Factorial analysis of the effect of sugarbeet vinasses on agroecological indices in horticultural field

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Abstract: The impact on soil of applying sugarbeet vinasses (V) was analyzed through a field experiment in horticulture greenhouse, arranged in a 2^3 factorial design. Two levels of three independent variables —application of V, use of polyethylene cover (PC) on the soil, and soil depth (D)— on various dependent variables were studied. Vinasses favored crop yield and reduced the number of Meloidogyne incognita juveniles in soil. The concentrations of N, P and K increased with the interaction VxD, with PC also increasing N concentration. The amounts of humic acids and humin decreased with D; fulvic acid concentration increased with V, but decreased with the interaction VxPC. Soil physical factors were improved mainly with D and V. Aromaticity of humic acid-like fractions increased with V. In general, V showed significant effects mainly on the topsoil, suggesting low leaching risks. The results indicate that the levels of the independent factors improving a group of variables were not the same that those contributing to another group. Therefore, their best combination should be determined for each scenario to achieve optimum agroecological performance.

Keywords: Aggregate Stability, Biodisinfestation, Humic Acid, Meloidogyne, Soil Organic Matter, Sugarbeet Vinasses

1 Introduction

The application to the soil of agricultural and industrial by-products which could exert a controlling effect on soil-borne pests is considered to produce the additional improvement of soil physical properties such as water retention and structural stability, and consequently, to lead to a greater resistance to degradation. This effect is particularly relevant in Mediterranean environments, with frequent and unpredictable rainfall events. Apart from this, the effect of incompletely composted —or immature organic matter (OM)— in the soil could contribute to the formation of humic substances. This would be the case with the so-called biofumigation or biodisinfestation practices which —combined or not with solarization under plastic cover— is being currently used for the management of plant-parasitic nematodes populations [1-4]. However, the success of the biodisinfestation treatment depends on a series of still not well-known factors, and the residual effects of such practices on, for instance, the productivity of the following crops or on soil physical and chemical properties, have not been sufficiently studied. In fact, several researchers have stated that the effects of biodisinfestation often rely on complex interactions between factors and processes that influence both the biophysical and chemical transformation of the amendment into the soil and its residual effect in the long term [5-8]. This work analyzes the factors affecting the effect on horticultural soil of applying liquid products (vinasses, V) from sugarbeet industry on a series of physical, chemical and biological properties of the soil.

2 Materials and Methods

2.1 Experimental design

A field experiment based on a 2^3 factorial design [9-10] was performed, studying the main effects and interactions between two levels of three independent variables with a presumable bearing on the performance of biodisinfestation practices: i) application or not of V, ii) use (i.e., biosolarization) or not of polyethylene cover (PC) on the soil, and iii) soil sampling depth (D). The dependent variables examined were grouped into three items: i) suppressive effect on plant-parasitic nematodes’ populations (Meloidogyne incognita), ii) soil physical properties, and iii) total amount and composition of soil OM. Also, iv) the yield of a chard crop after application of the treatments was recorded.

The main effects of the factors and their interactions [11] were calculated with the authors’ software. The effects and interactions between factors were considered to be significant when three different criteria
coincided: a) the comparison with the standard deviations computed from replicate runs (as in the case of the plant and soil experiments), b) the assumption of non-significant three-factor interactions and, c) the examination of the distribution of the effects and interactions on normal probability axes.[9]

2.2 Greenhouse field experiment
The field experiment was carried out in a greenhouse on horticultural soil at the Center for Agricultural Experimentation of Marchamalo (Guadalajara, Spain). The soil was a Typic Rhodoxeralf, a deep soil with clayey texture, and low OM content, characteristic of Mediterranean climates with xeric rainfall regimes. The treatments performed consisted of V addition to soil—or not—at 1.5 kg · m\(^{-2}\) prior to a chard (Beta vulgaris var. cicla) crop, and immediate placement—or not—of a polyethylene cover.

Along the chard crop cycle (about 90 days), data of productivity (kg · m\(^{-2}\)) in each plot were registered. At the end of the cycle, composite soil samples of each plot were collected from five at-random subsampling points (total soil weight ca. 2 kg), and at two depths (0–20, 20–40 cm). Before analysis, soil samples were air-dried, gently crushed and homogenized to 2 mm by sieving.

2.3 Nematological analyses
Nematodes were extracted from 100 cm\(^3\) of each soil sample using the sugar centrifugation method[12] and the number of alive M. incognita infective juveniles (J2) was counted under the stereomicroscope at 40× magnification.

2.4 Soil chemical analysis
Soil pH and electric conductivity were determined in a 1:2.5 (w:v) soil:water suspension; soil pH was measured using a Radiometer PHM61 pH-meter provided with a combined glass and calomel electrode for semisolids, whereas electric conductivity was measured with a conductimeter Crison 2000, the results being expressed in µS · cm\(^{-1}\). The soil C was determined by wet oxidation with K\(_2\)Cr\(_2\)O\(_7\) in acid medium followed by redox titration[14] and the total N after micro-Kjeldahl digestion. The available P was measured by the Bray and Kurtz method[15], available K, Ca and Mg were extracted with 1 M NH\(_4\)OAc (pH= 7) and the available micronutrients (Fe, Mn, Zn and Cu) with diethylenetriaminepentaacetic acid[16]. Sodium and K were determined by flame ionization spectroscopy, Zn, Mn and Cu by inductively-coupled plasma spectroscopy, and Ca and Mg by atomic absorption spectroscopy.

2.5 Soil physical properties
For the determination of the aggregate stability the Van Bavel’s method was used[17]. This is a single, easily comparable index of aggregate stability in dry soil—the so-called mean weigh diameter (MWD)—, which consists of treating a dry soil sample with continuous vibration in order to disrupt the less stable aggregates, and subsequently quantifying the particles of each different size obtained by sieving. In this work MWD was determined subjecting a 20 g soil sample (air-dried and homogenized to 2 mm) to a series of stacked sieves of 1, 0.5, 0.1, 0.08 and 0.05 mm, to which vibration was applied for 3 minutes. The mechanical effects of vibration broke the aggregates and the resulting particles passing through the sieves were recovered and weighed and the MWD was calculated using the equation

\[
\text{MWD} = \sum Wi \times Si
\]

Where Wi is the dry weight (as percentage referred to total weight) for each sieve size (Si) in millimeters. The soil water retention (WR) was determined as the difference in the water content between the water holding capacity at atmospheric pressure and at 0.1 bar negative pressure[18]. Water permeability was determined using Darcy’s law[19], which quantitatively describes saturated soils ability to transmit water when subjected to a hydraulic gradient and is expressed as saturated hydraulic conductivity (K).

2.6 Organic matter fractions
The quantitative determination of the particulate and extractable soil OM fractions was based on classical procedures[20-21]: the separation of the low density, particulate, non humic fraction (free organic matter, FOM) was carried out by flotation using 20 g soil samples suspended in 2M H\(_3\)PO\(_4\) (density 1.25 g · cm\(^{-3}\)). The soil suspension was stirred for 1 min, centrifuged at 1,935 g for 5 min, and the light floating fraction obtained was stored for analysis of its total C. The soil heavy fraction remaining after centrifugation was successively extracted by shaking with 0.1M Na\(_2\)P\(_2\)O\(_7\) followed by 0.1M NaOH (horizontal motion mechanical shaking for 3 h) and centrifuged. This treatment was repeated up to 5 times. The resulting
brow extract obtained corresponded to the total humic extract. Two aliquots were taken from this extract and one was precipitated with H$_2$SO$_4$ (1:1 by vol.) to determine the acid-insoluble fraction (humic acid, HA) and, by difference with the total humic extract, the amount of the acid-soluble fraction (fulvic acid, FA) was calculated. The soil residue after the alkaline extraction was washed with distilled water and desiccated at 40 °C. The C concentration in this residue was total humin.

2.7 Preparative isolation, de-ashing and spectroscopic characterization of the HA

The preparative isolation of the HA fraction was carried out by precipitating the total humic extract at pH=2 with 6M HCl, then redissolving the precipitate in 0.5M NaOH, followed by centrifugation at 43,500 g. The insoluble residue, consisting of mineral and organic particulate fractions, was discarded and the dark brown supernatant solution (sodium humate) was reprecipitated with 6M HCl. The resulting gel was subjected to dialysis in cellophane bags (Visking® dialysis tubing, molecular weight cut-off 12,000–14,000 Da; pore diameter ca. 25 Å) immersed into distilled water for one week, with periodical replacement of the water. After this period, the HA was desiccated at 35 °C.

The visible spectra were obtained from this HA solutions using a Hewlett Packard 8452A spectrophotometer. The visible spectroscopy of the HA is usually considered to reveal information about their aromaticity and polydispersity.[22f23] To determine optical density, HA solutions were prepared at a concentration of 66.6 mg L$^{-1}$ C (50% diluted as regards the concentration proposed by Kononova[24]) in 0.02 M NaOH. To determine the E4/E6 ratio, negatively related to the molecular size, the optical density was measured at 465 (E4) and 665 (E6) nm.

3 Results and Discussion

3.1 Crop yields and nematological analysis

The raw data (Table 1) indicated that V had a favourable effect on agronomical properties, increasing crop production and decreasing plant-parasitic nematodes populations in soil. The use of the PC showed detrimental effect on chard crop yield, but did not affect nematode populations.

<table>
<thead>
<tr>
<th>D</th>
<th>V</th>
<th>PC</th>
<th>Crop yield$^b$</th>
<th>No. J2$^c$</th>
<th>OM$^d$</th>
<th>C$^e$</th>
<th>N$^e$</th>
<th>pH</th>
<th>EC$^f$</th>
<th>P$_2$O$_5$ $^g$</th>
<th>K$^g$</th>
<th>Ca$^g$</th>
<th>Mg$^g$</th>
<th>Na$^g$</th>
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<tr>
<td>SD$^h$</td>
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<td>54</td>
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<td>115</td>
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<td>3.3</td>
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<td>8.3</td>
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Notes: $^a$D: depth (- 0–20 cm; + 20–40 cm), V: sugarbeet vinasses (- 0 kg · m$^{-2}$; + 1.5 kg · m$^{-2}$), PC: polyethylene cover (- not applied; + applied); Crop yield: harvested weight (kg · m$^{-2}$) of a chard crop planted after soil treatments; $^b$No. J2: Number of M. incognita infective juveniles; $^c$OM: organic matter; $^d$Expressed as g · kg$^{-1}$; $^e$EC: electric conductivity, expressed as µS · cm$^{-1}$; $^f$Expressed as mg · 100 g$^{-1}$; $^g$SD: standard deviation.
The results of the factorial analysis (Fig. 1) confirmed that V induced a suppressive effect on *M. incognita*, whereas D had a slighter and opposed effect, with higher number of J2 in the underlying soil layer compared to that of the topsoil. This fact suggests weak in-depth diffusion of V from the horizon where it is applied. Also, it does agree with previous findings on the mobility of the nematodes in the soil profile [25-26], which in this way escape from the suppressive effect of the treatment.

**Fig 1** Coefficients of the factorial functions obtained for the number of *M. incognita* juveniles (J2) on greenhouse horticultural soil subjected to two levels of three independent factors and their interactions. D: soil depth; V: sugarbeet vinasses application; PC: use of polyethylene cover.

### 3.2 Soil chemical analysis

Concerning chemical fertility, the factorial analysis showed that the interaction V×D was associated to significant increase in the concentration of macronutrients N, P and K, micronutrients Mg, Ca and Na, and EC (Table 1, Fig. 2).

**Fig 2** Coefficients of the factorial functions obtained for the chemical status on greenhouse horticultural soil subjected to two levels of three independent factors and their interactions. D: soil depth; V: sugarbeet vinasses application; PC: use of polyethylene cover.
3.3 Soil physical properties
As it is shown in Table 2 and Fig. 3, all the dependent factors related to soil physical quality were upgraded with V and D. In the case of the ratio MWD/C, the effect of D was particularly marked, as expected from the lower OM content in the underlying soil layer respect to the topsoil. Also, both MWD and K were improved, although to a lesser extent, by PC and the interaction V×PC. The interaction V×D also favoured soil K, whereas MWD was negatively affected by the interaction D×PC. These findings agree with previous considerations on the positive effect of organic amendments on soil physical quality, and would support the use of V as a valuable material to enhance soil physical status.

![Fig 3](image)

**Fig 3** Coefficients of the factorial functions obtained for soil physical factors on greenhouse horticultural soil subjected to two levels of three independent factors and their interactions. D: soil depth; V: sugarbeet vinasses application; PC: use of polyethylene cover.

3.4 Organic matter fractions and spectroscopic characterization of the HA
The separation of the OM fractions indicated the concentration of organic carbon in the uppermost soil layer for all treatments (Table 2, Fig. 4a). The distribution of the organic carbon between the fractions was also very similar, except in the case of the application of V, in which the proportion of HA, but mainly of FA, clearly increased, whereas the amount of humin was comparatively reduced. This fact suggests that although a labile nature of this water-soluble amendment is usually assumed, V probably represents a suitable material for the preferential formation of humic-like substances.

The results of the factorial analysis were in agreement with the raw data, showing a concentration of all organic fractions (FOM, HA, FA and humin) in the upper soil layer, i.e., a negative effect of D (Fig. 4b). Also, it was observed that V decreased FOM and increased FA. In the case of the HA, PC increased their concentrations but the interaction D×PC had a negative effect on it, whereas the amount of FA was mainly reduced by the interaction V×PC, and by PC.
### Table 2

<table>
<thead>
<tr>
<th>D</th>
<th>V</th>
<th>PC</th>
<th>Soil physical properties</th>
<th>Distribution of C (g·kg⁻¹) in soil organic matter fractions</th>
<th>Humic acids optical density</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>WR</td>
<td>K</td>
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<td>19.4</td>
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Notes: "D: depth (0–20 cm; + 20–40 cm), V: sugarbeet vinasses (0 kg·m⁻²; + 1.5 kg·m⁻²), PC: polyethylene cover (not applied; + applied); WR: water retention, determined as the difference in water content between the water holding capacity at atmospheric pressure and at 0.1 bar negative pressure, K: hydraulic conductivity, expressed in m·s⁻¹, MWD: mean weight diameter, MWD/C: ratio mean weight diameter to total organic C content; FOM: free organic matter, THE: total humic extract, HA: humic acids, FA: fulvic acids, HA/FA: ratio humic acids to fulvic acids; E4: optical density at 465 nm, E6: optical density at 665 nm, E4/E6: ratio E4 to E6; SD: standard deviation.

Concerning the HA quality, (Table 2, Fig. 5) the optical density, and mainly E4, was enhanced with V and in plots where the factors V×D and V×PC interacted. The E4/E6 ratio, which increases when the molecular size tends to decrease [22], decreased with D and the interaction V×PC, whereas PC and the interaction D×PC had an opposed effect on this index. This fact suggests the occurrence of condensation processes in soil OM, leading to more chemically-stable substances, mainly in the upper soil layer and in the case of the interaction V×PC. In general, the observed results of V on soil OM quality could be considered as a positive agrobiological feature, i.e. the increase in its aromaticity or structural condensation.
Fig 4 Organic matter fractions on greenhouse horticultural soil subjected to two levels of three independent factors. A. Organic matter fractions expressed as % of total soil organic. B. Coefficients of the factorial functions obtained for the organic matter fractions. CA: no vinasses application, no polyethylene cover, topsoil layer; CB: no vinasses application, no polyethylene cover, underlying layer; VA: application of vinasses, no polyethylene cover, topsoil layer; VB: application of vinasses, no polyethylene cover, underlying layer; PA: no vinasses application, use of polyethylene cover, topsoil layer; PB: no vinasses
Fig 5 Coefficients of the factorial functions obtained for the optical density of HA from greenhouse horticultural soil subjected to two levels of three independent factors and their interactions. E4: optical density at 465 nm, E6: optical density at 665 nm, E4/E6: ratio E4 to E6; D: soil depth; V: sugarbeet vinasses application; P: use of polyethylene cover.

4 Conclusion

The main effects and interactions between the studied factors indicate the possibility to optimize, through appropriate management of a series of independent variables, the effects exerted by the addition of V on important biological, chemical and physical soil properties. However, it must be highlighted that the levels of the factors affecting crop yields and the suppressing effect on soil-borne plant-parasitic nematodes populations and those levels contributing to the accumulation and quality of humic substances are not the same. This is also the case with the levels of the factors involved in the improvement soil physical properties. In this field experiment, V enhanced crop yields, suppressed M. incognita populations, improved soil physical status and lead to accumulation and condensation of humic substances, especially in the uppermost soil layer. This fact suggests the low mobility of this liquid amendment in soil and, as a consequence, a presumable low risk of leaching, although this point merits to be further studied. The positive effect of the interaction V×D was much more significant on soil available nutrients than the other factors studied, and in most cases it was associated to the OM content. The use of PC contributed only to increase the amount of some of the nutrients considered and to improve the physical properties, although to a lesser extent than D or V, and even was slightly detrimental for crop yield and MWD. Therefore, it could be concluded that, in our case study, the use of PC represents just an expense not an advantage. In general, the results suggest that, for each agroecological scenario, the best combination of factors should be determined in order to achieve the optimum performance at the different soil organizational levels.

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References


