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RESEARCH ARTICLE

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IN VITRO ASSESSMENT OF ALLELOPATHIC POTENTIAL OF OLIVE PROCESSING WASTE ON MAIZE (*ZEA MAYS* L.)

ABSTRACT:

The allelopathic potential of aqueous extract of air-dried olive processing waste (OPW) at four concentrations (1.0, 3.0, 6.0, and 9.0% w/v) on grain germination and seedling growth of *Zea mays* was studied under laboratory conditions. The germination of corn grains showed a relative tolerance to OPW aqueous extract. Increasing the concentration of OPW extract up to 3.0 % significantly improved the biomass of radicle and plumule thereafter; the fresh and dry weights were significantly reduced. A parallel result obtained for accumulation of soluble sugars and proteins. Thus, the effect of OPW aqueous extract may be promotive or inhibitory depending on the used concentration.

KEY WORDS:

Allelochemicals, Biomass, Germination, Olive processing waste, Proteins, Sugars, *Zea mays*.

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INTRODUCTION:

The olive oil production of Mediterranean countries represents about 98% of the entire worldwide production (Paredes *et al.*, 2005; Rossiter, 2013). Olive oil industry generates large quantities of solid and liquid wastes or by-products. Olive processing waste (OPW) can be a significant soil pollutant if released indiscriminately into the environment (Garcia *et al.,* 2000; Roig *et al.,* 2006). The antimicrobial properties of these by-products have been investigated (Kotsou *et al.,* 2004; Yangui *et al.,* 2010). OPW spreading on top soil may have beneficial effects such as nutrient availability for plant growth (Alburquerque *et al.,* 2006; Altieri and Esposito 2010). In addition, the phytotoxic effect of OPW on some crop plants and weeds was reported (Sampedro *et al.,* 2005; Cayuela *et al.,* 2008; Asfi *et al.,* 2012).

In fact, several authors attribute OPW toxicity to their phenolic content (Capasso *et al.,* 1992; Barakat *et al.,* 2010; García-Sánchez *et al.,* 2012). The chromatographic analyses of OPW showed the presence of several phenolic compounds including; *p*-tyrosol, hydroxytyrosol, protocatechuic, vainillic, *p*-coumaric, gallic, syringic, caffeic, ferulic, and hydroxybenzoic acids (Sampedro *et al.,* 2005; Hanifi and Hadrami, 2008; Azaizeh *et al.,* 2012). Phenolic compounds are one of the well-known allelochemicals (Inderjit and Duke, 2003). Allelochemicals are plant chemicals that released through leaching, root exudation, volatilization and residue decomposition into the environment and affect growth and development in natural and agro-ecosystems, a phenomenon known as allelopathy (Inderjit and Duke, 2003). The mode of action of these compounds on plant growth and metabolism has been summarized by many authors (Einhellig, 2002; Weidenhamer, 2004). They claimed that the joint action of several allelochemicals may cause alteration in plant metabolism owing to their interactions with vital growth processes and activities of many enzymes. The objective of this research was to assess the allelopathic potential of aqueous extract of air-dried oil processing waste (OPW) on grain germination and seedling growth of *Zea mays* under laboratory conditions.

MATERIAL AND METHODS:

Preparation of aqueous extract from OPW:

Solid OPW was collected from a modern olive mill at El-Arish city, North Sinai governorate, Egypt. One, 3.0, 6.0, or 9.0 g of air-dried OPW was mixed with 100 ml of distilled water in separate flasks to obtain final concentrations of 1.0, 3.0, 6.0, or 9.0%, respectively. The flasks were incubated overnight on shaker at room temperature. The mixture in each flask was filtered through Whatman No.1 filter paper and brought to its original volume.

Germination experiment:

The grains of *Zea mays* were surface sterilized using 0.1% (w/v) $HgCl_2$, washed several times under running water and finally washed in distilled water. Ten uniform grains placed in each of 25 clean, oven-dried Petri dishes which have been lined with 2 layers of filter paper. The Petri dishes divided into 5 groups. Petri dishes of the first group moistened with 15 ml distilled water to serve as the control, while the remaining groups moistened with 15 ml of 1.0, 3.0, 6.0, or 9.0% OPW aqueous extract. The Petri dishes were incubated at room temperature (28˚C) for two weeks. Emergence of 1 mm radicle was used as the criterion for germination. At the end of the incubation period, the length of plumule and radicle was measured in 5 seedlings picked up randomly. Plumule and radicle of each seedling were separated and their fresh weight determined, then oven-dried for dry weight measurements.

Extraction and determination of soluble reducing sugars:

Water-soluble carbohydrates were extracted by boiling a known weight of dry powdered tissues in distilled water for 1 h in a water bath. The extract was cooled and centrifuged at 5000 *g* for 10 min then the supernatant was completed up to known volume.

Reducing value of each sugar extract was determined according to the method adopted by Clark and Switzer (1977). One ml of each sugar extract was mixed with 1ml of freshly prepared Nelson's alkaline copper reagent. (Nelson's A:B; 25:1) and heated in a boiling water bath for 20 min, then rapidly cooled under running water. Nelson's A; 12.5 g of anhydrous Na_2CO_3, 12.5 g K, Na tartrate, 10 g $NaHCO_3$ and 100 g anhydrous Na_2SO_4 in 500 ml distilled water, Nelson's B; 7.5 g $CuSO_4$ in 50 ml distilled water. Thereafter, 1 ml of arsenomolybdate reagent (25 g ammonium molybdate in 450 ml distilled water mixed with 21 ml concentrated sulphuric acid and 3 g sodium arsenate in 25 ml distilled water) was added with several shaking to dissolve Cu_2O. When effervescence stopped, the mixture was made up to 10 ml with distilled water and its color intensity was measured at wavelength 540 nm against water-reagent blank treated in the same manner as samples. The content of reducing sugar was determined from glucose standard curve, and then calculated as mg sugar g^{-1} dry weight.

Extraction and determination of total soluble proteins:

Extraction of water soluble proteins was carried out according to the method described by El-Tayeb *et al.* (2006). Soluble protein was extracted by incubating 100 mg of dry powdered tissues in 10 ml distilled water for 2 h at 90°C. After cooling, the mixture was centrifuged at 5000 g for 10 min and the clear supernatant was completed upto known volume with distilled water.

Protein determination was carried out according to the modified Lowry method adopted by Hartree (1972). One ml of the clear protein extract was mixed with 0.9 ml of alkaline sodium carbonate solution and heated in a water-bath at 50°C for 10 min. After cooling, 0.1 ml copper sulphate-potassium sodium tartrate solution was added to the mixture and allowed to stand for 10 min at room temperature, followed by addition of 3 ml of 10% Folin-phenol reagent with immediate mixing. After 30 min, the absorbance of the blue colour was recorded at 750 nm against water reagent blank. The concentration of protein was determined using bovine serum albumin standard curve, then expressed as mg g^{-1} dry weight.

Statistical analysis:

Data analysed using the computer program SPSS (version 12). All the data were subjected to one-way analysis of variance (ANOVA) following a randomized complete block design. The treatment means were compared using Duncan's Multiple Range Test at P = 0.05, where needed, data were transformed by log (x+1) before statistical analysis.

RESULTS:

Effect of OPW aqueous extract on germination of corn seed grains:

As shown in figure 1, the lower concentrations (1.0 and 3.0%, w/v) of OPW aqueous extract did not significantly affect percent of germination of corn grains. On the other hand, the higher concentrations (6.0, 9.0% w/v) were inhibitory and the most suppressive concentration was the 9.0% that caused about 23% inhibition in grminability of corn grains.

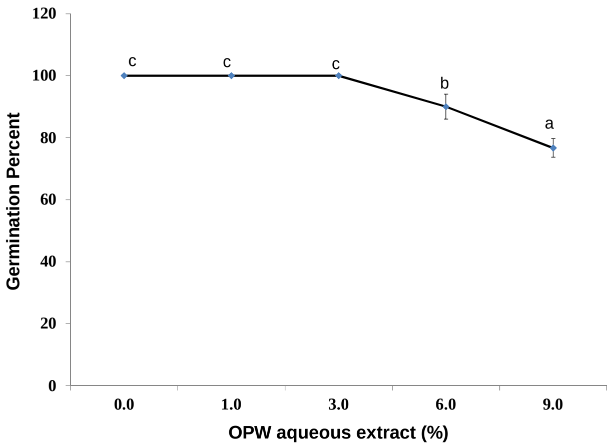

Fig. 1. Effect of Olive processing waste aqueous extract on germination of maize grains. Different letters above the bars indicate significant difference at the 0.05 level by Duncan's test.

Effect of OPW aqueous extract on radicle growth:

The aqueous extract of OPW at concentration of 3.0% significantly increased the length of corn radicle, while the highest concentration (9.0%) caused about 14% reduction in radicle elongation (Table 1). Concerning the biomass production, the higher concentrations (6.0 and 9.0%) significantly decreased fresh and dry weights of corn radicle, while the lower concentrations were stimulatory. The most promotive concentration was 3.0% that caused about 36 and 24% increase in the fresh and dry weights, respectively.

Table 1. Effect of Olive processing waste aqueous extract on radicle growth. All values are mean ± standard error of three independent replicates.

Extract Concentration (% w/v)	Length (cm)	Fresh wt (g)	Dry wt (mg)
0.00	8.96 ± 0.88^{ab}	0.30 ± 0.02^{ab}	309 ± 26^{ab}
1.00	9.99 ± 0.70^{ab}	0.34 ± 0.01^{ab}	337 ± 33^{ab}
3.00	11.04 ± 1.02^{c}	0.41 ± 0.04^{b}	384 ± 35^{b}
6.00	8.67 ± 0.82^{ab}	0.29 ± 0.04^{a}	290 ± 24^{a}
9.00	7.69 ± 0.61^{a}	0.25 ± 0.03^{a}	267 ± 27^{a}

Data in a column followed by different letters are significantly different at the 0.05 level by Duncan's test.

Effect of OPW aqueous extract on plumule growth:

Data presented in table 2 indicate that the 3.0% OPW extract significantly increased the plumule length, while the lower or higher concentrations did not exert significant effect on plumule length. Moreover, Increasing the OPW extract concentration up to 3.0% significantly improved the biomass of corn plumule thereafter; the fresh and dry weights were significantly reduced.

Table 2. Effect of Olive processing waste aqueous extract on plumule growth. All values are mean ± standard error of three independent replicates.

Extract Concentration (%, w/v)	Length (cm)	Fresh wt (g)	Dry wt (mg)
0.00	5.50 ± 0.72^{a}	0.19 ± 0.01^{b}	156 ± 33^{ab}
1.00	6.00 ± 0.25^{a}	0.21 ± 0.01^{bc}	173 ± 67^{ab}
3.00	6.55 ± 0.32^{b}	0.25 ± 0.02^{c}	191 ± 17^{b}
6.00	4.80 ± 0.15^{a}	0.17 ± 0.21^{a}	142 ± 10^{a}
9.00	4.15 ± 0.26^{a}	0.15 ± 0.01^{a}	137 ± 23^{a}

Data in a column followed by different letters are significantly different at the 0.05 level by Duncan's test.

Effect of OPW aqueous extract on soluble sugars content:

Accumulation of soluble sugars in tissues of both radicle and plumule was significantly improved with 3.0% of OPW extract that caused about 19 and 17% increase in the level of soluble sugars in radicle and plumule tissues, respectively (Fig. 2). On the other hand, the higher concentrations (6.0, 9.0%) caused significant lowering in the soluble sugars content.

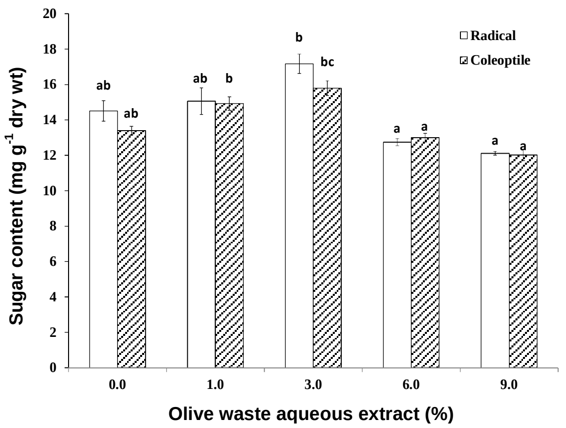

Fig. 2. Effect of Olive processing waste aqueous extract on soluble reducing sugars content in maize seedlings. Different letters above the bars indicate significant difference at the 0.05 level by Duncan's test.

Effect of OPW aqueous extract on soluble proteins content:

It is obvious from data presented in figure 3 that the lower concentrations (up to 3.0%) of OPW extract significantly increased the soluble protein content of radicle tissues, while the higher concentrations were inhibitory. Regarding the plumule tissues, all the tested concentrations had no obvious impact on the level of soluble proteins except for 9.0% that caused a significant reduction in the level of soluble proteins.

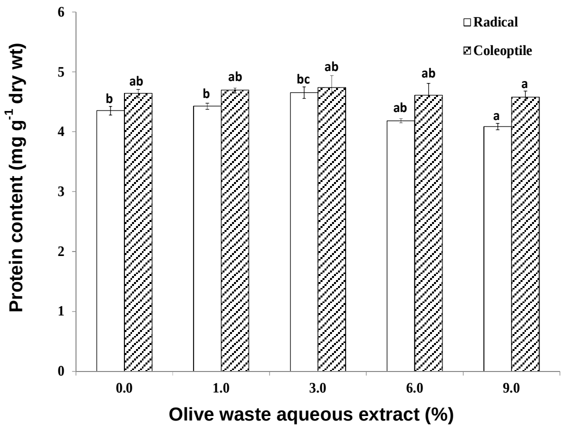

Fig. 3. Effect of Olive processing waste aqueous extract on soluble proteins content in maize seedlings. Different letters above the bars indicate significant difference at the 0.05 level by Duncan's test.

DISCUSSION:

The analysis of allelochemicals in OPW revealed that they are mainly phenolic compounds (Capasso *et al.,* 1992; Barakat *et al.,* 2010; García-Sánchez *et al.,* 2012). Phenolics are well known to interfere with several physiological processes associated with seed germination as well as plant growth and development (Inderjit and Duke, 2003; Weir *et al.,* 2004; Lara-Núñez *et al.,* 2009).

The present results revealed that the lower concentrations (up to 3.0%, w/v) of OPW aqueous extract have no effect on germination of corn grains. In accordance with our results, Hanifi and Hadrami (2008) reported a relative tolerance of maize germination to olive mill wastewaters (OMW). While, Asfi *et al.* (2012) demonstrated that OMW caused an evident concentration-dependent inhibition in germinability of spinach.

We demonstrated that concentrations of OPW aqueous extract up to 3.0% promoted the plumule and radical elongation as well as biomass production, but the higher concentrations had adverse effect on the measured growth parameters. The effect of OPW extract is more pronounced in radicle and plumule biomass than elongation. In this connection, Hanifi and Hadrami (2008) reported that OMW at concentration of 12.5% (v/v) inhibited the plumule and radicle elongation as well as the biomass of maize seedlings. In addition, Asfi *et al.* (2012) reported that spinach fresh weight as well as shoot and root length were reduced in response to OMW, where root was the most affected organ. He also recorded that fresh weight, was more affected than plant height. Moreover, Sampedro *et al.* (2005) reported that incorporation of olive-mill dry residue (DOR), at rate of 60 g kg^{-1} soil, reduced the root and shoot dry weights of tomato.

Several studies demonstrated the influence of allelochemicals on carbohydrate and protein metabolism (Baziramakenga *et al.,* 1997; Zhou and Yu, 2006). Our results revealed that OPW aqueous extract affected the soluble sugar and protein levels of maize seedlings and the effect was concentration dependent. In this connection, Mechri *et al.* (2011) reported that agronomic application of olive mill wastewater (OMW) significantly increased the soluble sugars in leaves of olive trees. In addition, El-Darier (2002) reported that treatment of maize seedlings with water extract of *Eucalyptus rostrata* leaf-litter leads to accumulation of sugars. Moreover, leaf leachate of *Acacia nilotica* significantly increased the soluble protein level in corn seedlings (El-Khawas and Shehata, 2005). It can be concluded that allelochemicals extracted from OPW can affect germination and seedling growth of *Zea mays* where the effect is concentration dependent.

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تقييم الجهد الاليلوباثى لمخلفات معاصر الزيتون على نبات الذرة الشامية

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اهتم البحث بدراسة التأثير الاليلوباثى للمستخلص المائى للمخلفات الصلبة الناتجه من معاصر الزيتون على عملية الانبات ونمو بادرات الذرة الشامية عند اربع تركيزات مختلفه (1 و 3 و 6 و 9 % وزن /حجم). أوضحت النتائج عدم وجود تأثير معنوى للمستخلص على عملية الانبات. كما أوضحت النتائج أن التركيزات المنخفضة (1 و 3 %) من المستخلص أدت إلى زياده ملحوظة فى طول الجذير والريشه وكذلك وزنهما الرطب والجاف بينما أدت التركيزات الأعلى (6 و9 %) إلى تثبيط عوامل النمو السابق ذكرها. وبالنظر إلى تأثير التركيزات المختلفة من المستخلص على البروتينات والسكريات الذائبه وجد أن زيادة تركيز المستخلص حتى 3 % كان له تأثير محفز بينما التركيزات الأعلى كانت مثبطه. وبذلك يمكن أن نستنتج أن تأثير المستخلص المائى لمخلفات الزيتون على نبات الذرة الشامية من الممكن أن يكون سلبياً أو إيجابياً اعتمادا على التركيز المستخدم.

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