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Original article

Effect of temperature treatments on vegetative and productive characteristics in garlic, 'Vietnamita' clone

Efecto de tratamientos de temperatura sobre características vegetativas y productivas en ajo, clon 'Vietnamita'

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ABSTRACT: Garlic (*Allium sativum* L.) is an annual plant; in our country it only reproduces asexually through bulbils from the previous harvest. This facilitates the transmission of systemic diseases to offspring and causes losses in terms of yield and quality of clones. Among the management strategies is the application of thermotherapy at high temperatures using air, hot water or water vapor. The objective of this work was to use different temperature treatments on vegetative and productive characteristics in Vietnamese clone garlic. For this, "teeth" from the 2019-2020 harvest were used, subjected to different degrees of temperature in a water bath. Planting was carried out 48 hours after drying the bulbs in the open air. A randomized block design with three replications was used, the data were processed using a Simple Ranking Analysis of Variance (ANOVA) and the differences between the means were determined using the Tukey test ($p \leq 0.05$). The best treatment (40 ºC/40 min) obtained 16.83 % sprouting at 14 days after planting, 7.82 leaves/plant at 120 days and 55.3 mm equatorial diameter of the bulb. In turn, significant differences were found in the different treatments, with 50 ºC/30 min there was a greater number of leaves at 90 and 120 days after planting with 11.27 and 8.40 leaves respectively, and 1.214 kg of yield, while that, with 30 ºC/50 min, 19.36 g of fresh mass was obtained in the bulb.

Key words: water, *Allium sativum* L., heat, yield, quality.

RESUMEN: El ajo (*Allium sativum* L.) es una planta anual, en nuestro país solo se reproduce de forma asexual mediante bulbillos provenientes de la cosecha anterior. Esto facilita la transmisión de enfermedades sistémicas a la descendencia y provoca pérdidas en cuanto a rendimiento y calidad de los clones. Dentro de las estrategias de manejo se encuentra la aplicación de termoterapia a altas temperaturas mediante aire, agua caliente o vapor de agua. El objetivo de este trabajo fue emplear diferentes tratamientos de temperatura sobre características vegetativas y productivas del ajo, clon 'Vietnamita'. Para ello, se emplearon "dientes" de la cosecha 2019-2020, sometidos a diferentes grados de temperatura en un baño de María. La plantación se realizó 48 horas después del secado de los bulbos al aire libre. Se utilizó un diseño de bloques al azar con tres réplicas, los datos se procesaron mediante un Análisis de Varianza de Clasificación Simple (ANOVA) y las diferencias entre las medias se determinaron mediante la prueba de Tukey ($p \leq 0,05$). Con el mejor tratamiento (40 ºC/ 40 min se obtuvo un 16,83 % de brotación a los 14 días después de la plantación, 7,82 hojas/planta a los 120 días y 55,3 mm de diámetro ecuatorial del bulbo. A su vez, se encontraron diferencias significativas en los diferentes tratamientos, con 50 ^{0}C/30 min se presentó mayor número de hojas a los 90 y 120 días de plantados, con 11,27 y 8,40 hojas respectivamente, y 1,214 Kg de rendimiento, mientras que, con 30 ^{0}C/50 min se obtuvo 19,36 g de masa fresca en el bulbo.

Palabras clave: agua, *Allium sativum*, calor, rendimiento, calidad.

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INTRODUCTION

Garlic (*Allium sativum* L.) is a plant of national and global importance due to its wide distribution and extensive cultivated area. At present, it is concentrated in the Asian continent in countries such as China, India, South Korea and Bangladesh. It also stands out in Spain on the European continent, and other nations such as Egypt, Argentina and Brazil in the rest of the world (1).

It is a crop very sensitive to environmental conditions and its development period is very limited in the year (2). In the edaphoclimatic conditions of Cuba, the most used clones are "Criollo" and "Vietnamita" with yields between 4-9 t ha^{-1} and average values of 6 t ha^{-1} in comparison with other producing countries that produce XXX (3).

Garlic is a species where all cultivars are sterile; reproduction is vegetative, by means of bulblets (cloves) that serve as propagation material (4), so “cloves” are planted from bulbs harvested in the previous season (5).

Like other vegetatively propagated crops, garlic is susceptible to systemic diseases caused by fungi, bacteria and viruses that spread to the rest of the crop, causing considerable losses in yield and bulb quality (6).

Among the management strategies is thermotherapy as a physical method (7). It consists of introducing the explants to treatment based on the application of dry or humid heat with variation of temperature and duration according to the treatment and plant species. The thermal treatments most frequently used in this crop are temperatures ranging between 35 and 38 °C (8).

It has been proven that garlic subjected to thermotherapy keeps better until the date of planting (generally between November and December). On the other hand, planting garlic cloves from bulbs treated with thermotherapy will generate plants with good sprouting and vegetative development (9).

The objective of this work was to use different temperature treatments on vegetative and productive characteristics in garlic (*Allium sativum* L.) "Vietnamita" clone.

MATERIALS AND METHODS

The experiment was carried out in two phases of development. The first phase was carried out in the Departments of Plant Physiology and Biochemistry and Plant Genetics and Improvement at the National Institute of Agricultural Sciences (INCA), Cuba. In this phase, the garlic “cloves” were subjected to temperature treatments.

The second phase was carried out in a family orchard located in the Batey “Curva Cabeza de Toro”, Melena del Sur municipality, Mayabeque province, where the previously treated agricultural seed was planted according to the growing season between October/February, the climatic data are shown in Table 1.

Plant material: Stored garlic bulbs of the Vietnamita clone (2019-2020) were used.

Experiment performed

Bulbs were subjected to different temperature treatments in a thermostated water bath without water recirculation, brand VWR (United Kingdom).

Treatments

1. Control (without temperature application).
2. Temperature 10 ºC for 70 min.
3. Temperature 20 ºC for 60 min
4. Temperature 30 ºC for 50 min
5. Temperature 40 ºC for 40 min
6. Temperature 50 ºC for 30 min
7. Temperature 60 ºC for 20 min

Seven days before planting, 10 garlic bulbs per treatment were randomly selected, shelled and subjected to heat treatment during the day.

The cloves were placed in gauze (5 cloves per gauze), to maintain the temperature as homogeneous as possible and then placed in a water bath. After the heat treatments were applied, the teeth were dried in the shade on “Whatman 42” filter paper.

The plant material was transferred to the field in individual envelopes, 48 hours before planting in a Ferrallitic Red compacted eutrophic soil according to the 1:250 000 map and the new version of Soil Classification (10).

The experiment was carried out in an area of 2.16 x 5.52 m, with a planting distance of 0.05 x 0.68 m.

Preparation of soil

Soil preparation was carried out according to the norms of the Technical Instructions for the production of this vegetable in the country (2) and consequently it was disinfected with *Trichoderma harzianum* Rifai, a biological control produced in the Center for Reproduction of Entomophagous and Entomopathogens (CREE) of Melena del Sur based on rice head (*Oryza sativa* L.), for the control of pathogenic fungi of the soil and some foliar diseases. Afterwards, background fertilization was carried out with the application of the complete NPK formula (9-13-17).

Table 1. Climatic data for the months of October 2020-February 2021 obtained at the Melena del Sur Experimental Station, Mayabeque, Cuba

Month	Average temperature (ºC)	Rainfall (mm)	Relative humidity (%)
October	26.2	120.7	86
November	24.9	30.0	72
December	24.3	20.0	73
January	23.2	25.0	72
February	24.1	24.0	71

A soil analysis was carried out, for which a representative sample was taken using the English Flag method for subsequent analysis in the INCA Chemical Analysis laboratory. It consisted of assimilable phosphorus (P_2O_5), exchangeable calcium (Ca^{2+}), exchangeable magnesium (Mg^{2+}), organic matter (M.O) and pH determinations. The chemical characteristics of the soil are shown in Table 2.

Table 2. Soil chemical characteristics in the planting area of garlic (*Allium sativum* L.) clone Vietnamita

Soil components	Values
pH	7.60
OM (%)	2.52
N (%)	1.46
P (ppm)	154.00
Ca (Cmol/Kg)	18.00
Mg (Cmol/Kg)	2.00

Cultivation work

Cultural attentions were carried out according to the Technical Instructions (2), and applications of *T. harzianum*, Abaco® (insecticide, acaricide with contact and stomach action) and NPK were also made, by means of fumigation backpacks, in all treatments. In addition, fumigations were made with BRAVO® (contact fungicide) and MANCOZEB® (contact fungicide).

Variables to be evaluated

The following evaluations were carried out seven days after planting:

- Sprouting (7 and 14 days after planting (dap).
- Plant height (cm) at 90, 100 and 120 dap.
- Number of leaves at 90 and 120 days dap.

Harvesting was performed when 75 % of the plants changed the color of their leaves from green to yellowish and the false stem lost its rigidity and bent. After harvesting, three bulbs per treatment were randomly selected and then the following evaluations were made:

- Bulb fresh mass (g)
- Bulb polar diameter (mm)
- Equatorial bulb diameter (mm)
- Number of teeth per bulb
- Pseudostem diameter (mm)
- Yield (kg experimental area^{-1})

After 30 days of sun-drying the garlic bulbs, 3 bulbs per treatment were randomly selected and the following evaluations were made:

- Bulb dry mass (g) [the same bulbs to which fresh mass was measured]. They were placed in an oven at 75 ºC for 72 hours until constant mass.
- Polar bulb diameter (mm)
- Equatorial bulb diameter (mm)
- Pseudostem diameter (mm)

Experimental design and data analysis

A randomized block design with three replicates was used. Data were processed by simple rank analysis of variance (ANOVA) and differences between means were determined by Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The data related to the percentage of sprouting are shown in Figure 1. At seven days, as can be seen, all plants showed 0 % sprouting. On the other hand, at 14 days, significant differences were observed, the best results were obtained by the plants of treatment 1 (Control) with 16.83 % of sprouting. The lowest result was obtained by the plants of treatment 7 (60 ºC/20 min) with 0 %. This variable was affected in the first week as a result of subjecting the garlic cloves to temperature changes that cause slight stress.

Several authors state that some plant species do not suffer serious lesions when subjected to high temperatures, while others may suffer negative effects due to severe damage and collapse at the cellular level in a matter of minutes (11).

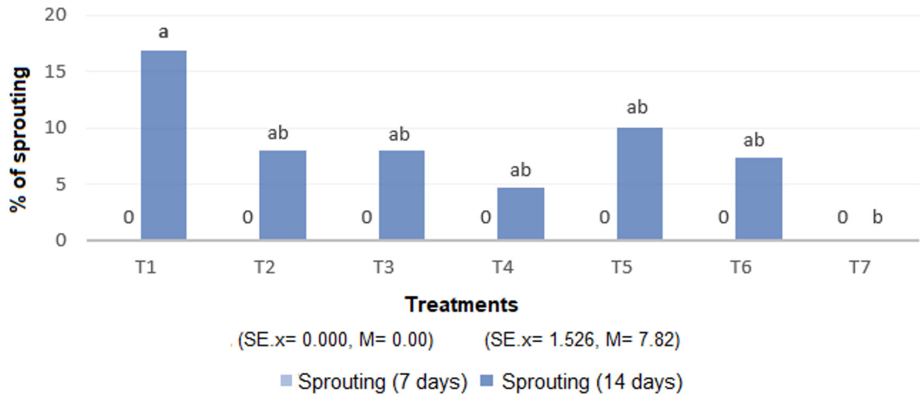
Some authors obtained superior results when applying thermotherapy to garlic cultivation. Seedling emergence was significantly higher than that of their controls in 9 of the 22 genotypes evaluated. However, in 50 % of the genotypes, no difference was detected in this variable; the range of emergence percentages was 20.8-93.3 and 5.6-89.6 % in thermo-treated bulb cloves and controls, respectively (12).

The use of high temperature to eliminate or reduce the "viral load" has been defined as the production of a progressively less suitable cellular environment for the viral agent(s) (13).

Other studies showed different results when bulblets were subjected to dry heat thermotherapy at 5 ºC for 20 days, the highest percentage of sprouting was reached at 12 days (dap) with 80 %, while the rest of the treatments (35-38-48-50 ºC) reached this percentage between 22-32 days (dap). However, it was remarkable the delay for this variable on bulblets submitted to thermotherapy by immersion in hot water at 48 ºC that only reached 40 % of sprouting at 22 days (dap), this result was similar after applying 60 ºC during 20 minutes and the sprouting started after 14 days of planting the crop (14).

With respect to plant height, there were no significant differences at 90 and 100 days of cultivation, while at 120 days there were differences between treatments (Figure 2). At 120 days, the control treatment with 42 cm was the most favorable. The lowest value was presented in treatment 7 (60 ºC/20 min) with 36.11 cm height. An adequate temperature can promote a more vigorous development of the plant.

Several authors suggest that thermotherapy has been proposed as an intermediate step in the process of obtaining virus-free seedlings, which could be translated into increases in the agro-morphological characteristics of the crop (15).

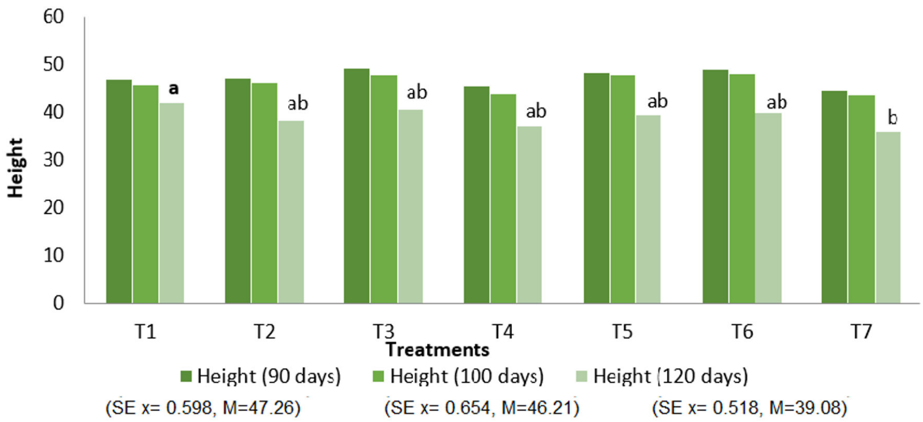


1- Control (without application of temperature). 2-Temperature 10 ºC for 70 min. 3-Temperature 20 ºC for 60 min. 4- Temperature 30 ºC for 50 min. 5-Temperature 40 ºC for 40 min. 6- Temperature 50 ºC for 30 min. 7-Temperature 60 ºC for 20 min

SE.x.-standard error of the mean M- mean

Means with different letters differ statistically according to Tukey's test (p≤0.05) (*** significant for p(0.001)

Figure 1. Percentage of sprouting of garlic (*Allium sativum* L.) 'Vietnamita' plants subjected to different heat treatments at 7 and 14 days after planting (n=21)



1- Control (without temperature application). 2- Temperature 10 ºC for 70 min. 3- Temperature 20 ºC for 60 min. 4- Temperature 30 ºC for 50 min. 5- Temperature 40 ºC for 40 min. 6- Temperature 50 ºC for 30 min. 7- Temperature 60 ºC for 20 min. SE x.- standard error of the mean .M.- mean. n- total explants of the experiment in the three replicates

Means with different letters differ statistically according to Tukey's test (p≤0.05) (*** significant for p≤0,001)

Figure 2. Height of garlic (*Allium sativum* L.) 'Vietnamita' plants treated or not with different temperature treatments at 90, 100 and 120 days after planting (n=146)

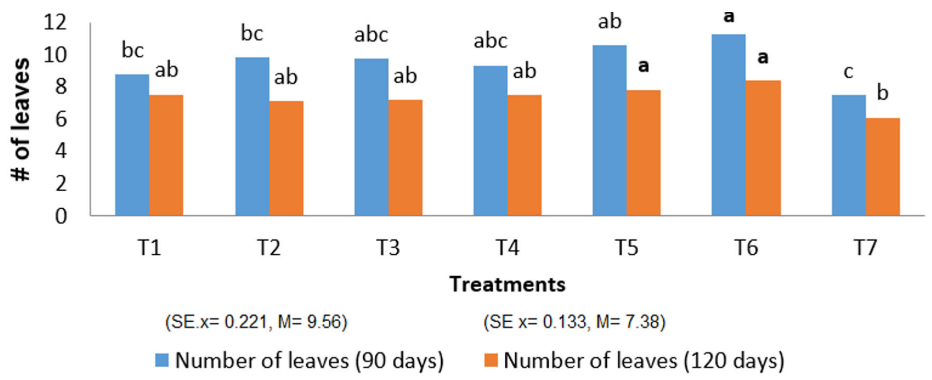
Some researchers achieved superior results by subjecting garlic to a storage temperature of 33 °C for six weeks. Plant height was significantly higher than that of their controls (marbled, white, and purple) in 13 of the 22 genotypes evaluated. In contrast, the remaining eight genotypes showed no differences for this variable in plants from heat-treated bulbs and their controls (12).

Different results obtained showed that the exposure of purple garlic “seed” to temperatures between 4-5 ºC for periods of time longer than 30 days significantly favored leaf length in less time (14).

With respect to the number of leaves per plant, there were significant differences among the treatments evaluated (Figure 3). The highest value at 90 days was obtained in treatment 6 (50 ºC/30 min) with 11.27 leaves/plant, while, at 120 days the most favorable results were reached in treatments 5 (40 ºC/40 min) and 6(50 ºC/30min) with 7.82 and 8.40 leaves/plant. Treatment 7 (60 ºC/20 min) presented the lowest values with 7.47 and 6.11 leaves/plant at 90 and 120 days. The use of adequate warm temperatures could favor this variable as shown in the results obtained.

Inferior results were shown with the application of thermotherapy, in seven of 22 garlic genotypes evaluated there were significant differences with respect to the number of leaves per plant. The highest values ranged between 5.3-4.3 leaves/plant (12).

Researchers state that heat treatment does not show differences on the number of leaves between treated and untreated plants (16). At the same time, other authors with different results showed that after applying thermotherapy with dry heat at 5 ºC for 20 days, the plants had a greater number of leaves at 90 days (dap) compared to the other treatments. In the following days, this variable was balanced among treatments (14).



1- Control (without temperature application). 2- Temperature 10 ºC for 70 min. 3- Temperature 20 ºC for 60 min. 4- Temperature 30 ºC for 50 min. 5- Temperature 40 ºC for 40 min. 6- Temperature 50 ºC for 30 min. 7- Temperature 60 ºC for 20 min

SE.x.- standard error of the mean .M.- mean. n- total explants of the experiment in the three replicates

Means with different letters differ statistically according to Tukey's test (p≤0.05) (*** significant for p(0.001)

Figure 3. Number of leaves per plant of garlic (*Allium sativum* L.) 'Vietnamita' treated or not with different temperatures at 90 and 120 days after planting (n=132)

Table 3. Fresh mass, dry mass, polar diameter, equatorial diameter and fresh and dry pseudostem diameter, number of cloves per bulb and clove length of garlic (*Allium sativum* L.) 'Vietnamita' plants treated or not with different temperature treatments (n= 21)

Treatments	Fresh bulb mass (g)	Bulb dry mass (g)	Polar diameter of bulb (mm)		Equatorial bulb diameter (mm)		Numbre of cloves per bulb	Diameter of pseudostem (mm)	
			Fresh	Dry	Fresh	Dry		Fresh	Dry
T1	**17.91 a**	**15.19 a**	43.3	41.0	**53.3 a**	51.3 ab	11	12.0	8.66
T2	16.68 ab	13.25 abc	43.0	41.3	48.3 ab	43.0 ab	10	12.6	9.00
T3	15.69 ab	9.18 bc	42.3	39.6	51.3 ab	49.3 ab	11	18.3	13.33
T4	**19.36 a**	13.50 ab	38.6	24.6	48.0 ab	46.6 ab	10	16.3	12.33
T5	15.72 ab	13.71 ab	42.3	40.3	**55.3 a**	**52.6 a**	12	17.3	10.33
T6	11.58 bc	11.02 abc	40.0	38.3	48.0 ab	46.0 ab	9	16.3	11.33
T7	8.83 c	7.54 c	39.3	37.6	41.3 b	39.0 b	9	13.6	10.33
S. Ex (±)	0.846	0.685	0.951	1.923	1.204	1.285	0.324	0.888	0.755
M	15.11	11.91	41.25	37.52	49.35	46.82	10.28	15.f2	10.75

1- Control (without temperature application). 2- Temperature 10 ºC during 70 min. 3- Temperature 20 ºC during 60 min. 4- Temperature 30 ºC during 50 min. 5- Temperature 40 ºC during 40 min. 6- Temperature 50 ºC during 30 min. 7- Temperature 60 ºC during 20 min. SE x.- standard error of the mean .M.- mean n- Total explants of the experiment in the three replicates. Means with different letters differ statistically according to Tukey's test (p≤0.05) (*** significant for p≤0.001)

Table 3 shows the results related to fresh and dry bulb mass content in garlic plants. As can be seen, in both cases there were significant differences between the treatments evaluated. For case one the most favorable treatments were T1 (absolute control) with 17.91 g and T4 (30 ºC/50 min) with 19.36 g, in the second case only T1 (absolute control) was the most favorable with 15.19 g. In turn, in both cases the lowest value was for T7 (60 ºC/20 min) with 8.83 g and 7.54 g, respectively.

The application of temperatures above 40 °C can impair bulb development in garlic plants, whereas, temperatures around 30 °C improve crop quality.

High temperatures and prolonged periods of thermotherapy can have a negative effect on plant development (11).

Other authors observed different results, thermotherapy treatments by dry heat at 35°C/12h and by immersion in hot water at 48 °C/30 min 150 days after planting, showed an accumulation of biomass in the bulb much lower than the other treatments (14).

Other authors reached inferior results, showing that the application of thermotherapy with dry heat at 5 ºC for 20 days showed the best result after the absolute treatment with 5.27 and 6.04 g, respectively, at 150 (dap). Plants treated with hydrotherapy showed a lower development during the evaluation (14).

With respect to the polar diameter of the bulb, there were no significant differences between the treatments evaluated, while for the equatorial diameter there were differences for both cases (Table 3). The highest values in case one were obtained in treatments 1 (absolute control) with 53.3 and 5 (40 ºC / 40 min) with 55.3 cm, which in turn was the most favorable in the second case with 52.6 cm. Likewise, in treatment T7 (60 ºC/20 min) reached the lowest values with 41.3 mm and 39.0 mm respectively.

The use of thermotherapy at appropriate temperatures improves the quality of garlic bulbs by reducing or eliminating any pathogen present in them and allows obtaining good postharvest results.

The decrease in diameter in symptomatic garlic plants demonstrates the effect of thermotherapy on this variable.

On this variable, it is suggested to use healthy garlic cloves in each production cycle, which can be achieved through thermotherapy (17).

Some authors obtained inferior results when sanitizing the 'Criollo-9' clone. This clone performed well in field conditions, with a polar and equatorial diameter of 3.9-4.05 and 3.4-3.9 cm (5).

Some studies indicate that in Argentina, Garlic virus A caused reductions between 6 and 11 % in the diameter of Morado-INTA and Blanco-IFFIVE garlic varieties (18).

Table 3 shows that there were no significant differences between treatments with respect to the number of cloves per bulb, which ranged from 9-12 cloves per bulb. The formation of teeth is not affected by applying thermotherapy; this technique only allows a more vigorous development of the plant.

Similar results show that in the reality of the field, thermotherapy has an effect against viruses, the garlic treated and then planted has a greener color, the spots of virosis decrease and they have a more vigorous growth (19).

Table 3 shows that there were no significant differences between treatments with respect to the diameter of the pseudostem. This does not interfere with plant development and allows plants to develop well by improving their quality.

Inferior results were achieved by some researchers with the use of thermotherapy on 22 garlic genotypes with respect to pseudostem diameter. It was shown that there were no significant differences in 17 genotypes, while in five of them there were differences with respect to this variable, the values ranged between 7.6 and 10.2 mm respectively (12).

Likewise, several authors' state that the application of thermotherapy (49 °C) does not show significant differences in garlic genotypes (Criollo and Chileno) when applying this technique (19).

When studying the multiplication of plants (16) through the *in vitro* culture system of the following garlic varieties: Burranquino, Napuri and Blanco Huaralino, different results were obtained and it is reported that heat treatment does not significantly affect the diameter of the pseudostem in the three varieties evaluated.

The results related to the yield of garlic bulbs treated or not by treatment are shown in Table 4. The highest values were reached at T4 (30 ºC/50 min) and T6 (50 ºC/30 min) with 1.574 and 1.214 kg, respectively. In turn, the lowest weight was obtained at T7 (60 ºC/20 min) with 0.257 kg. The exposure of the plant to a suitable temperature, either by means of water or dry heat, allows obtaining good yields.

The heat treatment allows obtaining more vigorous plants with better yields (20), referring that the thermotherapy is proposed as a step of control and obtaining virus-free seedlings, which in the case of garlic cultivation these accumulate in the bulblets and can cause severe damage to their plantations.

Tabla 4. Weight (kg) per treatment of garlic (*Allium sativum* L.) 'Vietnamita' plants treated or not with different temperature treatments (n=146)

Treatments	weight (Kg)
T1 (Control Absoluto)	0.369
T2 (10 ºC/70 min)	0.412
T3 (20 ºC/60 min)	0.973
T4 (30 ºC/50 min)	**1.574**
T5 (40 ºC/40 min)	0.587
T6 (50 ºC/30 min)	**1.214**
T7 (60 ºC/20 min)	0.257
Total	**5.386**

Inferior results were obtained after applying thermotherapy treatments at 49 °C in two genotypes (Criollo and Chileno), more favorable yields were achieved, with a more marked difference for the Chileno with superior results than the Criollo (19).

In all cases, the best results were achieved by plants with temperatures 30 - 50 ºC. 'Vietnamita' garlic plants were vigorous and resistant throughout their development. The application of temperature, either by immersion in water or by dry heat, is a technique that does not damage the plant material as long as it is used correctly. In this way, plants are obtained with a good birth and vegetative development, and being free of pathogenic organisms, it favors the achievement of superior values in terms of yield and quality.

CONCLUSIONS

1. Temperatures between 30 - 50 ºC, improved the growth and development of garlic (*Allium sativum* L.) 'Vietnamita' plants by being free of diseases.
2. The use of appropriate temperatures had a positive effect on morphological (sprouting, height, number of leaves, etc.) and physiological (fresh and dry mass, number of cloves, etc.) variables evaluated in garlic (*Allium sativum* L.) 'Vietnamita' plants.
3. Hydrotherapeutic treatment improves the yield of garlic (*Allium sativum* L.) 'Vietnamita' plants.

RECOMMENDATIONS

1. To apply temperatures between 30-50 ºC to determine its effect in garlic plants (*Allium sativum* L.) 'Vietnamita', as well as other clones.
2. To use hydrotherapeutic techniques in other crops of vegetative reproduction to establish an optimun temperature to improve its quality.

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