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Impact of olive pomace wastes and fungicide treatment on indigenous arbuscular mycorrhizal fungi associated with chickpea (*Cicer arietinum* L.) under field conditions

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Abstract

The effect of soil amendment with olive pomace wastes (OPW) on population and root infection of indigenous arbuscular mycorrhizal (AM) fungi associated with chickpea has been studied under field conditions. Yield components, phosphorus (P) percentage, AM spore density and root length colonization of AM fungi were recorded. We found that OPW increased biomass, seed yield, and shoot phosphorus concentration of chickpea plants. Our results also indicated that AM fungal spore population and colonization levels were substantially enhanced by the application of OPW. Fungicide applications significantly decreased both spore population and colonization length of AM fungi. We concluded that organic matter amendments potentially increased AM fungal association and hence might increase growth and yield of chickpea plants in the field.

Key words: Microorganisms; Organic matter; Olive pomace; Soil amendment; Spore populations.

Abbreviations: AM fungi**-** arbuscular mycorrhizal fungi; OPW- olive pomace wastes.

Introduction

Most legumes are symbiotically associated with Arbuscular mycorrhizal (AM) fungi and rhizobia. The beneficial effect of these symbiotic relationships on plant growth is well documented. However, in cultivated soils, the populations as well as the infectivity of AM fungi may be affected by cultural practices such as soil amendment with organic matter. It was reported that AM fungi usually increase as a result of soil amendment with organic matter which leads often to the benefit of plant from this relationship (Ryan et al., 1994; Douds et al., 1997). This result might be explained by the effect of organic matter on soil structure, water retention capacity, microbial activity, or chemicals released from organic matter (Ryan et al., 1994).

Olive pomace wastes (OPW) are produced in large quantities in many Mediterranean countries during November-February (Mechri et al., 2008). Once the olives have been ground into a paste, the paste is separated into three components; vegetable water, oil, and the husk. The husk (pomace) contains the skins, pulp and pit fragments. Olive pomace could be applied to the soil, either intentionally as soil amendment or unintentionally as a disposal method.
Al-Sakit and Al-Momani (1989) found that OPW treatment improved the growth of olive seedlings when applied as a fertilizer. However, it has been found that the yields of wheat and barley were decreased as a response to soil amendment with OPW and this was attributed to the presence of phytotoxic

Table 1. Plant height (cm) and number of leaves and branches per plant of chickpea as affected by different levels of olive pomace wastes (OPW) and soil treatments.

Soil treatments	OPW %	Stem height	Branches No.	Leaves No.
Fungicide	0%	41.7	3.0	102.7
	10%	45.3	3.3	121.7
	20%	54.7	3.7	151.3
	30%	46.0	3.7	175.0
Control	0%	42.7	3.7	136.3
	10%	62.7	3.7	157.7
	20%	54.0	3.0	198.7
	30%	57.3	4.3	207.7
Significance (P value)				
Soil treatment		0.0383	0.4754	<.0001
OPW		<.0001	0.3036	<.0001
Soil treatment x OPW		0.0003	0.2525	<.0001

compounds, Phenols (Aqeel, 2001). On the other hand, differential effects of OPW on different crops were also recorded (Aqeel, 2001; Al-Hassan, 2000). Previous investigations on the effects of OPW on plant growth and their association with AM fungi were done mostly on pot experiments with selected species and strains of AM fungi (Al-Sakit and Al-Momani, 1989; Al-Hassan, 2000). Therefore, investigating the influence of OPW amendments on plant growth and AM fungi under field conditions is still lacking.

The objective of the present experiment was to investigate the effect of soil amendment with OPW on indigenous AM fungi in terms of spore population and root infection. Another objective was to study the effect of OPW on the growth of chickpea (*Cicer arietinum* L.) under filed conditions.

Materials and methods

Study area

Field plots were located at the research station of Jordan University of Science and Technology located in the northern part of Jordan [32^o 30' N latitude, 35^o 59' E longitude, and 520 m altitude). The soil was sampled for analysis of physico-chemical properties and AM fungal population shortly prior to the initiation of the experiment. The soil was a silty clay loam with a pH 8.4 (1: 1; soil:water). Soil nutrient determinations included 0.24% total N, 9.9 ppm available P and 0.93% organic matter. The total indigenous AM fungal spore density prior to the start of the experiment was 223 spores 10g^{-1} soil.

Land Preparation and Application of OPW

Soil was prepared by plowing twice using disk plow followed by disk harrowing to mix and level soil surface. Experimental plots (2 x 1 m^2) consisted of five rows of 2 m long, 20 cm apart and 50 cm space between the plots. Soil was mixed with fresh OPW at four different levels: 0%, 10%, 20%, and 30%, on volume basis. OPW was surface applied and incorporated to 15 cm into the soil. Those levels were attained by adding 0 m^3, 0.015 m^3, 0.030 m^3, and 0.045 m^3 of OPW per 1m^2 of soil, respectively.

Soil Treatments and planting

After OPW incorporation, experimental plots were either treated with a systematic fungicide (Metalaxyl-50 g Kg^{-1}) or left untreated as control. Dry seeds chickpea were planted by hand at rate of 30 plants m^{-2}.

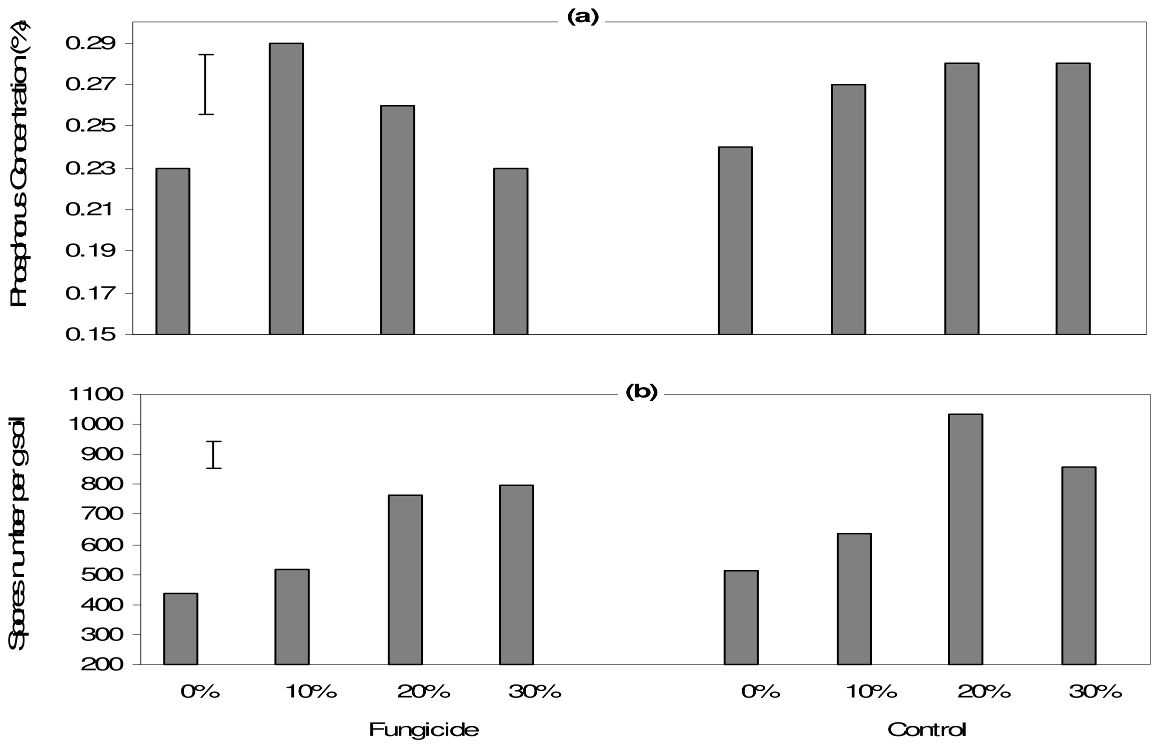

Fig 1. Effect of soil amendments with different levels of olive pomace wastes (OPW) and soil treatments on: (a) shoot phosphorus concentration (F=4.00, P= 0.0074 and (b) spore density (F=7.97, P=0.0001). Vertical bars represent LSD values at $P \leq 0.05$.

Harvest and measurements

Yield per unit area were obtained by harvesting the three central rows (150 cm x 60 cm = 0.9 m^2). Biomass was determined by weighing the total dry matter of the above-ground parts while seed yield was measured by weighting the seeds. In addition, five plants from each plot were dug out. Aboveground parts were used to measure plant height, number of leaves and number of branches; the average values were used for statistical analysis. Root system and surrounding soil were stored in cool chamber (4^oC) for mycorrhization assessments.

Phosphorus concentrations were determined for the harvested plants. Dried plant materials (only shoots) were finely ground using a scientific mill and analyzed for phosphorus concentrations using the method of Olsen (1954).

AM colonization and spore density

Roots were cleared with 10% KOH followed by acidification in HCl, and stained with 0.05 % Trypan Blue using the procedure of Phillips and Hayman (1970). The incidence of mycorrhizal colonization was estimated as the percentage of the length of each segment which is colonized according to the method of Bierman and Linderman (1981). Spores were extracted from 10g soil samples using the floatation-adhesive technique (Sutton and Barron, 1972). Number of spores was counted under a compound microscope.

Experimental design and Statistical Analysis

Field plots were laid out in split plot design. Soil treatments (fungicide and control) were occupied the main plots and OPW levels occupied the subplots. This resulted in twelve treatment combinations; each

Table 2. The main effects of soil treatments and olive pomace wastes (OPW) amendments on biomass, seed yield, and harvest index of chickpea.

Treatments	Biomass yield (kg ha^{-1})	Seed yield (kg ha^{-1})	Harvest index (%)
Soil Treatments			
Fungicide	5162	2260	43.9
Control	5707	2295	40.8
OPW %			
0%	4800	1988	41.4
10%	4858	2065	42.8
20%	5614	2349	42.0
30%	5838	2379	41.0
Significance (P value)			
Soil treatment	0.0135	0.0118	0.3408
OPW	<.0001	<.0001	0.6584
Soil treatment x OPW	0.0998	0.0907	0.0904

treatment was replicated three times. Considering the split-plot structure of the trial, data were analyzed with mixed model using the procedure MIXED in SAS (SAS Institute, 1996). The following model was fitted (same symbols as above): $C_{ijm} = \mu + F_i + S_j + (FS)_{ij} + R_m + (FR)_{im} + e_{ijm}$; where μ is general mean; F_i is soil treatments (i = 1 or 2); S_j is OPW levels (j = 1…4); $(FS)_{ij}$ is the interaction between both treatments; R_m is block effect (m= 1…3); (FR) is the interaction between soil treatment and block; and e is the residual term. Data presented are LSMEANS in the analysis.

Results

Plant characters

Chickpea phenology was significantly affected by OPW manuring and soil treatments (Table 1). Application of OPW at all rates significantly increased plant height and leaves number compared to 0% OPW (Table 1). However, application of OPW at the rate of 30% produced lower plant height compared to no OPW application for fungicide treatments, but not for the control treatment. Although higher number of branches was recorded with OPW application, treatment effects were not significantly different.

Yield and Phosphorus concentration

For biomass, seed yield, and harvest index, statistical analysis showed no significant interaction between soil treatments and OPW amendments; hence the results were shown separately for the two factors. Generally, yields were enhanced by OPW amendments (Table 2). For plants grown in soil amended with 30% OWP there were 20% and 22% increase in biomass and seed yield, respectively, than in 0% OWP. Soil fungicide treatment significantly decreased biomass as well as seed yield compared to the control treatment. On the other hand no significant differences were detected for harvest index in response to the applied treatments.

Chickpea plants grown in soils amended with OPW had slightly higher phosphorus concentrations compared to 0% OPW level (Fig. 1a). However, lower concentrations were associated with fungicide treatment than with control treatment.

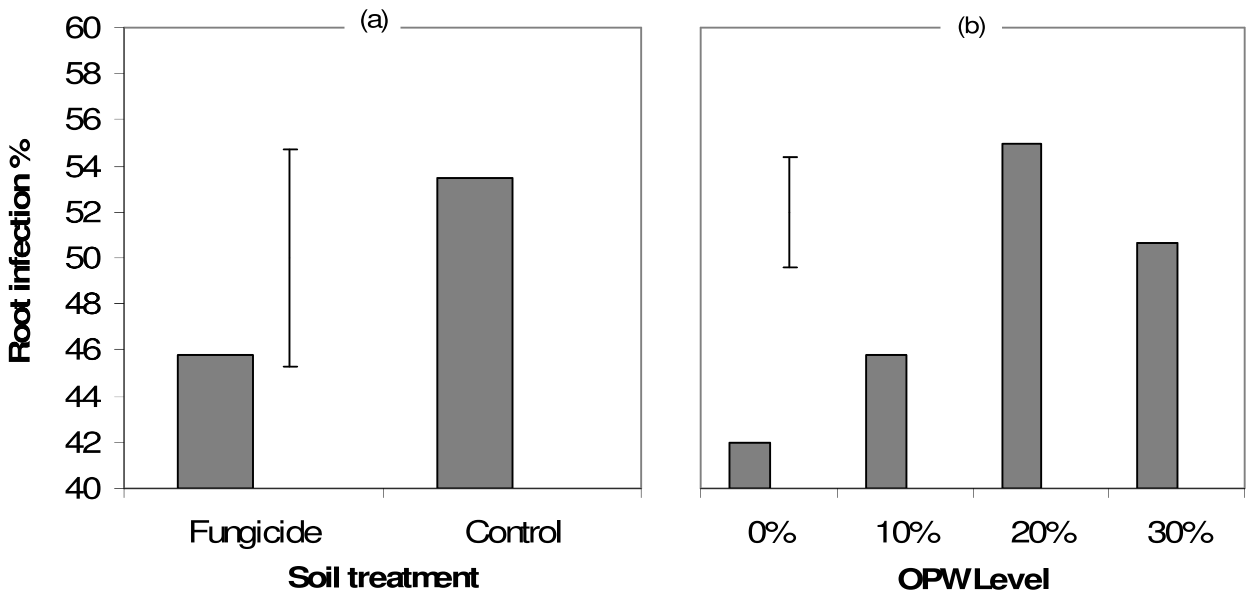

Fig 2. Length percentages of chickpea roots infected with arbuscular mycorrhizal (AM) fungi as affected by: (a) soil treatments ((F=3.65, P= 0.1335) and (b) olive pomace wastes (OPW) amendments at different levels (F=12.37, P= 0.0001). Vertical bars represent LSD values at P≤0.05.

AM colonization and spore populations

There were significant differences in AM infection percentages and spores density as a response to soil treatments and OPW amendments (Fig. 1 and 2). Spore density was positively correlated with OPW amendments, though this effect is less noticeable for the fungicide treatment than for the control treatment. On the other hand, significantly higher spores density was observed for the control treatment compared to the fungicide treatment.

Soil fungicide treatment slightly decreased root infection percentages with AM fungi compared to control treatments, although the differences between soil treatments were not significantly different. Regarding the main effects of OPW amendments, percent infection was significantly higher in soil amended with OPW at the rates of 10% and 20% compared to 0% level, and significant effect was not detected for the higher rate of OPW (30%) compared to 10% and 20% levels.

Discussion

Our results confirm previous work on plant growth response to organic amendments (Al-Abudlsalam and Garawani, 1998; Mandimba, 1998). It is generally recognized that the benefits of organic matter are not only due to supply of the nutrient elements, but also to the improvement of soil physical characters (McCoy, 1998). Our results showed that an enhanced mycorrhizal infection may account for enhanced plant growth through improved nutrient uptake (Muthukumar and Udaiyan, 2000).

Organic manuring tends to maintain soil structure, is less disruptive to the soil environment, encourages populations of beneficial soil microbes, facilitates crop rooting, improves water retention capacity and results in a more even distribution of nutrients in the soil profile (Arden-Clarke and Hodges, 1988). Groaker and Sreenivasa (1994) also reported an enhancement of growth, yield and root colonization in wheat as a result of organic amendment.

Soil amendment with OPW at the rate of 30% had negative effects on AM infection as well as spore density under control treatment. Two mechanisms could explain that response. First, OPW amendments might cause a reduction in plant growth, as a result of phenolic compounds, and hence might reduce soluble carbohydrates in the root system. Muthukumar and Udaiyan (2000) indicated that high carbohydrate concentrations in roots coincide with increased colonization levels. Second, the chemical composition and/or the decomposition products of the OPW amendment may have discourage mycorrhizal development in chickpea plants. However, this finding could be also attributed to the enhancement of soil microorganisms as a result of high percentage of organic matter, these microorganisms may compete or even parasitize mycorrhizal fungi (Sreenivasa and Bagyaraj, 1989).

The observed reduction in chickpea growth in response to high rates of OPW amendment was consistent with previous findings, indicated that fresh OPW contains high levels of phenolic compounds which might affect plant growth adversely (Aqeel, 2001; Al-Hassan, 2000). Pages et al. (1985) also indicated that OPW contains high levels of phytotoxic compounds, which may inhibit seed germination or reduce plant growth.

The higher shoot P concentrations found in chickpea grown on OPW amended soil is probably due to more efficient uptake of available P from the soil and manure and possibly to mineralization of organic phosphorus (Jayachandran et al., 1992) or due to the advantageous effect of the higher AM colonization (Tarafdar and Marschner, 1994; Verma and Arya, 1998).

AM soil infectivity fluctuates in relation to agricultural practices (Plenchette, 1989), amongst which soil sterilization and fungicide application are often considered to be the most detrimental, leading to a decrease in indigenous fungal populations (Trappe et al., 1984). Our results indicated that soil treatment with fungicide decreased AM fungi population and infection percentages, although the differences among infection percentages were not significantly different. Consequently, fungicide treatment might have weakened AM fungus propagules in treated soil and reduced their survival (Schreiner et al., 2001). However, soil fungicide treatment in our experiment did not completely eliminate AM fungi propagules. An explanation for this result could be that soil fungicide treatments under field conditions may adversely affect AM propagules in the surface soil (Menge, 1982), but not the deep soil layer.

We concluded that AM fungal spore population, colonization levels and the mycorrhizal response can be substantially altered by soil treatments and applications of different levels of OPW. Our results clearly showed that soil amendments with OPW significantly enhance number of AM spores as well as colonization percentages. OPW might have an indirect effect on AM fungi, through the effect of OPW on plant growth, or directly, through the effect of chemical compounds present in OPW on the growth of AM fungi itself. Future experiments should focus on the role of the chemical compounds contained in OPW on spore germination as well as the growth of AM hyphae to test if those compounds have direct effects on population and growth of AM fungi.

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