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Article

Sustratos orgánicos en la germinación y crecimiento de *Pinus ayacahuite* var. *veitchii* (Roezl) Shaw en vivero

Organic substrates in germination and growth of *Pinus ayacahuite* var. *veitchii* (Roezl) Shaw at the nursery

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Resumen:

En la región Montaña de Guerrero el acceso a sustratos comerciales es limitado y costoso, por lo que resulta pertinente generar alternativas para la producción de plántulas con materiales locales que complementen a la tierra de monte. En el presente estudio se evaluó el efecto de tres componentes orgánicos: tierra de monte (tm), lombricomposta (lc) y composta (cp) en la germinación y crecimiento de *Pinus ayacahuite* var. *veitchii* en vivero, en la localidad Plan de Guadalupe, Tlacoapa, Guerrero. Las semillas se depositaron en charolas de poliestireno expandido de 77 cavidades, distribuidos sobre mesas portacharolas bajo un diseño completamente al azar con cuatro tratamientos (T1, T2, T3 y T4) y un testigo (TG) con cuatro repeticiones. Las variables evaluadas fueron: porcentaje de germinación (%), germinación en tiempo (días), diámetro basal del tallo (mm) y altura de las plantas (cm). Los resultados del análisis de varianza mostraron diferencias estadísticamente significativas ($p < 0.05$) para todas las variables analizadas. Para obtener la mayor germinación de *P. ayacahuite* se recomienda utilizar el T1 (50 % tm + 50 % lc); no obstante, el crecimiento en diámetro, altura e índice de esbeltez, se obtiene al emplear el sustrato compuesto por 50 % tm + 20 % lc + 30 % cp (T4). En conclusión, es posible usar diferentes sustratos en cada etapa de producción de planta en vivero, para asegurar alta germinación, y un buen crecimiento, lo que resulta en plantas de calidad para su establecimiento en campo.

Palabras clave: Composta, desarrollo, mezcla de sustrato, *Pinus ayacahuite* Ehrenb. ex Schltdl., producción de planta, tierra de monte.

Abstract:

In the Region Mountain of *Guerrero*, access to commercial substrates is limited and expensive, so it is pertinent to generate alternatives in the production of seedlings with local materials that complement the forest soil. The present study evaluates the effect of three organic components —forest soil (fs), vermicompost (vc) and compost (cp)— on the nursery germination and growth of *Pinus ayacahuite* var. *veitchii*, in the locality known as *Plan de Guadalupe*, *Tlacoapa*, *Guerrero*. The seeds were sown in expanded polystyrene trays of 77 cavities, distributed on tables of racks under a fully random design with four treatments (T1, T2, T3 and T4) and a control (CT) with four repetitions. The evaluated variables were: germination percentage (%), germination in time (days), basal diameter of the stem (mm) and height of the plants (cm). The results of the variance analysis were statistically significant ($p <0.05$) for all the variables analyzed. To obtain the highest germination of *P. ayacahuite,* the use T1 (50 % fs + 50 % vc) is recommended; however, the maximum growth in diameter, height and slenderness index is attained by using the substrate composed of 50 % fs + 20 % vc + 30 % cp (T4). In conclusion, it is advisable to use different plants at each stage of nursery plant production, so as to ensure a high germination, but also good growth resulting in high quality plants for establishment in the field.

Keywords: Compost, development, substrate mix, *Pinus ayacahuite* Ehrenb. ex Schltdl., plant production, forest soil.

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Introduction

The type of substrate utilized in nurseries is one of the factors that influence the quality and production cost of a plant. Therefore, it is essential to seek options to reduce the costs and ensure the quality of the plants (Arteaga *et al.*, 2003). A way of reducing the costs from substrates is to mix different materials that will allow improving their physical, chemical and biological properties (Cruz-Crespo *et al.*, 2012).

Vermicompost is an organic substrate with a high content of nitrogen, potassium, phosphorus, magnesium and micro-nutriments (Ruíz, 2011). It highly miscible with soil for a gradual release of nutriments and accelerated plant production (Capistran *et al.*, 1999). Compost is a fertilizer obtained through the decay of various organic products; therefore, its physical and chemical characteristics vary (Burés, 1997). However, it contributes elements that are essential for the plant (Hicklenton *et al.,* 2001). Ground from the mountains is the most common substrate used in forest nurseries of Mexico that work with the traditional system (Reyes-Reyes *et al*., 2005).

Pinus ayacahuite Ehrenb. ex Schltdl. it is one of the most important species of conifers in Mexico, both for its ecological and economic value (Munive *et al*., 2008); which has led to enormous anthropogenic pressure on their populations (Musálem and Ramírez, 2003). Ecological and sylvicultural knowledge is essential to develop programs for the protection, conservation and promotion of this taxon (Jasso, 2005).

Studies on *Pinus ayacahuite* var. *veitchii* (Roezl) Shaw usually focus on the variation of cones and seeds among its populations (Munive *et al.*, 2008), as well as on the effect of vermicompost on *Pinus ayacahuite* (Altamirano, 2002).

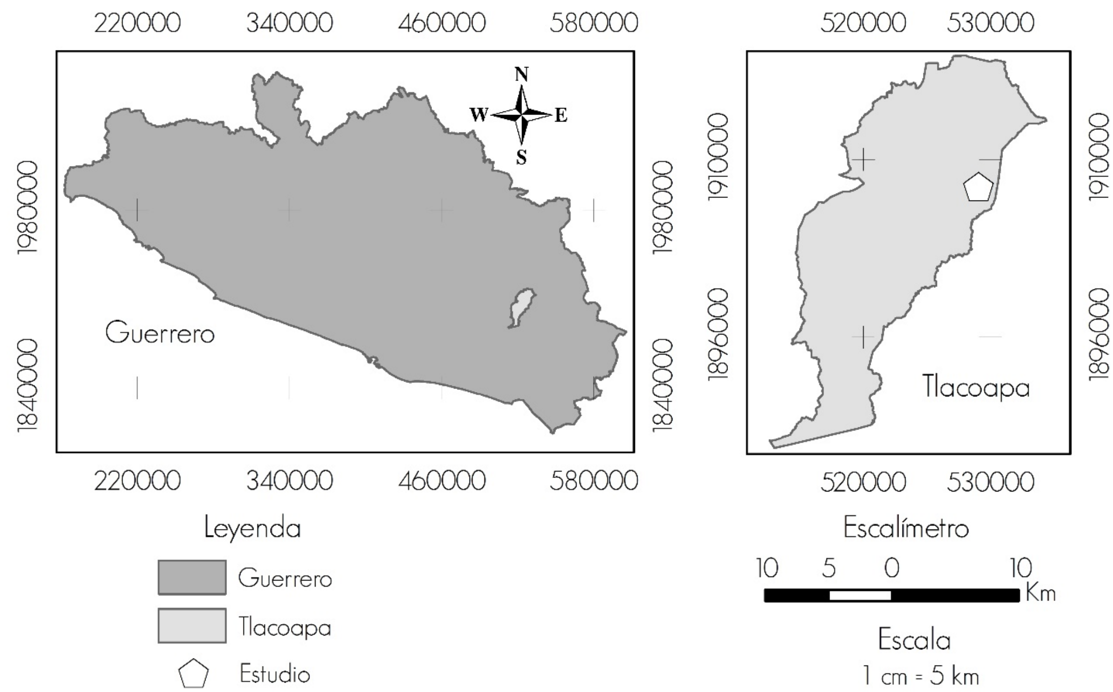
In regard to the use of alternative substrates, Mateo *et al.* (2011) assessed the effect of various doses of sawdust on the production of *Cedrela odorata* L.; Romero-Arenas *et al.* (2013) determined the germination capacity of *Pinus patula* Schiede ex Schltdl. & Cham. with a compost made with residues of walnut (*Juglans regia* L.) shells, and Sallesses *et al.* (015) tried using chicken manure-based compost to produce a hybrid of *Eucalyptus grandis* W. Hill ex Maiden ×*Eucalyptus camaldulensis* Dehnh.

For the state of *Guerrero*, and specifically the Mountain Region, *Pinus ayacahuite* var. *veitchii* populations are restricted to individuals; for this reason, there is a need to implement reproduction and propagation strategies that may ensure the continuity of the species.

The objectives of this research were: a) to evaluate the effect of various combinations of organic components on the germination of *Pinus ayacahuite* var. *veitchii*, b) to identify the substratum with the best influence on the growth (in both height and diameter) of this species, and c) to determine its Slenderness ratio.

Materials and Methods

The experiment was carried out at the forest nursery of the *"Unión Campesina Independiente de Protectores de Bosques"* ("Independent Farmers' Union of Forest Guardians"), located at the geographical coordinates 17°15'20.98'' N and 98°43'35.18'' W, at 1 830 m, in the community known as *Plan de Guadalupe*, in *Tlacoapa, Guerrero* (Figure 1). The climate is A(C)w_2(w), *i.e.* semi-warm subhumid (INEGI, 2008). The predominant soil type is a eutric Regosol with a coarse texture (INEGI, 2014). The vegetation corresponds primarily to a low deciduous forest and a pine-oak forest (Inegi, 2016).



Leyenda = Leyend; Escalímetro = Scale

Figure 1. Location of the study area.

Germplasm and substrate

The germplasm of *Pinus ayacahuite* var. *veitchii* was acquired at the seed bank of the *Protectora de Bosques del Estado de México* (Forest Protection Agency of the State of Mexico) (Probosque); the seeds were collected, on April 3rd and 4th, 2014, at *La Ciénaga, in Santa Cruz Pueblo Nuevo, Tenango del Valle*, State of Mexico, with the batch number 216104914 GC- 2015. The germination percentage once out of the bank was 93.33 to 99.86 %.

The compost was made four months in advance, using straw, weeds, goat manure, and green and dry leaves; the vermicompost consisted of cow dung from a livestock farm located in the city of *Tlapa de Comonfort*, *Guerrero*; and the forest soil was collected at a pine-oak forest of *Xocoapa*, in *Tlacoapa, Guerrero*, eight days before planting. Copper Block expanded polystyrene containers with 77 cavities, with a volume of 170 cm^3, a length of 60 cm, a width of 35 cm, and a height of 16 cm.

Sowing and irrigation

The substrate was irrigated at field capacity 12 hours before the sowing, which was carried out on October 20th, 2015; for this purpose, a seed was manually placed in each cavity at a depth of 1 cm (Musálem and Ramírez, 2003). The first irrigation was applied on the day of the planting; after the seedling emerged, it was watered every other day (Romero-Arenas *et al.*, 2013), with an aspersion system installed at the nursery.

Experimental design

The experimental design was fully random, with four treatments and a control, with four repetitions of each treatment. Each was applied in a tray with 77 cavities, during the germination phase, for a total of 20 experimental units. The substrate mix for each treatment was formulated based on three components: forest soil (fs); vermicompost (vc) and compost (cp); CT (100 % fs); T1 (50 % fs + 50 % vc); T2 (50 % fs + 50 % cp); T3 (50 % fs + 30 % vc + 20 % cp), and T4 (50 % fs + 20 % vc + 30 % cp). During the growth phase, 120 seedlings per treatment, and 30 per tray were selected at random; a period of 174 days was considered as the lowest survival.

Germination, growth and slenderness ratio

Germination in *Pinus ayacahuite* var. *veitchii* had a duration of 30 days; in order to keep better control of the experiment, periodical quantifications were carried out at 5, 10, 15, 20, 25 and 30 days. The growth of the seedlings was assessed on April 11, 2016, *i.e.* 174 days after the planting. The height was measured in centimeters (cm) with an ACME DR30 aluminium rule calibrated in centimeters from the substrate level to the tip of the plant; the diameter was measured in millimeters (mm) from the stem base with a Mitutoyo CD-6"CSX digital vernier caliper. The slenderness ratio was calculated based on the height quotient in cm divided by the stem diameter in mm (Mateo *et al.*, 2011).

Statistical analysis

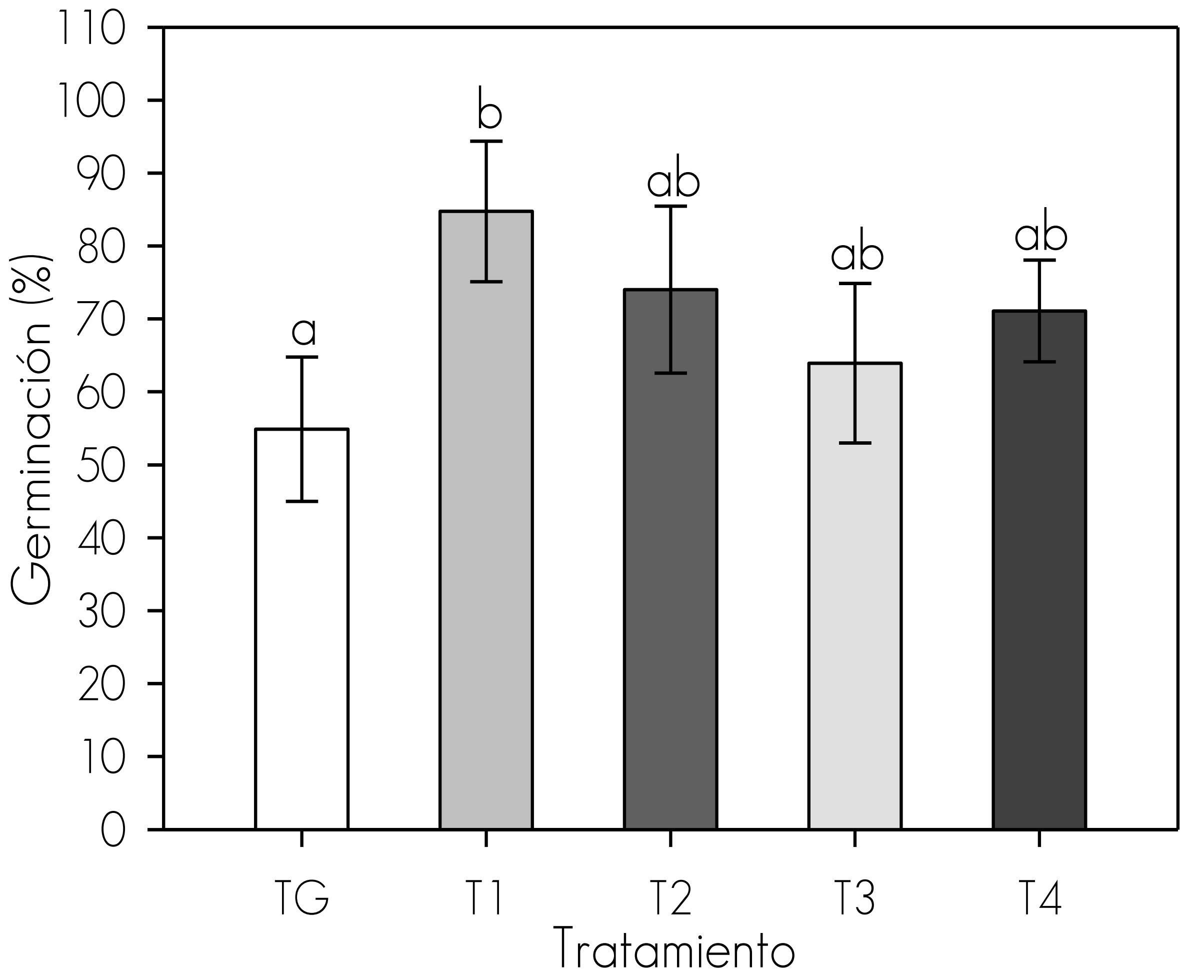
Variance analyses were carried out for the variables germination (%), stem diameter (mm), height (cm) and slenderness ratio. When significant differences ($p < 0.05$) between treatments were observed, a Tukey's mean comparison test was carried out (Steel and Torrie, 1980). All analyses were performed using the IBM SPSS Statistics software, version 20 (SPSS, 2011).

Results and Discussion

Germination

In general, *P. ayacahuite* var. *veitchii* completed its germination at 30 days. The variance analysis indicated a difference between treatments ($p = 0.009$) (Table 1). T1 (50 % fs + 50 % vc) registered the highest germination percentage, *i.e.* 84.73 % (Figure 2); this value is similar to that cited by Villagómez and Carrera (1985), of 80 %. The result of Romero-Arenas *et al.* (2013), who used a substrate of 50 % walnut shell compost + 25 % agrolite + 25 % vermiculite for the germination of *Pinus patula*, was 84.25 %. In contrast, Quiroz *et al.* (2008) obtained 73.2 % germination in *Pinus pinea* L. with the use of composted pine bark.

On the other hand, the CT reached only 54.70 % of germination, a much lower percentage than the high germination values registered by Aparicio *et al.* (1999), with 100 % forest soil, for *Pinus patula* (92 %), *Pinus montezumae* Lamb. (82 %) and *Pinus pseudostrobus* Lindl. (78.2 %). For T2 (50 % forest soil + 50 % compost) had 74.03 % germination, while *Jatropha* spp. had 95 % (Martiñón and Aragón, 2014).



Germinación = Germination; *Tratamiento* = Treatment

The vertical lines represent the standard deviation; different letters above the bars correspond to statistical differences (p≤0.05).

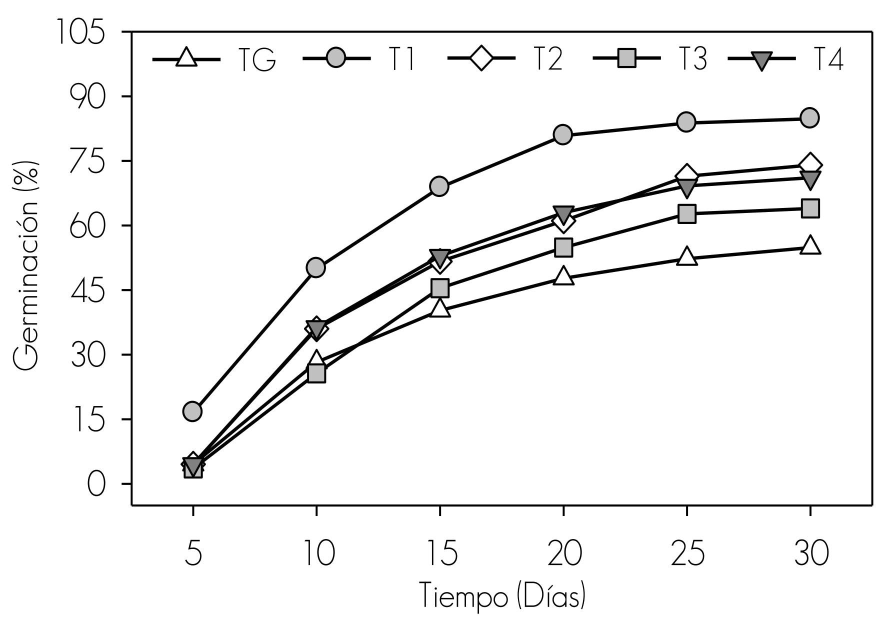
Figure 2. Germination of *Pinus ayacahuite* var. *veitchii* (Roezl) Shaw at 30 days.

The variance analysis by time point indicated significant differences only at 5 days (p<0.05), for the rest of the time germination was statistically equal (Table 1); notably, T1 had a higher percentage (16.56 %), above the mean of the other treatments, which attained a mere 6.57 % at 5 days (Figure 3).

Table 1. Result of the variance analysis for germination by time intervals and in general for *Pinus ayacahuite* var. *veitchii* (Roezl) Shaw.

Time (days)	Statistics	
	F Value	**P**
5	3.531	0.032*
10	1.651	0.213
15	0.950	0.462
20	0.577	0.375
25	1.142	0.375
30	0.897	0.490
General	5.088	0.009*

*= Significant (p≤0.05).



Germinación = Germination; *Tiempo (días)* = Time (days)

Figure 3. Accumulated germination in *Pinus ayacahuite* var. *veitchii* (Roezl) Shaw at 5, 10, 15, 20, 25 and 30 days.

As shown in Figure 3, the most active period in the germination phase occurred at 10, 15 and 20 days, while the growth became stabilized at 25 and 30 days, when the last germinations took place, until the process was completed, at 30 days. The first germinations occurred on the third day, unlike the 5 and 6 days registered by Villagómez and Carrera (1985) for the first germinations of the same taxon.

T1 remained the best treatment throughout the experimental phase, while CT began slightly above T3, at 5 and 10 days, and was surpassed by T3, at 15, 20, 25 and 30 days; eventually, CT was below all other treatments (Figure 3).

Diameter, Height and Slenderness ratio

Generally, *Pinus ayacahuite* var. *veitchii* registered a growth in diameter of 2.07 mm, a height of 5.096 cm and a Slenderness ratio of 1.865; differences between treatments occurred at 174 days for the three variables, according to the variance analysis (Table 2).

Table 2. Results of the variance analysis for the assessed variables in the growth of *Pinus ayacahuite* var. *veitchii* (Roezl) Shaw at 174 days.

Variable	Statistics		
	Mean	**F value**	**P**
Diameter	2.072±0.3672 mm	16.259	< 0.001
Height	5.096±1.5432 cm	16.092	< 0.001
Slenderness ratio	1.865±0.4814	6.031	<0.001

In the Tukey mean comparison test, T4 had the largest diameter, of 2.85 mm, followed by T3, with 2.78 mm, while T2, T1 and CT were statistically equal, with diameters of 2.71, 2.66 and 2.58 mm, respectively (Table 4). The values for T4 are similar to those cited by Chávez *et al.*

(2014), of 2.9 mm at 10 months in *Pinus patula* with a composted pine bark substrate; conversely, Altamirano (2002) registered the highest growth rates (3.46 mm), with 30 % mine sand + 20 % forest soil + 50 % vermicompost, seven months after the transplanting of *Pinus ayacahuite*. Finally, Sáenz *et al.* (2010) registered values of 5.2 to 5.6 mm in annual production cycles (75 % forest soil + 25 % compost) for *Pinus ayacahuite*; the difference is that fertilization was applied in this research, and the plants were produced in 10 cm × 20 cm black polyethylene bags.

T4 (50 % fs + 20 % vc + 30 % cp) exhibited the largest height, of 5.73 cm; in contrast, the lowest measures, of only 4.82 and 4.58 cm, were for T1 and CT, respectively (Table 4). Altamirano (2002) documented a height of 15.68 cm, with 30 % mine sand + 20 % forest soil + 50 % vermicompost, seven months after the transplanting of *Pinus ayacahuite* in 18 × 25 cm black polyethylene bags. In the present study, the CT yielded lower values than those obtained by Altamirano (2002) when assessing the initial growth of *Pinus oaxacana* Mirov. and *Pinus rudis* Endl., of 6 and 5.16 cm, respectively. Values of 20.88 and 24.34 cm in height were cited for the annual production cycle in *Pinus ayacahuite*, when fertilized every two weeks, from January to May, with 18-46-00 and 46-00-00, each time in doses of 4 kg 200 L^{-1} of water (Sáenz *et al.*, 2010).

The Slenderness ratio between treatments ranged between 1.72 and 2.00, with a marked statistical difference in T1 and T4 (Table 3), which suggests a greater size and quality of the seedlings in T4. This is similar to the ratio of 2.01 cited by Sallesses *et al.* (2015), with a 100 % chicken-manure compost substrate, for the hybrid of *Eucalyptus grandis* × *Eucalyptus camaldulensis*. In contrast, Sáenz *et al.* (2010) cite high values of 4.16 to 4.18 for the annual production of *Pinus ayacahuite* with fertilizers 18-46-00 and 46-00-00, applied every two weeks.

Table 3. Mean comparison test of diameter, height and slenderness ratio in *Pinus ayacahuite* var. *veitchii* (Roezl) Shaw.

Treatment	N	Variables		
		Diameter (mm)	Height (cm)	Slenderness ratio
CT	120	2.58 a	4.82 ab	1.83 ab
T1	120	2.66 ab	4.58 a	1.72 a
T2	120	2.71 ab	5.22 bc	1.92 bc
T3	120	2.78 bc	5.12 b	1.84 abc
T4	120	2.85 c	5.73 c	2..00 c

Different letters indicate significant differences.

Conclusions

The use of a 50 % fs + 50 % vc substrate for the germination of *Pinus ayacahuite* var. *veitchii* is viable, given that the result was satisfactory, compared to all other treatments, which ensured a certain degree of uniformity at this phase.

In regard to growth, the substrate mix of 50 % fs + 20 % vc + 30 % cp yielded better results for diameter, height and slenderness ratio, all essential characteristics for the field establishment of *Pinus ayacahuite* var. *veitchii*.

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Conflict of interests

The authors declare no conflict of interests.

Contribution by author

Bernardo López López: drafting of the manuscript and research development; Paula Gálvez Arce: research development and field work; Beatriz Calleja Peláez: in-field support and mapping; Jorge Méndez González: review and editing of the manuscript; Juan Manuel Ríos Camey: review of the manuscript and statistical analysis.

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