Original Article

Germination, phytotoxicity, and nematicidal effect of wine vinasse, a by-product of the wine industry

Germinación, fitotoxicidad y efecto nematicida de la vinaza de vino, un subproducto de la industria vinícola

https://eqrcode.co/a/fciPy5

Iván Castro-Lizazo[1], Miguel Ángel Diez-Rojo[2], María Rosa González-López[3], José Antonio López-Pérez[2*]

[1]Facultad de Agronomía, Universidad Agraria de La Habana (UNAH), San José de las Lajas, Mayabeque, Cuba.
[2]Centro Investigación Apícola y Agroambiental. Instituto Regional de Investigaciones Agroalimentarias y Forestales, JCCM, Camino de San Martín s/n, Marchamalo, (IRIAF, JCCM), Marchamalo, 19180 Guadalajara, Spain.
[3]Museo Nacional de Ciencias Naturales (MNCN, CSIC), 28006 Madrid, Spain.

ABSTRACT: The objective of this study was to determine the effect of wine vinasse (WV), the final byproduct obtained from distillation of the fermentation wine, on plants and nematodes through germination, phytotoxicity and nematode survival tests. Before conducting the bioassays, fertility parameters of two WV samples from different years were analyzed and compared. Six WV solutions were applied to obtain the most effective ones with nematicidal properties without compromising plant growth. For this purpose, two very different plant-parasitic nematodes, *Meloidoyne incognita* and *Xiphinema index*, both widespread in vineyards were analyzed. The effects of these WV doses were tested on seed and plant growth in germination and phytotoxicity bioassays. The doses 1, 3 and 5 % were applied to soils naturally infested by root-knot nematodes for biodisinfestation in the laboratory. As a result, the number of *Meloidogyne* juveniles was significantly reduced, while other non-parasitic nematodes and enchytraeids were less affected. Marmande tomato plants grown in biodisinfested soil showed a reduction in galling index (0 at 3 & 5 % solutions). Our results show that WV contributes to reducing plant-parasitic nematode populations without phytotoxic side effects. The fertilizing effect of WV on Marmande tomato and white mustard plants indicates that it can be introduced into horticultural cropping systems. The valorization of WV as an organic resource for biodisinfestation of agricultural soils may help to solve accumulation problems and contribute to a circular economy by reducing waste. The characterization of both WV and agrarian soil will help to define the suitability of such actions prior to applying this organic amendment.

Key words: Agroindustrial by-products, biodisinfestation, plant-parasitic nematodes, root-knot nematodes.

RESUMEN: El objetivo de este estudio fue determinar el efecto de la vinaza de vino (VV), el subproducto final obtenido de la destilación del vino de fermentación, sobre plantas y nematodos mediante pruebas de germinación, fitotoxicidad y supervivencia de nematodos. Antes de realizar los bioensayos, se analizaron y compararon los parámetros de fertilidad de dos muestras de VV de diferentes años. Se aplicaron seis soluciones de VV con el fin de obtener las más eficaces con propiedades nematicidas sin comprometer el crecimiento de las plantas. Para ello, se analizaron dos nematodos fitoparásitos muy diferentes, *Meloidoyne incognita* y *Xiphinema index*, ambos muy extendidos en los viñedos. Se probaron los efectos de estas dosis de VV sobre el crecimiento de las semillas y las plantas en bioensayos de germinación y fitotoxicidad. Las dosis 1, 3 y 5 % se aplicaron a suelos infestados naturalmente por nematodos formadores de nódulos para su biodesinfestación en laboratorio. El resultado fue que el número de juveniles de *Meloidogyne* se redujo significativamente, mientras que otros nematodos no parásitos y los enquitréidos se vieron menos afectados. Las plantas de tomate Marmande, cultivadas en suelo biodesinfectado, mostraron una reducción del índice de nodulación (0 en soluciones al 3 y al 5 %). Estos resultados muestran que VV contribuye a reducir las poblaciones de nematodos parásitos de las plantas sin efectos fitotóxicos secundarios. El efecto fertilizante de VV en las plantas de tomate Marmande y mostaza blanca indica que puede ser introducido en los sistemas de cultivos hortícolas. La valorización del VV como recurso orgánico para la biodesinfestación de suelos agrícolas puede ayudar a resolver los problemas de acumulación y contribuir a una economía circular al reducir los residuos. La caracterización de ambos, VV y suelo agrario, ayudará a definir la idoneidad de tales acciones antes de aplicar esta enmienda orgánica.

Palabras clave: subproductos agroindustriales, biodesinfestación, nematodos fitoparásitos, nematodos formadores de nódulos.

INTRODUCTION

The largest extensions of vineyard worldwide are found in Central Spain, 443,347 ha of vineyards in Castille-La Mancha (1). These vineyards are associated with an important industry related to the production of wine and its derivatives, generating different by-products from the winery, grape pressing, fermentation and distillation processes. The wine vinasse (WV), the final by-product obtained from distillation of the fermentation wine, is generated in large quantities and can end up being an environmental problem. Some by-products have become a source of organic matter (OM) and their beneficial effects derived from their decomposition when applied to the soil are the object of study (2, 3). WV could be useful in biodisinfestation as other OM sources have already been (4, 5). There are previous experiences with WV used as a fungicide (6).

*Corresponding author: *José Antonio López Pérez.* E-mail: jalopezp@jccm.es
Received: 16/02/2021
Accepted: 26/09/2021

Thus, there is much interest in finding alternative applications that contribute to the valorization of this by-product and methods that solve the problems associated with the seasonal nature of the distillery industry, specifically related to its composition and the volumes generated (7, 8).

Prior to use these by-products, it is necessary to rule out any possible toxicity and to test that there are no phytotoxic effects on crops. Winery-sludge was seen not to affect the germination response of *Lepidium sativum* L. (9), and such germination studies are useful to define any possible toxicity of different substances (10, 11) and to assess the suitability of by-products to select those that are innocuous.

The objective of this work was to test WV generated by distilleries as a source of OM to control plant-parasitic nematodes. First, by testing the effect on seed germination and plant growth, and, then, determining the optimal doses by studying the *in vitro* response of two phytopathogenic nematodes, a root-knot nematode (RKN-*Meloidogyne incognita* (Kofoid and White, 1919) Chitwood 1949) and *Xiphinema index* Thorne & Allen 1950. Finally, WV was assessed as an organic source for biodisinfestation of a naturally RKN infested soil. The data obtained shed further light on the use of by-products like WV for biodisinfestation.

MATERIALS AND METHODS

The experiments were carried out at the Laboratory of Agroecology at the former Centro de Ciencias Medioambientales, now Instituto de Ciencias Agrarias (ICA) of the Spanish National Research Council (CSIC, Madrid), Spain.

Main characteristics of wine vinasse used in this work

Wine vinasse provided by ALTOSA distillery (Tomelloso, Ciudad Real) was used to develop the assays. Its chemical composition was analysed (Técnicas Reunidas SA) (Table 1). Sample A (2007) was used to test toxicity in the assays with: i) phytoparasitic nematodes, ii) seed germination, and iii) crop seedlings. Sample B (2015) was used for the biodisinfestation assay with naturally infested soils obtained from a greenhouse at Marchamalo (Guadalajara).

Wine vinasse bioassessments

The toxic effect on the reproductive (germination rate of cress seeds) and vegetative (root and shoot elongation of cress, tomato and mustard plantlets) growth was assessed.

- **Seed germination (CEN 2012).** Ten cress seeds (*Lepidium sativum* L.) were spread evenly on filter paper moistened with 2 mL of 1, 3, or 5 % of the vinasse (sample A) placed in 7 cm diameter Petri dishes, and distilled water was used as control (test repeated 5 times with 4 replicates for each concentration, 160 cress seeds per test). After three days in darkness in a temperature-controlled chamber (24 ± 1ºC), the seeds with rupture of seed coats and visible protrusion of about 1 mm of radicle were counted.
- **Phytotoxicity assessment of WV on commercial crop seedlings.** Root and shoot elongation of cress as toxicity control and two important plant species was studied: tomato (*Solanum lycopersicon* L. cv. Marmande), a good host for RKN; and white mustard (*Sinapis alba* L.), a Brassicaceae plant with biofumigant properties (12). A hundred seeds were placed on pleated filter paper. Each paper was placed in separate trays, one tray per concentration, and watered with 100 mL of the corresponding solution every 48 hours. The four trays, with 1, 3, and 5 % of the vinasse A and distilled water, were kept in a growth chamber for 15 days. The bioassay was repeated twice. The results were expressed with indicators based on the relative seed germination (RSG), relative root elongation (RRE) (10) and relative shoot elongation (RSE), according to the formulas below:

$$\text{RSG (\%)} = \frac{\text{Number of seeds germinated in the sample extract}}{\text{Number of seeds germinated in the control}} x\ 100$$

$$\text{RRE}\left(\%\right) = \frac{\text{Mean root elongation in the sample extract}}{\text{Mean root elongation in the control}} x\ 100$$

$$\text{RSE}\left(\%\right) = \frac{\text{Mean shoot elongation in the sample extract}}{\text{Mean shoot elongation in the control}} x\ 100$$

Nematode analysis

The bioassays focused on two of the main phytopathogenic nematodes present in Central Spain, *Meloidogyne incognita* (RKN) and *Xiphinema index* (a virus-transmitting nematode). Although these nematodes are both very common in vineyard soils, their body size, life cycle, and reproductive strategy mean, they are not equally susceptible to environmental disturbances (13). *M. incognita* was isolated from soil samples obtained from greenhouses located at Villa del Prado (Madrid, Central Spain). *X. index* was obtained from a population kept on soil around a fig tree located at the Instituto de Ciencias Agrarias (ICA, CSIC, Madrid).

The soil from a greenhouse at Marchamalo (Guadalajara) was used for the biodisinfestation at the laboratory. This soil was clay loam alfisol, pH almost neutral (7.7) with 3.6 % of organic matter content. Nematodes were extracted following the sieving and the Baermann-Funnel technique (14).

- *In Vitro* response of *M. incognita* and *X. index* to WV

In order to find the minimum effective dose, different concentrations of WV, ten mobile and infective *Meloidogyne* second-stage juveniles (J_2) were exposed to 5 mL of different concentrations of WV diluted in distilled water or the vehicle alone (distilled water) as a control in each Petri dish (50 mm diameter). Four replicates per solution were maintained in a dark chamber at room temperature (21ºC) with the lids of the Petri dishes left on. The nematodes were observed every 15 minutes, probing them with a needle to confirm they were alive. The assay was repeated twice and the same method was followed to test *X. index*.

- *In lab* assay with horticultural soil. Soil biodisinfestation

Soil samples were taken with a hoe (5-30 cm depth) around tomato plant roots grown under greenhouse conditions at Marchamalo (Guadalajara). The soil sampled was mixed and homogenised, divided among four polyethylene plastic bags (32 x 50 cm), one for each dose of WV (500 g dry equivalent of soil, volume/mass soil), and sealed. A set of four control bags with soil exposed to tap water instead of WV was also stored with the rest of the replicates in a dark chamber at 30ºC. This temperature has been shown effective for biofumigation experiments with plant residues and animal manure (15) and is below those reached with biosolarization experiments designed to obtain thermal stress (16).

In addition to the plant-parasitic nematodes, the rest of the nematodes present was identified to the genus level and assigned to the trophic groups typical of plant-soil systems (17): microphages (bacterial or fungal feeders), predators, and omnivores (18). Enchytraeids (Annelida: Oligochaeta) were included in the edaphic microcosms as they are sapro-microphytophagous, i.e., they feed on the dead soil OM that exists in agrarian soils, including the fungi and bacteria attached to this material (19).

The amount of water applied was calculated by previously establishing the field capacity of the infested soil (120 ml). This volume of by-product solution was applied to each set of bags and it was mixed homogeneously with the soil. After 20 days, the plastic bags were removed from the chamber and the nematodes were extracted from a 100 g soil sub-sample using Baermann funnel method. Another part of the remaining soil (300 g) was placed in a plastic pot in which a tomato plant (cv. Marmande) with two true leaves was planted. These plants acted as a target to assess how biodisinfestation affected juveniles and eggs of *Meloidogyne* in the soil tested, evaluating their presence by the galling index produced in the tomato roots.

Response of target plants on treated soil

The effect of WV on RKN susceptible tomatoes was studied through plant growth parameters and its effect on the plant roots. The tomato plants were grown in wet vermiculite in pots within a growth chamber (24 ± 1ºC), and their roots were rinsed and cleaned before they were transplanted to the control or WV-treated soils. The plants were measured after 45 days in the growth chamber, sufficient time to check for the presence of RKNs through the galls they produce on the roots. A galling index from 0 to 10 was established, where 0 meants no galls, 5, that approximately half of the root system was parasitized, and 9 or 10 implied that the root was totally deformed and the plant could even be dead due to RKN parasitization (20). The shoot of each plant was cut off at the soil level and the roots were washed free of soil. The plants were dried and growth parameters, such as height, weight, and leaf number, were measured.

Statistical analysis

In order to check the effect of WV on *M. incognita* and *X. index* individuals, a Kaplan-Meier survival analysis was established. The nematofauna and enchytraeid population densities were compared by a non-parametric Mann-Whitney U Test. Mean values of germination and phytotoxicity Tests, and plant growth parameters were compared by analysis of variance (ANOVA), when the *F* ratio was significant, least significant differences between means of significant main factors were analysed with the Tukey *HSD* test ($p<0,05$). Data analysis was developed with SPSS v. 17.0 software for Windows.

RESULTS AND DISCUSSION

Characterization of wine vinasses

Fertility parameters of two samples extracted at different seasons were studied (Table 1): WV sample A from 2007, and sample B from 2015. The parameters evaluated were homogeneous, except for the variation in K and SO_4 content, mainly due to their low values in the 2015 WV.

Previous studies of wastewater and vinasse samples from different wineries and alcohol distilleries across Spain (21) showed great variability in the data observed. As a result, it was recommended that winery by-products should be tested before using them as soil amendments. The fertility values of the different WV analysed in this study proved to be very similar and close to the range of WV values reported elsewhere (7, 21, 22). The final composition of WV is defined by the processes involved in the wine production and in the processes used in the distillery.

Table 1. Chemical composition of the wine vinasses. / *Composición química de las vinazas de vino*

Wine Vinasse	Sample A (2007)	Sample B (2015)
Humidity (g L^{-1})	977	980
pH	3,45	3,71
OM (g L^{-1})	20,40	17,14
Total N (g L^{-1})	0,12	0,.28
P (g L^{-1})	0,185	---
Ca (g L^{-1})	0,067	0,04
Mg (g L^{-1})	0,10	0,01
Na (g L^{-1})	0,038	0,03
K (g L^{-1})	1,71	0,04
SO_4 (g L^{-1})	0,239	0,09
Pb (mg L^{-1})	< 0,5	< 0,5
Zn (mg L^{-1})	0,20	0,024
Fe (mg L^{-1})	0,302	0,04
Cu (mg L^{-1})	--	0,40
Mn (mg L^{-1})	0,013	0,012

-- not measured

Germination and phytotoxicity

The preliminary germination test with cress seeds showed no significant differences with the control ($p < 0.05$), and seed germination was over 80 % in all cases (range of F values: 0.206-1.769; data not shown). The phytotoxicity test did not show any detrimental effect on the three plant species. All plants germinated similarly, the relative germination values (RSG) were over 90 % in all cases. WV significantly improved shoot length in all plants. With the exception of tomato plants, root growth was shorter with the most concentrated WV solution (Fig. 1).

Germination and plant growth are the most common tests in bioassays for checking phytotoxicity (10). Some winery by-products are known to have a negative effect on plant germination and growth. For instance, winey wastewater decreases the germination index of *Lepidium sativum* L. seeds (23), delays seed germination, and also inhibits the vegetative growth of several plants (24). Here, WV had no effect on germination, which was always over 70 % with any of the solutions tested, and it did not affect seed emergence, data that coincides with those obtained with similar doses (25).

After ruling out any toxic effect of the WV solutions on germination, two important species were used to test its influence on plant growth. Plant species may differ substantially in their sensitivity to compounds (26), yet our data show that seed germination was not affected (RSG > 80 %). WV improved the growth of these plants, producing an increase in the aerial part of the plant (RSE > 100 %) and a reduction of the plant rootlet as the doses increased. No phytotoxicity was noted, which confirmed earlier results on tobacco and soybean plantlets (27). The enhanced growth of the aerial part of mustard seedlings is a particularly interesting effect, as this increase in biomass will enhance biofumigation if this is used as green-manure (28). Hence, complementary techniques may be applied to improve the physicochemical and biological properties of agrarian soils (4).

Effect on plant-parasitic nematodes

WV was tested separately for its *in vitro* effects on RKN and *X. index* individuals. In a second assay, three WV dosages were applied to naturally infested soils from Marchamalo (Guadalajara).

- *In Vitro* assays

M. incognita **(** Fig. 2 **):** WV diminished the survival of at least 50 % of the individuals over 360 minutes at all the concentrations used, except at the lowest concentration of 0.2 %. The Kaplan-Meier survival analysis showed significant differences among the solutions (Table 2A), as also seen in the Log-Rank Test. Whilst juveniles placed in the Control and 0.2 % solutions behaved similarly, they behaved distinctly to those maintained in other WV concentrations.

X. index **(** Fig. 3 **):** The highest concentration of WV employed (3 %) was lethal to these individuals after 45 minutes, whereas at the other concentrations, 100 % mortality was obtained after 75 minutes. The Log-Rank Test showed significant differences among the solutions (Table 2B).

The WV solutions above 0.2 % effectively reduced the number of the two plant-parasitic nematodes tested *in vitro*, producing 50 % mortality after 360 mins for RKN and after 75 mins for *X. index*. The higher susceptibility of *Xiphinema* than *Meloidogyne* is most likely due to differences in cuticle structure and the secretory-excretory systems, which are more developed in Secernentea (*Meloidogyne*) than in Adenophorea

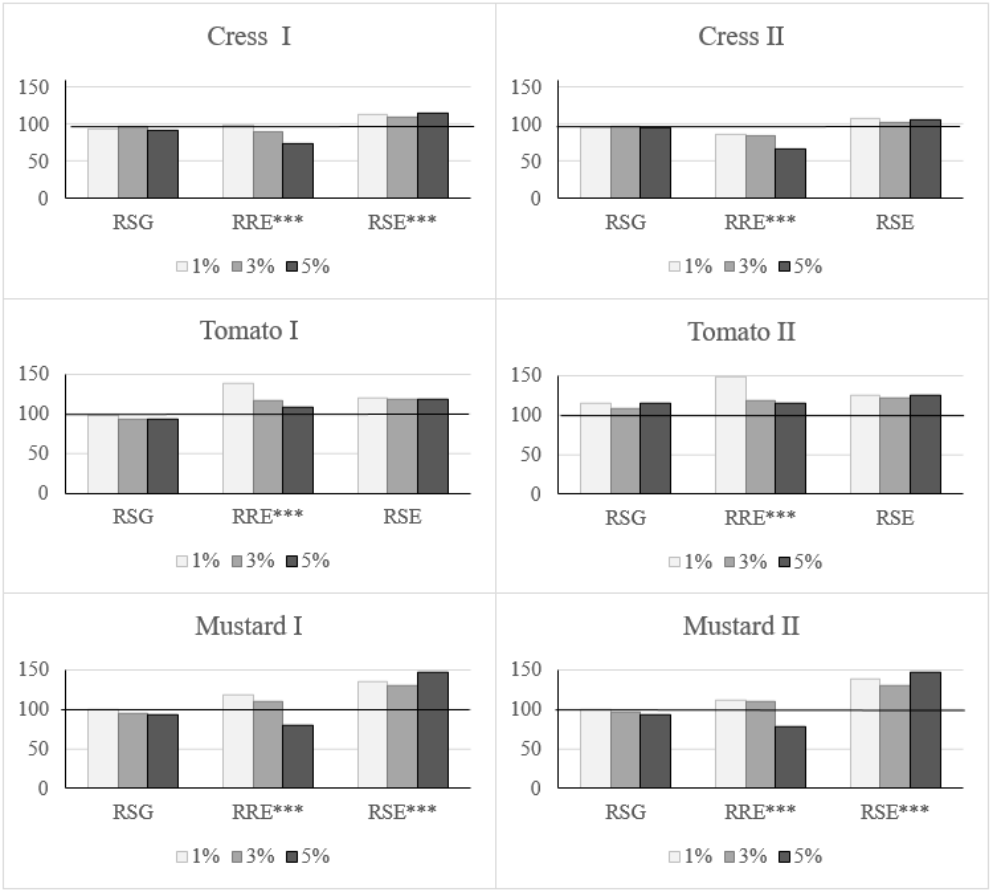

Figure 1. Phytotoxicity assessment of WV on cress, tomato and mustard plants (mean values, n= 100 plantlets, two replicates) with three wine vinasse dilutions (1, 3, and 5 %). Relative seed germination (% RSG), Relative root elongation (% RRE) and Relative shoot elongation (% RSE) (mean ± S.E., ***$p<0.001$). Black line at 100 %, indicates the same value as the control. / *Evaluación de la fitotoxicidad de WV en plantas de berro, tomate y mostaza (valores medios, n =100 plántulas, dos réplicas) con tres diluciones de WV (1, 3 y 5 %). Germinación relativa de semillas (% RSG), alargamiento relativo de la raíz (% RRE) y alargamiento relativo del tallo (% RSE) (media ± Error Estándar, *** p<0,001). Línea negra al 100 % indica el mismo valor que el testigo.*

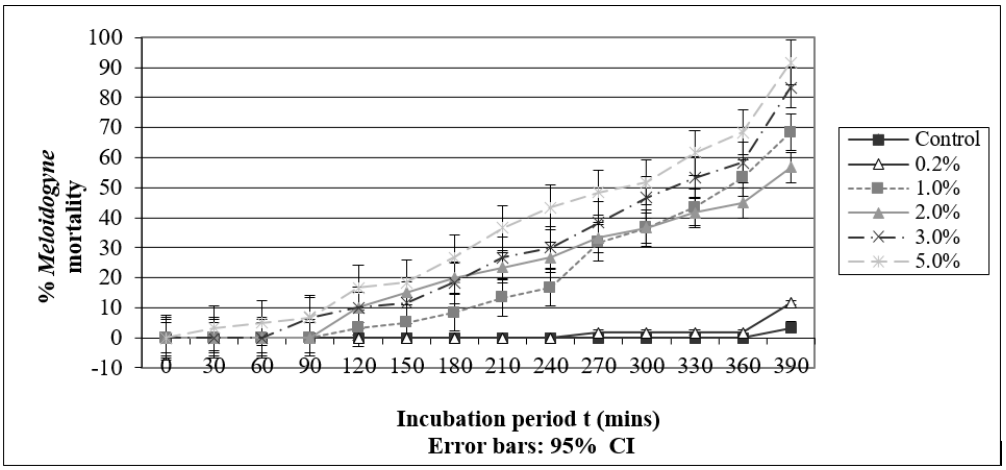

Figure 2. Accumulated mortality of *Meloidogyne incognita* (ten individuals per replicate) at different WV concentrations (mean values of four replicates, the bars indicate the standard error). / *Mortalidad acumulada de Meloidogyne incognita (10 individuos por réplica) a diferentes concentraciones de WV (valores medios de 4 réplicas, las barras indican el error estándar).*

(*Xiphinema*: Wright (29)). These differences would also explain *Xiphinema´s* higher susceptibility to environmental disturbances. WV has been used to combat fungi, inhibiting the growth of several phytopathogenic fungi *in vitro* at a concentration of 5-7 %, and *Sclerotinia sclerotiorum* and *Fusarium oxysporum* f. sp. radices-cucumerinum at higher doses (10-15 %: Santos (6)). Yet, nematodes appear to be more susceptible to WV as 50 % mortality was reached with concentrations around 1.0 %.

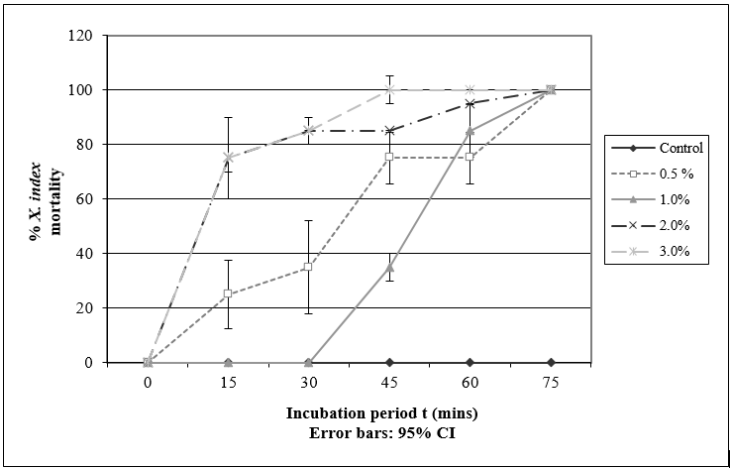

Figure 3. Accumulated mortality of *Xiphinema index* (five individuals per replicate) at different WV concentrations (mean values of four replicates, the bars indicate the standard error). / *Mortalidad acumulada de Xiphinema index (5 individuos por réplica) a diferentes concentraciones de WV (valores medios de 4 réplicas, las barras indican el error estándar).*

Table 2. Mean survival time and Log-Rank Test: A. *Meloidogyne incognita*; B. *Xiphinema index*. / *Tiempo de supervivencia media y prueba de rango logarítmico: A. Meloidogyne incognita; B. Xiphinema index. 95 % C.I.*

A.	Means			
Treatment	**Estimate**	**Std. Error**	**Lower Bound†**	**Upper Bound†**
Control	--			
0.2 %	--			
1.0 %	360,0	15,5	329,7	390,3
2.0 %	390,0	16,5	357,8	422,2
3.0 %	330,0	29,0	273,2	386,8
5.0 %	300,0	31,7	237,9	362,1
Overall	390,0			
Overall Comparisons		Chi-Square	df	Sig.
Log Rank (Mantel-Cox)		161,95	5	0,000

† 95 % C.I.

B.	Means			
Treatment	**Estimate**	**Std. Error**	**Lower Bound†**	**Upper Bound†**
Control	--			
0.5 %	45,0	3,63	38,90	52,12
1.0 %	60,0	2,40	55,31	64,70
2.0 %	30,0	2,66	24,80	35,22
3.0 %	30,0	2,66	24,80	35,22
Overall	30,0	3,00	24,16	35,84
Overall Comparisons		Chi-Square	df	Sig.
Log Rank (Mantel-Cox)		43,9	3	0,000

† 95 % C.I.

- *In lab* assay with horticultural soil. Biodisinfestation of naturally infested soil

Natural RKN infested soil from Marchamalo (Guadalajara, Central Spain) was used in the laboratory assays. In the light of the *in vitro* assays, the doses of WV studied were 1, 3, and 5 %, and when the fauna was assessed after incubation in the 30ºC chamber, biodisinfestation with WV mainly affected phytoparasitic RKN **(** Table 3 **)**, remaining only in the control conditions after incubation. There were no differences in the other faunal group.

As a biodisinfestant, WV diminished the presence of RKN when applied to naturally infested soil. Biodisinfestation has been successful employed in soil (2, 5) and here, treatment appeared to be effective against RKN infestation of Marchamalo soil. There were fewer microphagous individuals, omnivores and enchytraeids as the WV concentration increased, although only the numbers of microphagous individuals were significantly different. More enchytraeid worms were found in conjunction with organic farming, whereas they were virtually absent in association with conventional management (30), as evident in the Marchamalo soil (around a hundred per sample).

Table 3. Effect of three doses of WV on soil from Marchamalo (Guadalajara, Spain). 100 cm^{-3} soil†. / *Efecto de tres dosis de WV en suelo de Marchamalo (Guadalajara, España). 100 cm^{-3} suelo †*

	Nematodes						Annelids	
Treatment	**Plant-parasitic RKN (J_2)**		**Omnivores**		**Microphages**		**Saprophages Enchytraeids**	
Control	62 ± 13	b	136 ± 37	a	1291 ± 117	b	81 ± 17	a
1 %	22 ± 9	ab	300 ± 101	a	1123 ± 135	b	158 ± 79	a
3 %	27 ± 12	ab	268 ± 74	a	672 ± 57	a	111 ± 35	a
5 %	3 ± 2	a	89 ± 20	a	377 ± 42	a	30 ± 12	a
Asyntotic sig.	0.009		0.119		0.00		0.274	

† The same letters in a column indicate there were no significant differences ($p \leq 0.05$): mean values ± standard error (n = 4).

Table 4. Effect of soil biodisinfestation with three doses of WV on the growth of tomato cv. Marmande†. / *Efecto de la biodesinfección del suelo con tres dosis de WV en el crecimiento de tomate cv. Marmande †.*

	Wine vinasse dose								***F* value**
	Control		**1 %**		**3 %**		**5 %**		
Soil from Marchamalo (Guadalajara)									
Height (cm)	26.65 ± 4.5	a	32.1 ± 1.43	a	28.8 ±2.07	a	28.9 ±3.75	a	0.498
Total weight (g)	1.90 ± 0.16	a	2.16 ± 0.44	a	2.46 ±0.46	a	2.53 ±1.27	a	0.416
Shoot weight(g)	1.44 ± 0.12	a	1.66 ±0.38	a	1.88 ±0.33	a	2.05 ±0.35	a	0.571
Root weight (g)	0.46 ± 0.04	a	0.49 ±0.06	a	0.58 ±0.14	a	0.47 ± 0.15	a	0.224
# Leaves	8.0 ± 0	a	8.5 ± 0.29	a	8.5 ±0.29	a	8.0 ± 0.41	a	1.000
Galling index	1.75 ± 0.25	a	0.5 ± 0.29	b	0 ± 0	b	0 ± 0	b	18.714

†The numbers in the rows followed by the same letter are not significantly different ($p \leq 0.05$): mean values ± standard error (n = 4)

Growth of tomato plants on treated soil. The growth parameters of tomato cv Marmande plants did not show any significant differences when exposed to any dose of WV. The galling indices were significantly different in plants treated with WV (Table 4).

There was a significant decrease in RKNs, which was corroborated by the significant reduction in the root-galling index. In assessing the effects on tomato plant growth it is important to discriminate the influence on plant growth produced by the WV solution and the benefits from growing the plants in soil biodisinfested with this by-product (soil fertility effect), as well as the possible influence on RKN growth (reduction of root damage).

The low doses of WV applied here were sufficient to affect the plant-parasitic nematodes but they did not increase the bacterial and fungal contents of the soil. When higher doses of WV were tested (6), the bacterial and fungal components of the microbiota were enhanced. Repeated WV application and higher doses would possibly improve the biological effects of the WV used, as recommended previously (30).

The virus-transmitting nematode *X. index* is usually associated with vineyards, so that the possibility of applying WV to these crops to reduce nematode populations would be a particular benefit. RKN is also associated with this crop, which is a problem for vine replantation (31). The fact that WV is a liquid means it can be easily applied with standard irrigation systems, reducing the use of heavy machinery and making *X. index*, which can inhabit profound depths, suitable to be treated. This species has been found at depths between 30 and 50 cm in permeable soils (32), although it can survive at depths up to 3.5 m (33). Hence, a treatment with a liquid, which extends and disperses readily, will reach the nematode more easily than, for instance, an alternative treatment with a solid substance.

Our results suggest that WV can enhance the growth of two important plants for horticulture and could be used to reduce phytopathogenic nematodes.

CONCLUSIONS

WV appears to represent a suitable tool to be applied in agricultural soils, showing that: (i) germination process of cress, tomato, and mustard is not affected by WV; (ii) shoot elongation in all plants and root length in tomato plants at all the doses tested were improved; (iii) the *in vitro* survival of *X. index* and *M. incognita*was diminished, with a faster effect on *Xiphinema*; (iv) biodisinfestation with WV reduced RKN significantly, with a smoother effect on microphagous and omnivorous nematodes.

The WV analysis and the bioassays carried out here help to better understand the potential of this winery by-product when applied to agricultural soils. In fact, its fertilizing effect on tomato and mustard plants indicates that it could be introduced into horticultural cropping systems.

The characterization of both WV and agrarian soil will help define the suitability of such actions prior to applying this type of organic amendment. Finally, the valorization of this resource may contribute to

reducing the possible impact of its accumulation and WV integration into the environment. Its use as a soil improver and a tool to reduce phytopathogenic nematode populations may be useful in organic farming, enhancing soil conservation. Further studies should focus on its effect on soil fertility properties.

ACKNOWLEDGEMENTS

This study was financed by INIA (RTA2007-00099-00-00 and RTA2015-00013-C03-01), MMARM grants (OTT2007X1317), and an INCRECYT contract (PCYTA-JCCM) held by Dr. López-Pérez. We wish to acknowledge the assistance of C. Martínez in the soil sampling and analysis. We want to express a special gratitude to our mentor, Professor Antonio Bello, who encouraged this work and from whom we learned of his vast knowledge in agroecology and nematology.

REFERENCES

1. MAPA (Ministerio de Agricultura, Pesca y Alimentación). Anuario de Estadística 2019. Viñedo: Análisis provincial de superficie total y según clases. https://www.mapa.gob.es/estadistica/pags/anuario/2019/CAPITULOSPDF/CAPITULO07/pdfc07_11.1.5.pdf.
2. Oka Y. Mechanisms of nematode suppression by organic soil amendments - A review. Appl. Soil Ecol. 2010;44:101-115.
3. Renčo M. Organic amendments of soil as useful tools of plant-parasitic nematodes control. Helminthologia. 2013;50:3-14.
4. Larregla S, Guerrero MM, Mendarte S, Lacasa A. Biodisinfestation with organic amendments for soil fatigue and soil-borne pathogens control in protected pepper crops. In: Meghvansi, M.K., Varma, A., Organic Amendments and Soil Suppressiveness in Plant Disease Management. Springer International Publishing Switzerland. 2015;Volume 46 of the series Soil Biology: pp 437-456.
5. Núñez-Zofio M, Larregla S, Garbisu C, Guerrero MM, Lacasa CM, Lacasa A. Application of sugar beet vinasse followed by solarization reduces the incidence of *Meloidogyne incognita* in pepper crops while improving soil quality. Phytoparasitica. 2013;41:181-191.
6. Santos M, Diánez F, Cara M de, Tello JC. Possibilities of the use of vinasses in the control of fungi phytopathogens. Bioresource Technol. 2008;99:9040-9043.
7. Bueno PC, Rubi JAM, Giménez RG, Ballesta RJ. Impacts caused by the addition of wine vinasse on some chemical and mineralogical properties of a Luvisol and a Vertisol in La Mancha (Central Spain). J. Soil Sediment. 2009;9:121-128.
8. Mateo JJ, Maicas S. Valorization of winery and oil mill wastes by microbial technologies. Food Res. Int. 2015; 73:13-25.
9. Saviozzi A, Levi-Minzi R, Riffaldi R, Cardelli R. Suitability of a winery-sludge as soil amendment. Biores. Technol. 1994;49: 173-178.
10. Luo Y, Liang J, Zeng G, Chen M, Mo D, Li G, *et al.* Seed germination test for toxicity evaluation of compost: Its roles, problems and prospects. Waste Manage. 2018;71:109-114.
11. USEPA. Seed germination/root elongation toxicity test, US Environmental Protection Agency, Washington DC. 1992.
12. Berlanas C, Andrés-Sodupe M, López-Manzanares B, Maldonado-González MM. Gramaje D. Effect of white mustard cover crop residue, soil chemical fumigation and *Trichoderma* spp. root treatment on black-foot disease control in grapevine. Pest Manag Sci. 2018;74: 2864-2873. DOI 10.1002/ps.5078
13. Ferris H, Bongers T. Nematode indicators of organic enrichment. J. Nematol. 2006;38:3-12.
14. Barker KR. Nematode extraction and bioassays. In: Barker KR, Carter CC, Sasser JN. (Eds.), An Advanced Treatise on *Meloidogyne*: Methodology, vol. 2. North Carolina State University Graphics, Raleigh, 1985:pp.19-35.
15. López-Pérez JA, Roubtsova T, Ploeg A. Effect of three plant residues and chicken manure used as biofumigants at three temperatures on *Meloidogyne incognita* infestation of tomato in greenhouse experiments. J. Nematol. 2005;37:489-494.
16. Achmon Y, Fernández-Bayo JD, Hernandez K, McCurry DG, Harrold DR, Su J, *et al.* Weed seed inactivation in soil mesocosms via biosolarization with mature compost and tomato processing waste amendments. Pest Manag. Sci. 2017;73:862-873. https://doi.org/10.1002/ps.4354
17. Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH. Ecological linkages between aboveground and belowground biota. Science. 2004;304:1629-1633.
18. Yeates GW, Bongers T, De Goede RGM, Freckman DW, Georgieva SS. Feeding habits in soil nematode families and genera-an outline for soil ecologists. J. Nematol. 1993;25:315-331.
19. Didden WAM. Ecology of terrestrial Enchytraeidae. Pedobiologia. 1993;37:2-29.
20. Bridge, J., Page S. L. J., 1980. Estimation of root-knot nematode infestation levels on roots using a rating chart. Trop. Pest Manage. 26;296-298.
21. Bustamante MA, Paredes C, Moral R, Moreno-Caselles J, Pérez-Espinosa A, Pérez-Murcia MD. Uses of winery and distillery effluents in agriculture: characterisation of nutrient and hazardous components. Water Sci. Technol. 2005;51:145-151.

22. Vlyssides A, Barampouti EM, Mai S. Wastewater characteristics from Greek wineries and distilleries. Water Sci. Technol. 2005;51:53-60.
23. Oliveira M, Queda C, Duarte E. Aerobic treatment of winery wastewater with the aim of water reuse. Water Sci. Technol. 2009;60:1217-1223.
24. Mosse KPM, Patti AF, Christen EW, Cavagnaro TR. Winery wastewater inhibits seed germination and vegetative growth of common crop species. J. Hazard. Mater. 2010;180:63-70.
25. Kannabiran B, Pragasam A. Effect of distillery effluent on seed germination, seedling growth and pigment content of *Vigna mungo* Hepper. Geobios (Jodhpur). 1993;20:108-112.
26. Ravindran B, Sheena Kumari SK, Stenstrom TA, Bux F. Evaluation of phytotoxicity effect on selected crops using treated and untreated wastewater from different configurative domestic wastewater plants. Environ. Technol. 2016;37:1782-1789.
27. Castro Lizazo I. Biodesinfección de suelos en relación con la diversidad en hortalizas y platanera. [Tesis en opción al grado de Dr. en Ciencias], Univ. Almería. 2010: 282 pp.
28. Muiru WM, Ogumo E, Kimenju JW, Mukunya D. Potential of green manure crops in suppressing root knot nematodes in French beans. Int. J. Agron. Agri. R. 2017;11:90-97.
29. Wright DJ. Respiratory physiology, nitrogen excretion, and osmotic and ionic regulation. In: R. N. Perry and D. J. Wright (Eds). The physiology and biochemistry of free-living and plant-parasitic nematodes. CAB International, Wallingford, UK. 1998.103-131.
30. Bonanomi G, Lorito M, Vinale F, Woo SL. Organic amendments, beneficial microbes, and soil microbiota: toward a unified framework for disease suppression. Annu. Rev. Phytopathol. 2018;56. doi.org/10.1146/annurev-phyto-080615-100046.
31. López-Pérez JA, Escuer M, Díez-Rojo MA, Robertson L, Piedra Buena A, López-Cepero J, *et al*. Host range of *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Nematoda: Meloidogynidae) in Spain. Nematropica. 2011;41:130-140.
32. Weischer B. Ecology of *Xiphinema* and *Longidorus*. In: F. Lamberti, C.E. Taylor and J.W. Seinhorst (Eds). Nematode Vectors of Plant Viruses. Plenum Press, London-New York. 1975. 291-306.
33. Raski DJ, Hewitt VB, Goheen AC, Taylor CE, Taylor RH. Survival of *Xiphinema index* and reservoirs of fanleaf virus in fallowed vineyard soils. Nematologica. 1965;11:349-352.

Declaración de conflicto de intereses: Los autores declaran que no existe conflicto de intereses

Contribución de los autores: **Iván Castro Lizazo:** participó en la búsqueda de información, en la toma de muestras de suelo, extracción y recuento de nematodos, ensayos con plantas y nematodos, colaboró en la preparación de los datos para su análisis estadístico. **Miguel Ángel Díez Rojo:** participó en el diseño del trabajo, búsqueda de información, toma de muestras de suelo, extracción y recuentos, ensayos con nematodos, análisis estadísticos. **María Rosa López-González:** participó en la búsqueda de información, en los ensayos con semillas y plantas y en la preparación de los datos para su análisis estadístico. **José Antonio López-Pérez:** participó en el diseño del trabajo, en la búsqueda de información, recuento de nematodos y enquitréidos, coordinación del artículo y su escritura.