated with 1.25 mg of wax/g of soil according to control (0.9 ± 0.1 ng/ml and 0.7 ± 0.20 ng/ml respectively). Similar results were found by Ami et al. in sorghum plants using UPLC MS/%S assay [16]. For our best knowlege and lettirature survey, this is the first report on auxin enhancement in plants by wool wax fatty acids. Our results are similar to those found by Bach et al. in 2010 [17]. Auxin or indole-3-acetic acid (IAA) is a key plant growth hormone, involved in processes as diverse as branching, gravitropism, phototropism, and seed development [18]. However, the biosynthetic pathways leading to the main auxin in plants, are not well understood. Although there is good evidence that the amino acid tryptophane is an early precursor [19]. Wounding and/or stress of plant tissues can have a major impact on auxin biosynthetic route used by plants [20].

Conclusion

Industrial production of wool generates a significant release of liquid waste which ranks among the main environmental hazards in the whole Mediterranean region and not only in Tunisia. These dangers have been aggravated especially with mismanagement and have been causing release of bad smells and thus threatening the contamination of the entire environment.

Our study focused on valorizing these liquid effluents in agriculture, whose main objective was to provide farmers with an efficient biological product. Wool wax, rich on fatty acids, has shown no phytotoxic effects on olive plants and could contribute to an improvement of growth by enhancing of phytohormone production as auxin. This leads us to the valuate wool wax as natural product in agricultural and fertigation practice.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest

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Data availability statement

All data generated or analysed during this study are included in this article and its supplementary information files

Statement of Novelty

In this work, we showed for the first time that wool wax can be valorized in agriculture to promote germination and growth of olive (*Olea europea*). Wool wax is rich on fatty acids which can act on phytohormones, as auxin by enhancing its production.

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Figures

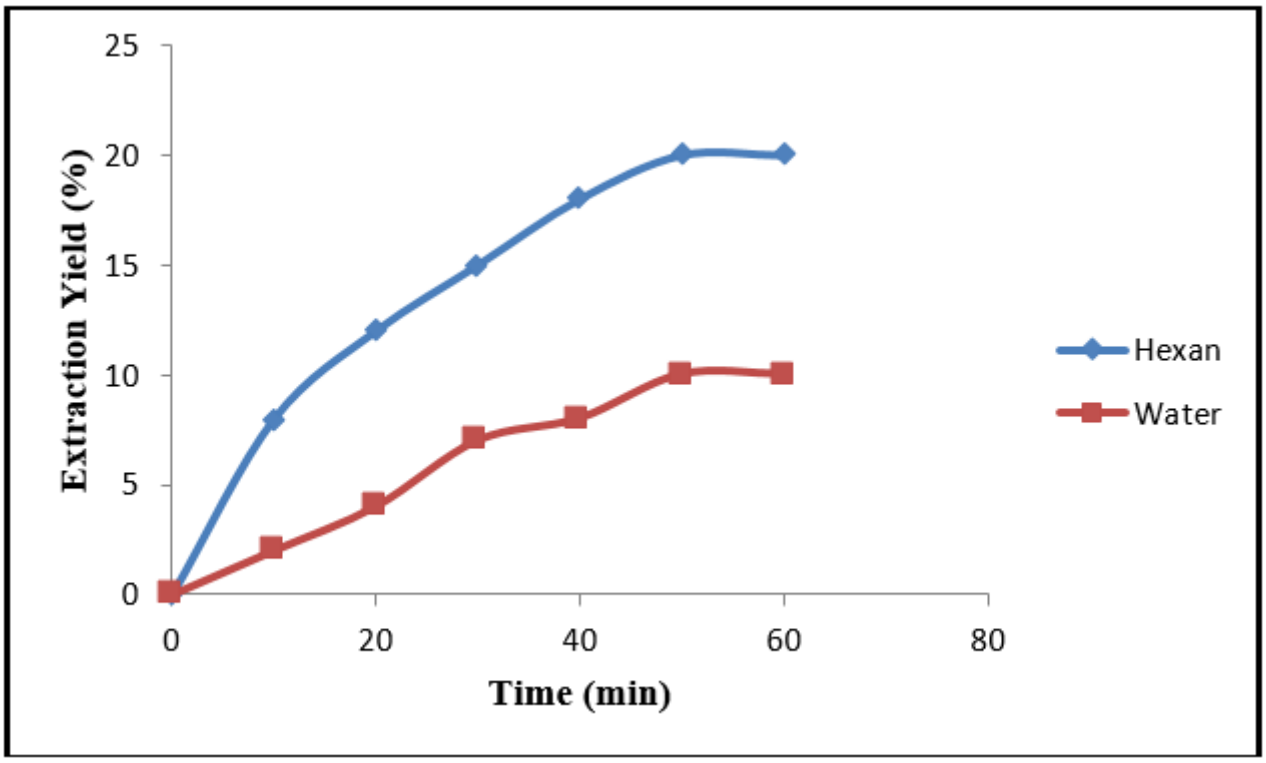

Figure 1

Kinetics of wax extraction yield from wool using water and hexan

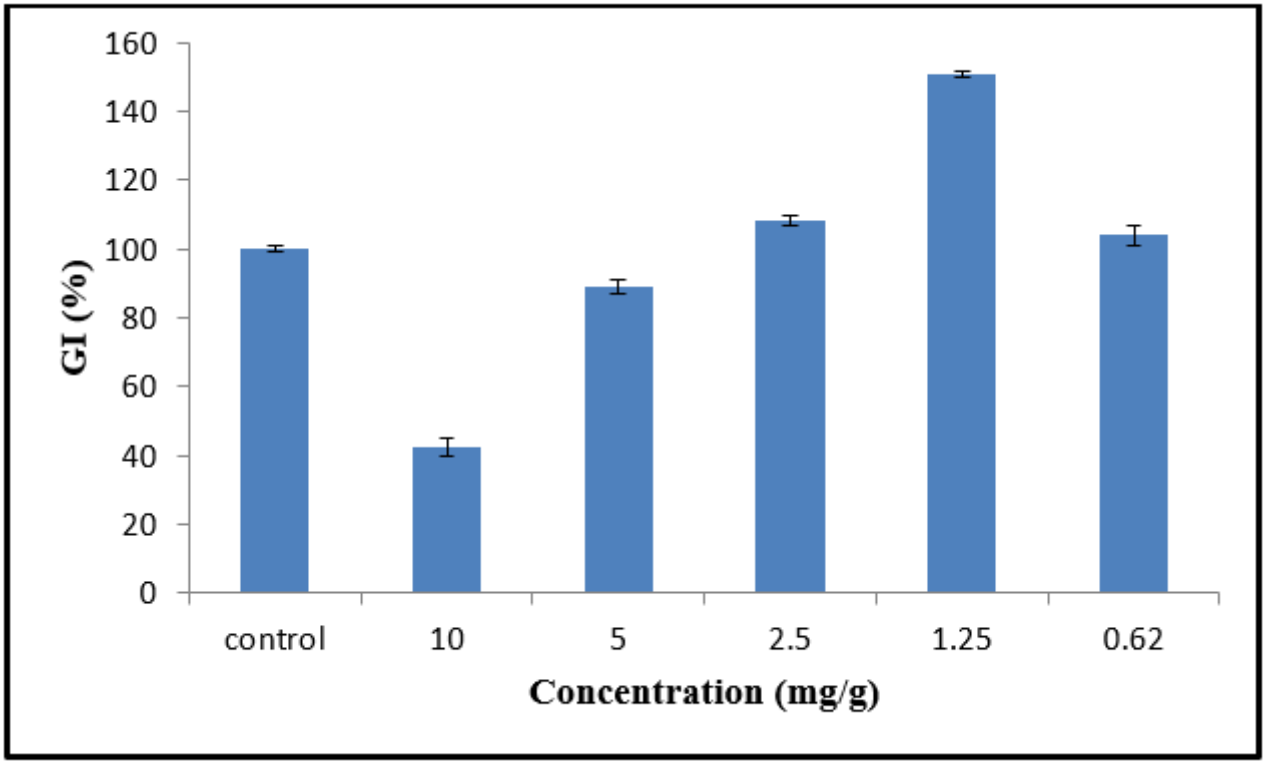

Figure 2

Germination index of olive seeds using different concentrations of wool wax

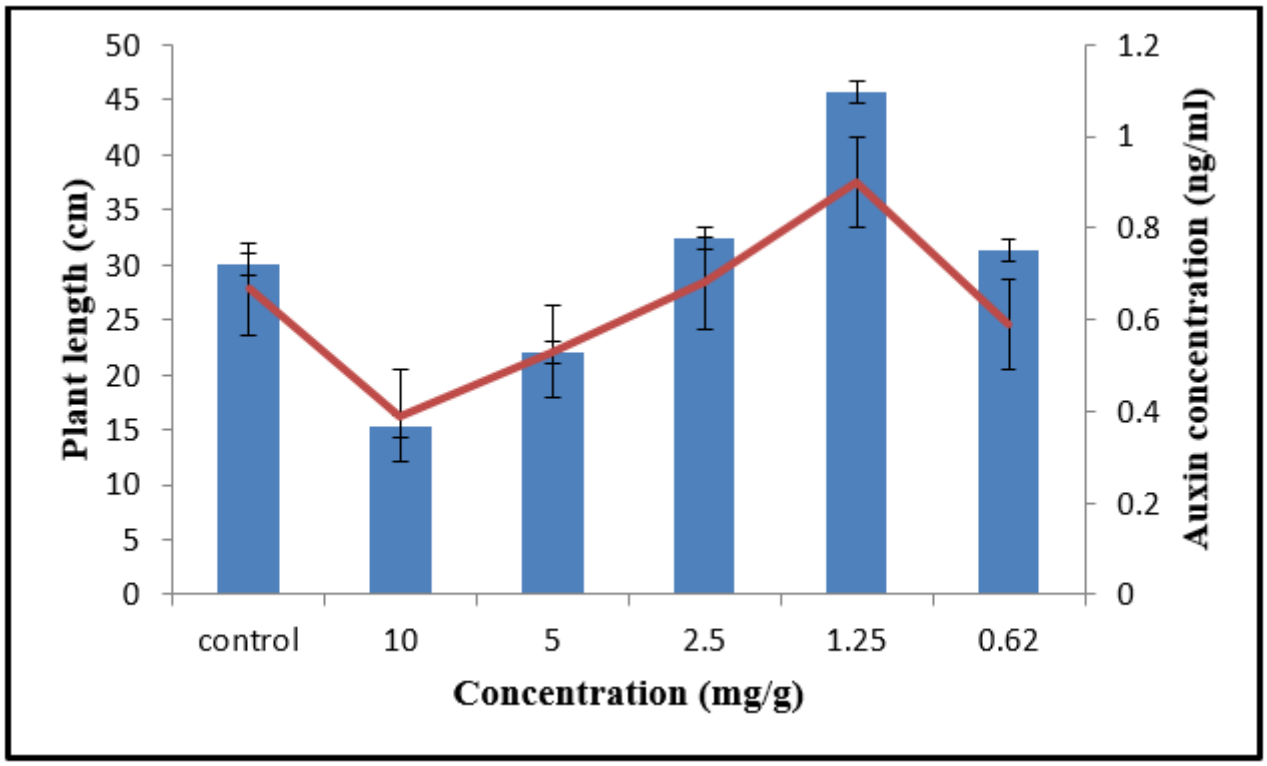

Figure 3

Variation of plant length using different concentrations of wool wax and correlation with auxin level

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