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Potential agricultural use of a sub-product (olive cake) from olive oil industries composting with soil

[Uso agrícola potencial de un subproducto (alperujo) de las industrias del aceite de oliva compostado con suelo]

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Abstract

Context: Olive cake and olive mill wastewater are by-products of olive oil industries. Olive cake is not currently being exploited.

Aims: To evaluate the cytotoxicity and genotoxicity induced in *Allium cepa* root meristems by olive cake in various preparations (aqueous dilutions of olive cake and mixtures of olive cake with soil before and after a composting process).

Methods: Olive cake aqueous dilutions as well as fresh and composted olive cake - soil mixtures were obtained. Samples were assayed on *Allium cepa* L roots and the phenolic content was also determined.

Results: Aqueous dilutions showed acute genotoxicity with a mitotic index dramatic reduction and a high number of cell abnormalities. Olive cake induced chromosome aberrations such as bridges, stickiness, and laggard chromosomes and cell aberrations such as strap, folded, giant and binucleated cells. Anomalies increase with polyphenol concentration, both in aqueous dilutions and in mixtures of olive cake – soil without composting. Composted mixtures did not exhibit cell toxicity up to 10% of olive cake although they can decrease the mitotic index, which would produce a reduction in plant growth.

Conclusions: Results indicate the varied behavior of olive cake according to sample preparation and suggest the possible use of this by-product incorporated to soils and subjected to composting.

Keywords: *Allium cepa*; genotoxicity; olive cake-soil mixture; olive cake; polyphenols.

Resumen

Contexto: El alperujo es un subproducto de las industrias del aceite de oliva, el cual, actualmente, no está siendo explotado.

Objetivos: El objetivo de este estudio es evaluar la citotoxicidad y genotoxicidad inducida en meristemas de raíces de *Allium cepa* por alperujo en diferentes preparados (diluciones acuosas de alperujo y mezclas de alperujo con suelo, antes y después de un proceso de compostado).

Métodos: Se prepararon diluciones acuosas de alperujo así como mezclas frescas y compostadas de alperujo-suelo. Las muestras fueron analizadas sobre raíces de *Allium cepa* L. y se determinó, además, el contenido de compuestos fenólicos.

Resultados: Las diluciones acuosas mostraron genotoxicidad aguda con una reducción drástica del índice mitótico y un alto número de anomalías celulares. El alperujo indujo aberraciones cromosómicas, tales como puentes, pegajosidad y cromosomas rezagados y aberraciones celulares como células alargadas, plegadas, gigantes y binucleadas. Las anomalías aumentaron con la concentración de polifenoles tanto en diluciones acuosas como en mezclas de alperujo-suelo sin compostaje. Las mezclas compostadas no mostraron toxicidad celular hasta el 10% de la torta de oliva, aunque disminuyeron el índice mitótico, lo cual produciría una disminución en el crecimiento de plantas.

Conclusiones: Los resultados indican el diferente comportamiento del alperujo según la preparación de las muestras e indican el posible uso de este subproducto incorporado a suelos y sometido a compostaje.

Palabras Clave: *Allium cepa*; alperujo; genotoxicidad; mezclas alperujo-suelo; polifenoles.

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INTRODUCTION

Olive cake is a by-product generated during olive oil production. Olive cake annual production in the Mediterranean countries reached 3 million tons, while in Latin America 77 thousand tons, of which approximately 70% comes from Argentina, mainly from the Cuyo and NOA regions (COI, 2018). Olive-mill wastes represent a significant environmental problem due to their high production during a short period of time. Their high polyphenol, lipid and organic acid content turns them into phytotoxic wastes (Saadi et al., 2007; El Hassani et al., 2009; Buchmann et al., 2015). Seed germination was reported to be inhibited by the cytotoxic effects of olive-mill wastes on *Lycopersicon esculentum*, *Vicia faba*, *Helianthus annuus* L. and *Medicago sativa* (Alburquerque et al., 2006; Mekki et al., 2006; Garrido et al., 2012; Hammann et al., 2014). Olive cake phytotoxicity is essentially linked to its compounds content of difficult biodegradability (Dermeche et al., 2013; Aggoun et al., 2016).

Garrido et al. (2012) have demonstrated some alternations caused by oxidative stress in sunflower plant roots exposed to olive cake such as root length and meristematic size decrease by detention of cell division, and an increase in the number of cortical cell layer. Aybeke et al. (2008) showed in the wheat (*Triticum aestivum* L.) root apical meristem exposed to olive mill wastewater, local disintegrations of cell walls and nuclear membrane, irregular nucleus and dense nucleoli.

The *Allium cepa* test has been used to detect a wide variety of contaminants in water samples, soils and municipal sludge (Fiskesjö, 1985; Liu et al., 1995; Gana et al., 2008; Qin et al., 2010; Adhikari et al., 2014). An advantage of the technical procedure is the possibility to expose the test organism directly to complex mixtures without previous treatment (Rank, 2003). *Allium cepa* test has been considered one of the most promising tests for evaluating environmental mutagenicity, since it is effective, simple and fast (Cardozo et al., 2006). This test showed a dose-dependent relationship with mitosis inhibition as well as the capability to characterize aneugenic and clastogenic agents and micronucleus induction (Ateeq et al., 2002; Seth et al., 2008; Srivastava and Mishra, 2009; Kumari et al., 2011; Wang et al., 2014).

The aim of this study is to evaluate the cytotoxicity and genotoxicity induced in *A. cepa* root meristems by olive cake in various preparations (aqueous dilutions of olive cake and mixtures of olive cake with soil, before and after a composting process).

MATERIAL AND METHODS

Chemicals

Folin- Ciocalteu reagent, sodium carbonate, Orcein, formic acid, gallic acid and $HgCl_2$ were supplied from Sigma Aldrich Company (St. Louis, MO, USA). Glacial acetic acid, acetone and hydrochloric acid were provided from Cicarelli Reagents S.A. (San Lorenzo, Sta. Fe, Argentina)

Olive cake samples

Olive cake samples were obtained from olive oil extraction of a pilot plant located at EEA-INTA Sumalao (Catamarca, Argentina), during the 2014-2015 production period.

Three types of sample preparations were carried out: aqueous dilutions (AD) and mixtures of fresh (FM) and composted (CM) olive cake-soil.

AD of fresh olive cake was made with distilled water at 0, 5, 10 and 20% v/v. The samples were kept at 4°C.

Soil samples from "A" horizon (15 cm deep) of a foothill that sustains a secondary succession of Arid Chaco Forest from San Fernando del Valle de Catamarca (28°27'25.8''S, 65°45'35.1''W) were taken to make olive cake-soil mixtures.

FM and CM mixtures were prepared at 0, 5, 10 and 20% v/v, mixing both components in 50 L containers. FM samples were prepared and used immediately. CM samples were kept at room temperature for 40 days and were mixed every week for their aeration.

The samples taken for the assays both from soil alone and from FM/CM mixtures consisted of homogenous mixtures from representative subsamples, by using the quart method (Vita Serman and Matías, 2013).

Polyphenol determination

Polyphenolic compounds were extracted from 5 g of AD, FM and CM. Samples were homogenized for 10 minutes by ultrasound in 30 mL of water acidulated with 5% formic acid/acetone (1:1, v: v) and kept for 12 h at 4°C. Then, the medium was centrifuged, and the supernatant stored at 4°C. Each extraction was conducted by triplicates.

Polyphenolic compounds content was analyzed by using the Folin–Ciocalteu reagent according to Singleton et al. (1999). Each extract sample was mixed with 100 µl of Folin-Ciocalteu reagent, 400 µL of sodium bicarbonate (15.9%) and maintained for 20 min, at room temperature. Absorbance was measured at 765 nm in an UV-Visible spectrophotometer (200-1000 nm, 2100C Unicom Optics). The polyphenol concentration was expressed as µg equivalent of gallic acid/ g fresh weight. Calibration equation for total phenol determination was found as $y = 0.049x$ ($r^2 = 0.923$). Polyphenol quantitative analyses were repeated three times.

Allium cepa assays

Bulbs of *Allium cepa* L. were purchased from the local market at San Fernando del Valle de Catamarca city. The plant material was identified by Dr. Marta Arias, of the Center for Studies of Plant Species of Regional Interest (CEVIR), Faculty of Exact and Natural Sciences, National University of Catamarca, Catamarca, Argentina. Healthy and similar-sized onion bulbs, which had not started any growth, were selected as materials. Before starting the experiments, dry cataphiles were removed from bulbs.

For *A. cepa* test, bulbs grew for 48 h in plastic containers by dipping the base in distilled water for activating root growth. Ten bulbs for each treatment with root lengths between 2–2.5 cm were used. Each bulb was transferred to a plastic container of 250 cm^3 containing 200 mL of AD at 5, 10 and 20% (v/v). Distilled water was used as a negative control and $HgCl_2$ solutions (1 mg/L and 3 mg/L) as positive mutagenic controls (Banerjeei et al., 2010).

For assays with FM and CM, each bulb was planted in a plastic container of 250 cm^3 containing 200 cm^3 of FM or CM at 5, 10 and 20% (v/v). There were irrigated with distilled water to maintain the substrate moisture. Soil was used as a negative control.

After 72 h of exposure for *A. cepa* test, and 120 h of exposure for FM and CM assays, root tips were removed from the bulbs, washed, fixed in ethanol: glacial acetic acid (3:1, v/v) and stored at 4°C.

For the observation of changes in cell division and chromosome disorder, fixed root tips were hydrolyzed in 1 N hydrochloric acid for 5 min at 60°C, washed and squashed in orcein solution for 7 min (Li, 1989). The Mitotic Index (MI) represents the total number of dividing cells in relation to the number of analyzed cells in the cell cycle. A minimum of 1,000 cells were scored for MI and expressed as a percentage of total number of examined cells. The frequency of chromosomal aberrations and cytological abnormalities were expressed as the number of aberrant or abnormal cells, respectively, per 1,000 cells examined by each one of six slides in each sample (Fiskesjö, 1985). For a cytogenetic examination, an optic microscope (Carl Zeiss) at 1000X magnification was used. Photomicrographs were taken with the help of a digital camera (Olympus) fixed on a microscope that was connected to a computer in order to transport images.

Statistical analysis

All tests were conducted in triplicates. Each experimental value is expressed as the mean ± standard deviation (SD). The statistical software InfoStat (Student Version, 2015, Di Rienzo et al., 2015) was used to evaluate the significance of differences between groups. The one-way ANOVA with Tukey posttest at a confidence level of 95% was used for comparisons between groups. Differences were considered significant at p values of < 0.05.

RESULTS

Polyphenols in olive cake samples

Table 1 shows the total soluble polyphenols in AD, FM and CM olive cake samples. Polyphenols in AD and FM showed a sustained and proportional increase to the olive cake percent, reaching values up to 522 µg/g with the highest concentration in AD and 503 µg/g in 20% FM. On the other hand, in the CM samples, polyphenols levels were lower than those of AD and FM at the same olive cake proportion.

Table 1. Polyphenol content in samples of aqueous dilutions (AD), fresh mixtures (FM) and composted mixtures (CM).

Olive cake	AD	FM	CM
	(µg gallic acid / g FW)		
0%	-	23.87 ± 0.20^{f}	23.87 ± 0.20^{f}
5%	130.61 ± 0.41^{c}	137.68 ± 0.37^{c}	35.17 ± 0.51^{e}
10%	261.23 ± 0.72^{b}	215.83 ± 0.39^{b}	41.14 ± 0.86^{e}
20%	522.46 ± 0.98^{a}	503.42 ± 0.92^{a}	52.68 ± 0.96^{d}

Same letters are not significantly different at ($p<0.05$)

Effects of olive cake on cell division

Acute genotoxicity was observed when root tips were treated with AD (Table 2). The MI in root tip decreased dramatically between 98-99% with the exposure to AD (5, 10 and 20%). All mitotic cells in AD were found in the metaphase. This result is very similar to that of positive controls with $HgCl_2$ solutions where cellular division was totally inhibiting (Table 2).

MI of root tip cell in the presence of FM and CM samples was less affected than AD. In the presence of FM, MI decreased with all the tested concentrations, 20% FM being the most affected, with a decrease of 50% respect to the soil control. MI of CM samples at 5% was not affected, soil control being significantly higher (Table 2).

The controls (water and soil) did not show significant differences in both MI (49%) and index per phase, with a 79% in the prophase stage and between 6 and 9% in the other stages (metaphase, anaphase and telophase). Mitotic phase was affected in a different way, depending on the type (FM or CM) and of mixture concentration. Prophase decreased in all mixtures respect to control. Metaphase was about 3-fold higher in 10 and 20% CM than the control. Only 10 and 20% FM showed significant changes in the anaphase, with a 40% decrease and 80% increase, respectively. In the telophase, only 20% FM did not show significant differences with the control (Table 2).

Cytogenetic effects of olive cake

Chromosome aberrations and cellular abnormalities induced by AD, FM and CM olive cake were observed such as chromosome stickiness, chromosomal bridges, and laggard and vagrant chromosomes, strap, giant, binucleated and folded cells. However, some abnormalities were characteristic according to the type of samples. In AD samples, strap cells were observed with strap nuclei or with displaced nuclei, whereas in FM and CM, they were not present. Between chromosome aberrations, only chromosomal sticky was found in AD, while in FM and CM bridges, laggard and vagrant chromosomes were found (Table 3 and Fig. 1).

In general, after treatment with 5% and 10% AD, the total abnormalities (cellular and chromosomic) decreased significantly with respect to the positive controls and increased at the maximum concentration (20%) at the same level of mutagen control with highest concentration. A similar pattern was observed in 5 and 10% FM; however, 20% FM does not show significant differences with respect to the positive control with lowest concentration (1 mg/L). The CM showed the lowest cellular toxicity, with a strong decrease of the abnormalities respect to the positive control and to AD and FM.

Although the percentage of chromosome aberrations, in general, was low except in 20% CM. In addition, vagrant chromosomes were observed in all CM and in 20% FM.

In FM, only at 20%, strap cells increased more than twice the positive control. Ten and 20% FM showed higher values of folded and binucleated cells than the positive control. FM showed giant

cells in highest proportion respect to other samples. In AD and FM, giant cells with displaced nuclei were observed (Table 3).

Fig. 2 shows polyphenol content and total abnormalities, in an overlapping way, of all the samples. As can be seen, the CM samples showed lowest values of both polyphenols and abnormalities respect to AD and FM samples. AD samples showed a proportional increase with concentration in both variables, Pearson's correlation coefficient being of 0.96. Polyphenols content in 20% FM was similar to 20% AD however aberrations level was lower (Table 3, Fig. 2).

DISCUSSION

Roots are the first organs that come into contact with toxic substances that can affect cell division in the meristematic regions, which depend on the growth and development of plants. Therefore, in the present study we used *A. cepa* for a rapid detection of cytotoxicity and genotoxicity of different samples using olive cake.

The mitotic index is considered to reliably identify the presence of cytotoxic agents in the environment (Radić et al., 2010). Liman rt al. (2011) reported that MI values lower than negative controls may indicate an affectation in the growth and development of the exposed organism caused by the evaluated agent; in that sense, all the treatments with olive cake (AD, FM and CM) caused a decrease of the mitotic index, except in 5% CM where it increased. A decrease in MI of more than 50% commonly has sublethal effects, but if IM decreases below 22% compared to negative controls, it can have a lethal impact on the organism (Antonsiewicz, 1990). In FM and CM, there were no decreases in the MI higher than 50% in comparison with the negative controls; although they did not reach sublethal values, they could be indicators of impairments in the growth and development induced by the olive cake.

Table 2. Effects of aqueous dilutions (AD), fresh mixtures (FM) and composted mixtures (CM) on mitotic index in root tip cells of *Allium cepa.*

Treatment	Mitotic index	Mitotic index per phase			
		Prophase	Metaphase	Anaphase	Telophase
$HgCl_2$ 1 mg/L	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
$HgCl_2$ 3 mg/L	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Water	47.80 ± 2.00^{b}	78.66 ± 0.21^{a}	5.65 ± 0.20^{e}	8.57 ± 0.20^{b}	7.11 ± 0.21^{d}
AD 5%	0.50 ± 0.05^{f}	0.00 ± 0.00	100.00 ± 0.00^{a}	0.00 ± 0.00	0.00 ± 0.00
AD 10%	0.50 ± 0.02^{f}	0.00 ± 0.00	100.00 ± 0.00^{a}	0.00 ± 0.00	0.00 ± 0.00
AD 20%	1.00 ± 0.20^{f}	0.00 ± 0.00	100.00 ± 0.00^{a}	0.00 ± 0.00	0.00 ± 0.00
Soil	47.90 ± 1.05^{b}	78,70 ± 0.20^{a}	5.64 ± 0.21^{e}	8.56 ± 0.21^{b}	7.10± 0.20^{d}
FM 5%	30.20 ± 1.00^{d}	66.89 ± 0.12^{e}	8.61 ± 0.38^{c}	7.95 ± 0.28^{b}	16.55 ± 0.22^{a}
FM 10%	39.16 ± 1.04^{c}	73.91 ± 0.22^{b}	5.08 ± 0.31^{e}	5.08 ± 0.31^{c}	15.93 ± 0.42^{a}
FM 20%	24.00 ± 1.00^{e}	69.58 ± 0.74^{d}	7.92 ± 0.28^{d}	15.42 ± 0.16^{a}	7.08 ± 0.29^{d}
CM 5%	51.03 ± 0.57^{a}	71.99 ± 0.19^{c}	8.68 ± 0.19^{c}	8.88 ± 0.19^{b}	10.45 ± 0.19^{b}
CM 10%	32.16 ± 1.04^{d}	62.46 ± 0.46^{f}	18.77 ± 0.08^{b}	8.00 ± 0.21^{b}	10.77 ± 0.17^{b}
CM 20%	38.17 ± 1.04^{c}	63.08 ± 0.47^{f}	19.38 ± 0.07^{b}	8.31 ± 0.21^{b}	9.23 ± 0.19^{c}

Same letters in same column are not significantly different at ($p<0.05$).

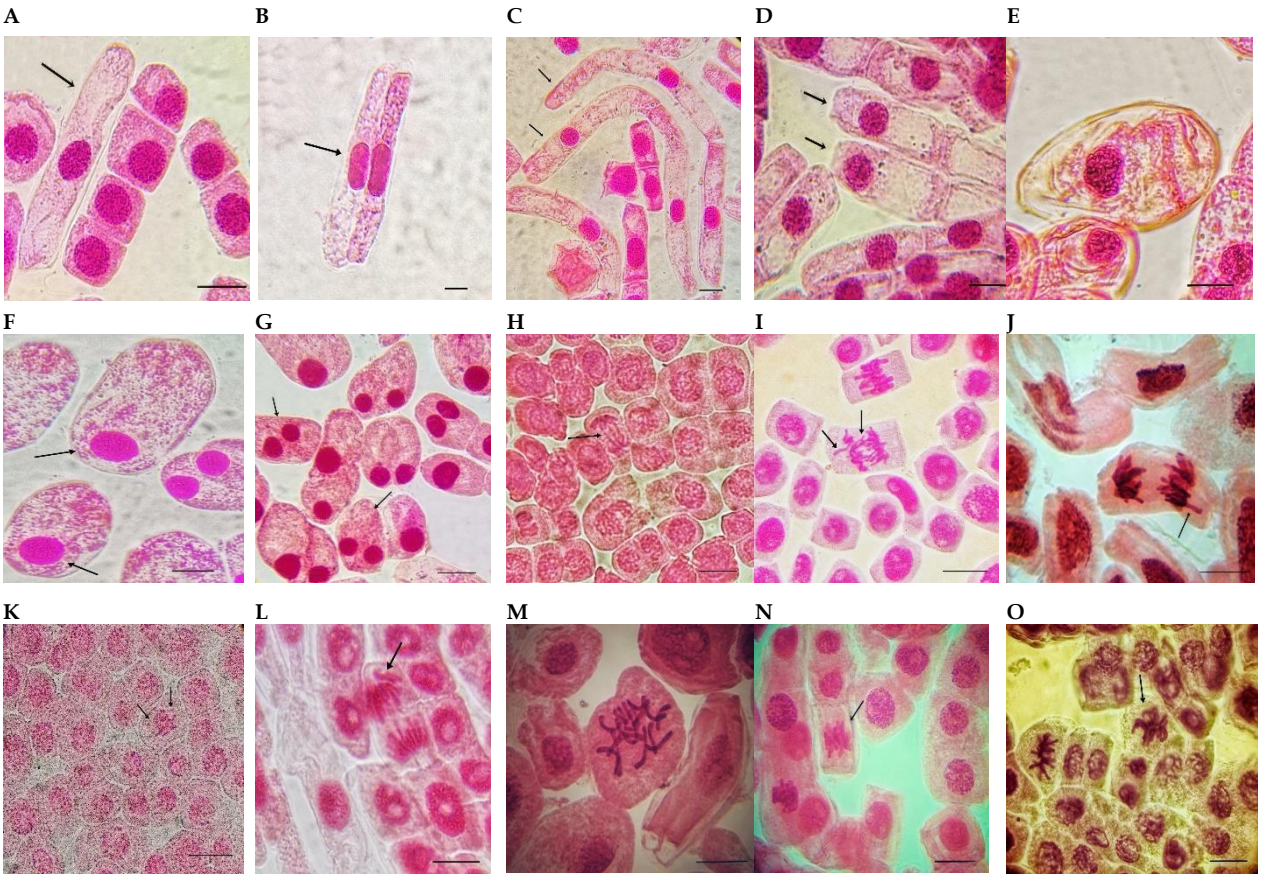

Figure 1. Aberrations induced by olive cake samples (AD, FM or CM) on *A. cepa* root tip cells.

(A) strap cell; **(B)** strap cells with strap nuclei; **(C)** folded cells; **(D)** strap cells with displaced nuclei; **(E)** giant cell; **(F)** giant cells with displaced nuclei; **(G)** binucleated cells; (H) bridges; **(I)** laggard chromosome and bridge in telophase; **(J, K)** laggard chromosomes in different mitosis phases; **(L)** vagrant chromosomes; **(M, N** and **O)** sticky chromosomes. Bar, 20 µm.

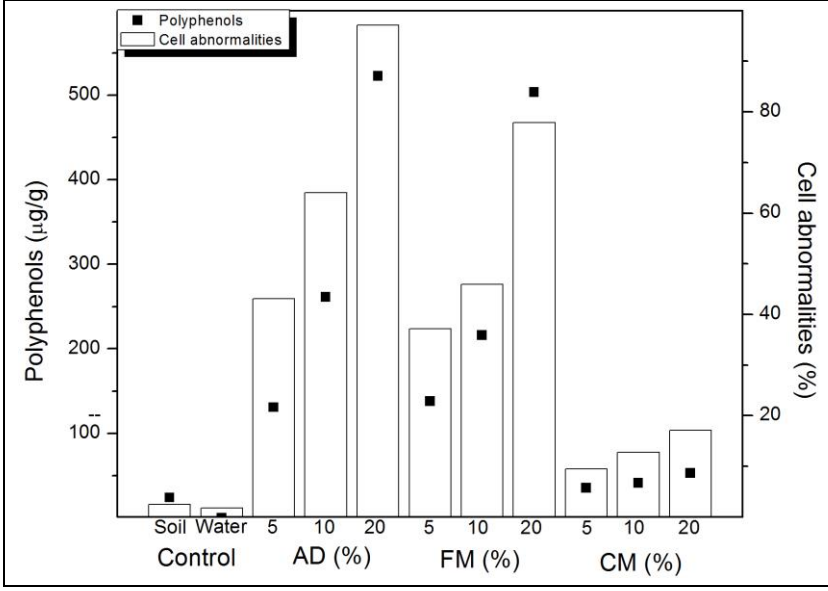

Figure 2. Polyphenol content in samples of aqueous dilutions (AD), fresh mixtures (FM) and composted mixtures (CM) and total cellular abnormalities of root tips of plants developed in each treatment.

The values are mean, standard deviations are shown in Tables 1 and 3.

Table 3. Effects of aqueous dilutions (AD), fresh mixtures (FM) and composted mixtures (CM) on chromosome aberrations and cellular abnormalities in the root tip cells of *Allium cepa.*

Treatment	Chromosome aberrations per 1000 cells (CA)				Total CA (%)	Cellular abnormalities per 1000 cells (Ab)							Total Ab (%)	CA+Ab (%)
	Bridge	Sticky	Vagrant	Laggard		Strap cells	Strap cells/ strap nucleus	Strap cells/ displaced nucleus	Giant cells	Giant cells/ displaced nucleus	Binucleate dcells	Folded cells		
$HgCl_2$ 1mg/L	wm	wm	wm	wm	wm	164^{d}	2^{d}	135^{b}	236^{b}	234^{a}	2^{e}	56^{d}	82.90	82.90 ± 2.97^{b}
$HgCl_2$ 3mg/L	wm	wm	wm	wm	wm	196^{c}	178^{a}	98^{c}	267^{a}	145^{b}	35^{b}	40^{e}	95.90	95.90 ± 1.04^{a}
Water	1^{b}	1^{g}	0	1^{b}	0.30	0	1^{d}	0	20^{h}	0	0	0	2.10	2.40 ± 0.20^{h}
AD 5%	0	5^{e}	0	0	0.50	74^{e}	31^{c}	67^{d}	96^{e}	98^{d}	32^{b}	28^{f}	42.60	$43.10 \pm 2.80^{d-e}$
AD 10%	0	5^{e}	0	0	0.50	123^{d}	94^{b}	106^{c}	131^{d}	119^{c}	36^{b}	26^{f}	63.50	64.00 ± 1.03^{c}
AD 20%	0	10^{d}	0	0	1.00	220^{b}	150^{a}	196^{a}	151^{c}	150^{b}	8^{d}	86^{c}	96.10	97.10 ± 0.60^{a}
Soil	2^{b}	0	0	2^{b}	0.40	4^{f}	0	0	4^{i}	0	0	6^{g}	0.14	1.80 ± 0.03^{h}
FM 5%	0	1^{g}	0	0	0.10	3^{f}	0	0	214^{b}	99^{d}	10^{c}	45^{e}	37.1	37.20 ± 1.23^{e}
FM 10%	2^{b}	13^{c}	0	5^{a}	2.00	15^{f}	0	0	228^{b}	15^{e}	82^{a}	100^{b}	44.00	46.00 ± 2.67^{d}
FM 20%	0	6^{e}	3^{b}	0	0.90	315^{a}	0	0	230^{b}	0	95^{a}	130^{a}	77.00	77.90 ± 0.47^{b}
CM 5%	0	3^{f}	4^{a}	0	0.70	9^{f}	0	0	48^{g}	0	8^{d}	23^{f}	8.80	9.50 ± 0.35^{g}
CM 10%	0	16^{b}	2^{c}	0	1.80	12^{f}	0	0	55^{f-g}	3^{f}	15^{c}	25^{f}	11.00	$12.80 \pm 0.91^{f-g}$
CM 20%	13^{a}	35^{a}	3^{b}	2^{b}	5.30	1^{f}	0	0	79^{f}	0	32^{b}	6^{g}	11.80	17.10 ± 1.00^{f}

Same letters in same column are not significantly different at ($p<0.05$) wm: without mitosis.

The decrease in the MI below 22% in all AD accounts for the potential cytotoxicity and suggests the mitodepressive and antiproliferative effects on *A. cepa* root tip meristematic cells. The content of polyphenols in FM and AD was similar, however the amount of abnormalities found in AD was highest and mitosis was strongly inhibited. This could be due to some chemicals, which are available in AD, while in FM it may be associated to soil. In relation to the latter, Leme and Marín Morales (2009) found similar effects working with municipal mud. Crude extracts, such as fresh olive cake, are composed of complex mixtures of phytochemicals, which may work synergistically, additively or antagonistically. The increased mitotic index in 5% CM treatment as well as the higher values of FM and CM in comparison to AD mitotic index may be the result of the interaction of olive cake with soil and the composting treatment. Those factors which decreased the levels of toxic components could be responsible for the diminished observed genotoxic effects.

In AD and FM elongated and giant cells were mainly found. This may be due to alterations in the signals for cellular elongation and growth, which occur in the roots above the meristematic cells. Those effects over the cells was observed by Zabka et al. (2010) in hydroxyurea and caffeine-treated *Allium cepa*, indicating a delay in DNA replication and an increase in cell growth. Prajitha and Thoppil (2016) demonstrated giant a cell formation induced by yellow and orange- red synthetic lemon food colorants. Giant cells are formed when cells try to undergo mitosis and fail to complete cytoplasmic division. Only in AD, analyses did reveal changes in the nuclear morphology of elongated cells, finding elongated and displaced nuclei, with a greater variability in nuclear modeling resulting from exposure to olive cake dilutions, which confirms its strong toxicity. The proportion of giant cells in AD that had displaced nucleus was 50%, while that percentage was much lower in MF and even lowest in MC, which indicates a decrease in the toxic effect. CM samples had a lower

content of polyphenols and the fewest values of elongated and giant cells. However, these residual polyphenols could be the most recalcitrant (Min et al., 2015), which could account for the presence of cellular abnormalities.

The cytological analysis of root apical meristems showed a dramatic increase in the chromosomal aberrations in 20% MC. The different types of chromosomal aberrations allowed to get a general idea of the effects of olive cake in all phases of the cell cycle. Stickiness is considered to be a common sign of toxic effects on the chromosomes that probably leads to cell death (Fiskesjö, 1997, Renjana et al., 2013), in all AD cells found in mitosis showed stickiness, whereas it was only found in MF and MC from 10% olive cake. Chromosome bridges result from chromosome and/or chromatid breaks, indicating the clastogenic effect, whereas both vagrant and sticky chromosomes increase the risk for aneuploidy (Leme and Marin-Morales, 2009; Radić et al., 2010). Pronounced stickiness of the chromatin matrix often resulted in atypical metaphase and anaphase. Increased stickiness also leads to the formation of sticky bridges in anaphase and telophase, thereby preventing normal cytokinesis. Sticky chromosomes indicate that toxic agents are affecting the chromatin organization. This effect is related to a disturbed balance in the quantity of histones or other proteins responsible for controlling the structure of nuclear chromatin (Kurás et al., 2006). Olive cake phytotoxicity would be essentially linked to its high phenolic compound content (El Hassani et al., 2009).

CONCLUSIONS

In summary, the objective of this study was to evaluate the different forms of agricultural application of olive cake for its use and revaluation. Aqueous dilutions showed acute genotoxicity with a dramatic reduction of the mitotic index with a high level of cellular abnormalities. The fresh olive cake-soil showed genotoxicity with a reduction of a half the mitotic index and a high level of cellular aberrations. Results indicate that the incorporation of olive cake composting with soil in proportions of up to 10% is not cytotoxic or genotoxic, although they can decrease the mitotic index, which would produce a decrease in plant growth. For this reason, complementary studies to evaluate the growth of plants of agricultural interest would be necessary.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Hammann A	Ybañez LM	Isla MI	Hilal M
Concepts or ideas	x	x	x	x
Design	x	x	x	x
Definition of intellectual content	x	x	x	x
Literature search	x	x	x	x
Experimental studies	x	x		
Data acquisition	x	x	x	x
Data analysis	x	x	x	x
Statistical analysis	x			
Manuscript preparation	x	x	x	x
Manuscript editing	x	x	x	x
Manuscript review	x	x	x	x

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