Growth and Foliar Alkaloid Content of *Clitoria ternatea* under Different Irrigation Frequencies

Desarrollo y contenido foliar de alcaloides de la conchita azul (*Clitoria ternatea*) con diferentes frecuencias de riego

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ABSTRACT

Clitoria ternatea is widely used in tropical livestock farming. This study evaluates the agronomic performance and alkaloid content of *C. ternatea* under different irrigation frequencies in protected cultivation. A randomized block design with controlled manual irrigation was implemented with five treatments, four replicates, and five experimental units per treatment. Treatments included daily irrigation (T0), irrigation every two days (T1), every three days (T2), every four days (T3), and every five days (T4). We evaluated plant height, leaf and internode number, leaf area, green and dry matter yield, and alkaloid content. Irrigation every three or four days generally resulted in the highest values for the measured variables compared to the control. Reduced irrigation frequency affects the agronomic performance of *C. ternatea* in protected cultivation. Agronomic indicators were highest under irrigation every three or four days. Alkaloid content did not vary with irrigation frequency.

Keywords
alkaloids • height • leaf area • number of leaves • yield

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Resumen

La *Clitoria ternatea* constituye una de las especies de mayor uso en la ganadería del trópico. El objetivo de este trabajo es evaluar el comportamiento agronómico y los alcaloides de la *C. ternatea* al aplicar diferentes frecuencias de riego en cultivo protegido. Se empleó un diseño en bloques al azar. Se emplearon cinco tratamientos con cuatro repeticiones cada uno, con cinco unidades experimentales cada una. Los tratamientos consistieron en diferentes frecuencias de riego, sin riego (T0), cada dos días (T1), cada tres (T2), cuatro (T3) y cada cinco (T4). Se evaluaron las variables altura de la planta, número de hojas y entrenudos, área de la hoja, rendimiento de materia verde y seca de la planta, así como los alcaloides. Las frecuencias de riego después de tres y cuatro días mostraron los mayores valores para las diferentes variables evaluadas, con respecto al control y resto de los tratamientos. La disminución de la frecuencia de riego no afectó el comportamiento agronómico de la *C. ternatea* en cultivo protegido. Los indicadores agronómicos fueron mayores para las frecuencias de riego de tres y cuatro días. Por otra parte, el contenido de alcaloides no varió en dependencia de la frecuencia de riego.

Palabras clave

alcaloides • altura • área de la hoja • número de hojas • rendimiento

Introduction

In Latin America and the Caribbean, legumes are grown with economically important crops to promote sustainable agricultural and livestock production systems (14, 20). *C. ternatea* (Fabaceae) is widely used in tropical livestock farming due to its adaptability, productivity, and nutritional value (22). These benefits improve animal diets and help meet feed demand during the dry season. They also promote the use of sustainable animal production techniques compatible with environmental protection.

However, in the tropics, the agronomic performance, yield, and chemical composition of this species are highly variable. This variability is influenced by plant age, management practices (especially irrigation and fertilization), and edaphoclimatic conditions (3, 23).

A previous study in Ecuador showed that plant quality decreases with age (10). However, as this research was conducted during the rainy season, it did not address irrigation effects. Furthermore, scarce information describes how irrigation affects the development and foliar alkaloid content of *C. ternatea* in Ecuador's various climatic conditions. Thus, this study aims to evaluate the agronomic performance and alkaloid content of *C. ternatea* under different irrigation frequencies in protected cultivation.

Materials and methods

Site and Experiment

The study was conducted at the Technical University of Cotopaxi's experimental area, El Triunfo sector, La Maná Cantón. This site has a maximum temperature of 30°C, a minimum of 17°C, relative humidity of 86.83%, average annual rainfall of 3029.30 mm, 735.70 light hours per year, and sandy loam soil (7). The experiment lasted 70 days, and irrigation was applied manually.

Seed Collection and Storage

Seeds were collected at the Sacha Wiwa Experimental Center, treated, and stored in the Germplasm Bank of the Technical University of Cotopaxi Extension, La Maná. Seeds were stored at 6.50% humidity and 4°C until sowing.

Soil Preparation, Sewing and Transplanting

Soil in the research area contains 6.5% organic matter and has a pH of 6.5; therefore, no amendments were necessary. Seeds were sown during the dry season (July) in polystyrene trays with 200 cells filled with common substrate from the experimental center. One seed was placed in each cell. Twenty days after sowing, seedlings with two to three true leaves were transplanted into polyethylene bags, one seedling per bag. These were immediately arranged according to the experimental design with guides for their climbing nature. All this work was carried out at the final study site. Weeds were controlled manually, and the area was disinfected by solarization (19).

The crop was protected with transparent plastic to prevent rain from interfering with treatments. Sides were covered with dark saran. Treatments were arranged on tables, one meter above the ground. Each table was divided into five sections, each representing a replicate. Variables were measured 10 days after transplanting.

Experimental Design and Treatments

The structured design with controlled manual irrigation had five treatments, four replicates and five experimental units, totalizing 100 plants. Treatments included daily irrigation (T0), irrigation every two days (T1), every three days (T2), every four days (T3), and every five days (T4).

Plant Height, Number of Leaves, and Leaf Area

Plant height was measured from the stem base to the tip of the terminal bud using a measuring tape, expressed in cm. Leaf number was averaged from 10 plants randomly selected per treatment. Leaf area was determined using the Smart Petiole application, calculating leaf area from scanned images. These variables were evaluated at 10, 20, 30, 40, and 50 days.

Node Number, Green and Brown Matter, and Alkaloid Content

Internode number was determined at 40 and 50 days after transplanting. Green and dry matter yields were measured by weighing 10 randomly selected plants per treatment at 50 days after emergence. Fresh weights were measured using a semi-analytical balance (precision: 0.01 mg). Dry matter was determined in an air-circulation oven (1). Results were expressed in tons (2).

Alkaloid content was determined using Dragendorff's reagent by mixing (a) 0.8 g pentahydroxy bismuth nitroxide in 40 ml distilled water and 10 ml acetic acid, and (b) 0.8 g potassium iodide in distilled water. The bismuth nitrate solution was prepared by dissolving 10 mg $Bi(NO3)3·5H2O$ in 5 mL concentrated nitric acid, then diluting to 100 mL with distilled water. Additionally, 3% thiourea and 1% disodium sulfide were required.

Alkaloid solutions were diluted in acidic, acid-alcoholic, and basic-alcoholic media (21). All solutions were centrifuged, and the precipitate was washed with alcohol and re-centrifuged. The resulting precipitate was mixed with 5 mL thiourea solution, and the compound formed was measured spectrophotometrically. Results were expressed as moles of alkaloids per gram (21).

Statistical Analysis

Analysis of variance (ANOVA) was performed, and means were compared using Tukey's test ($p \leq 0.05$). Normality was assessed using the Kolmogorov-Smirnov test, and homogeneity of variances was tested using Bartlett's test.

Results

Plant Height

At 10 days, treatment T4 showed the greatest plant height ($p<0.05$), with significant differences compared to other treatments. T1, T2, and T3 did not differ significantly, with values ranging from 10.36 to 10.60 cm. The control treatment had the lowest height, significantly different from the others (table 1, page 4).

Different letters in the same column indicate statistical differences for p<0.05.

T0= daily irrigation, T1= irrigation every two days, T2= irrigation every three days, T3= irrigation every four days, T4= irrigation every five days.

Letras desiguales en una misma columna difieren significativamente para p<0,05.

T0= irrigación diaria, T1= irrigación cada dos días, T2= irrigación cada tres días, T3= irrigación cada cuatro días, T4= irrigación cada cinco días.

Table 1. Height of *C. ternatea* (cm) according to evaluation days.

Tabla 1. Altura de la *C. ternatea* expresada en cm según días de evaluación.

Treatments	Age (days)				
	10	**20**	**30**	**40**	**50**
T0	9.30c	10.60c	13.05c	25.90c	40.45c
T1	10.36b	13.76b	17.89b	38.50a	48.82b
T2	10.52b	14.92a	17.04b	17.30d	30.71d
T3	10.60b	12.00b	21.54a	36.50ab	40.25c
T4	12.401a	15.01a	17.87b	33.80b	70.80a
EE±	0.89	0.45	1.23	2.83	3.57
P	0.0002	0.0012	0.0023	0.0001	0.0001

At 20 days, T2 and T4 reflected the highest values ($p<0.05$). T1 and T3 showed no differences between while T0 presented the lowest value (10.60 cm), significantly different from the rest (Table 1). At 30 days, T3 reached 21.54 cm (highest, significant value). Treatments T1, T2 and T4, ranging between 17.0 and 17.89 cm, did not differ from each other. The lowest value was shown by T0 (table 1).

Treatment T1 yielded the highest value at 40 days (38.50 cm), significantly differing from the others, while T3 and T4 did not differ from each other, and T2 showed the lowest height (17.30 cm). However, at 50 days, T4 reached the highest value, T0 and T3 ranged between 40.25 and 40.40 without significant differences, and T2 was the shortest (table 1).

Number of Leaves

At 10 days, treatment T3 had the greatest number of leaves ($p<0.05$) with 11.60, not significantly different from T2. T1, T2, and T4 did not differ significantly. The control (T0) had the lowest number of leaves, although this was not significantly different from T1 (table 2). After 20 days, the highest value was reached by T3 with significant differences compared to the rest. Treatments T0, T1, T2 and T4 showed no differences between themselves, with values between 11 and 12.40.

Different letters in the same column indicate statistical differences for p<0.05

T0= daily irrigation, T1= irrigation every two days, T2= irrigation every three days, T3= irrigation every four days, T4= irrigation every five days.

Letras desiguales en una misma columna difieren significativamente para p<0,05.

T0= irrigación diaria, T1= irrigación cada dos días, T2= irrigación cada tres días, T3= irrigación cada cuatro días, T4= irrigación cada cinco días.

Table 2. Number of leaves of *C. ternatea* according to evaluation days.

Tabla 2. Número de hojas de *C. ternatea* según días de evaluación.

Treatments	Age (days)				
	10	**20**	**30**	**40**	**50**
T0	9.00c	12.00b	14.20d	24.60b	41.20b
T1	9.40bc	12.40b	16.40c	27.60a	38.40b
T2	10.20ab	11.00b	18.60b	18.201c	27.40c
T3	11.60a	14.50a	22.20a	20.80c	48.00a
T4	9.40bc	12.40b	17.00bc	27.80a	41.40b
EE±	0.45	1.78	2.23	2.11	3.56
P	0.0001	0.0002	0.0019	0.0029	0.0013

Treatment T3 reached the highest value ($p<0.05$) at 30 days, with significant differences in contrast to the rest. However, T2 and T4 did not show differences between themselves, the control reflected the lowest height and was differentiated from the rest. At 40 days, T1 and T4 reached the highest numbers of leaves with significant differences compared to the rest. Consequently, T2 and T3 were the lowest, without significant differences between them. Thus, at 50 days, T3 reached the highest value (48), with significant differences from the rest. However, T0, T1 and T4 showed no differences, while T2 showed the lowest number of leaves (table 2, page 4).

Leaf Area

At 10 days, T2 and T3 showed the greatest leaf area ($p<0.05$), although T2 did not differ from T4. The lowest leaf area values were observed in T0 and T1, ranging between 3.28 and 3.64 cm^2. At 20 days, T2 and T3 did not show differences showed the highest values, while T4 did not present significant differences compared to T3 (table 3). The lowest value was from T0 with 3.35 cm^2.

Treatment T4 reflected the highest value leaf area at 30 days (8.13 cm^2) with significant differences with respect to T0, T1 and T3. T2 did not show differences with respect to T3, showing values between 6.99 and 7.79 cm^2. The smallest area was reflected by T0. At 40 days, T2 and T3 did not show significant differences, ranging between 14.90 cm^2 and 16.02.

At 50 days, control and T4 reflected the lowest values, 8.90 and 9.74 $cm^{2,}$ respectively, while T3 showed the significantly highest leaf area (table 3).

Table 3. Leaf area according to evaluation days.

Tabla 3. Área de las hojas según días de evaluación.

Treatments	Leaf area (cm^2) Age (days)				
	10	20	30	40	50
T0	3.28±0.10c	3.35±0.33c	4.14±0.34d	5.36±0.63c	8.90±0.62c
T1	3.64±0.24c	5.14±0.53b	5.18±0.54c	8.95±0.82b	13.12±2.27b
T2	5.67±0.23ab	6.75±0.35a	7.79±0.42ab	14.90±0.58a	14.40±1.67ab
T3	6.38±0.41a	6.54±0.32ab	6.99±0.31b	16.02±0.59a	16.84±3.78a
T4	5.19±0.12b	5.84±0.74b	8.13±0.83a	7.29±0.66bc	9.74±2.89c
EE±	0.24	0.32	0.89	1.89	1.58
P	0.0029	0.0034	0.0012	0.0002	0.0041

Different letters in the same column indicate statistical differences for $p<0.05$.

T0= daily irrigation, T1= irrigation every two days, T2= irrigation every three days, T3= irrigation every four days, T4= irrigation every five days.

Letras desiguales en una misma columna difieren significativamente para $p<0,05$.

T0= irrigación diaria, T1= irrigación cada dos días, T2= irrigación cada tres días, T3= irrigación cada cuatro días, T4= irrigación cada cinco días.

Internode Number

At 40 days, T4 had the maximum internodes (6.70, $p<0.05$), significantly different from the other treatments, which ranged from 4.70 to 5.26. At 50 days, internode number was not significantly different among treatments, ranging from 5.58 to 6.68 (figure 1, page 6). At 50 days, the number of knots did not reflect differences between treatments, with values between 5.58 and 6.68 (figure 1, page 6).

Yield and Alkaloid Content

At 50 days, treatment T2 achieved the highest green matter yield, although not significantly different from T0 and T4. For dry matter, treatments T0, T1, and T2 did not differ significantly. The lowest yields were observed in T3 and T4 (figure 2, page 6).

Different letters in bars differ significantly for $p<0.05$.
Letras distintas en columnas difieren significativamente para $p<0,05$.

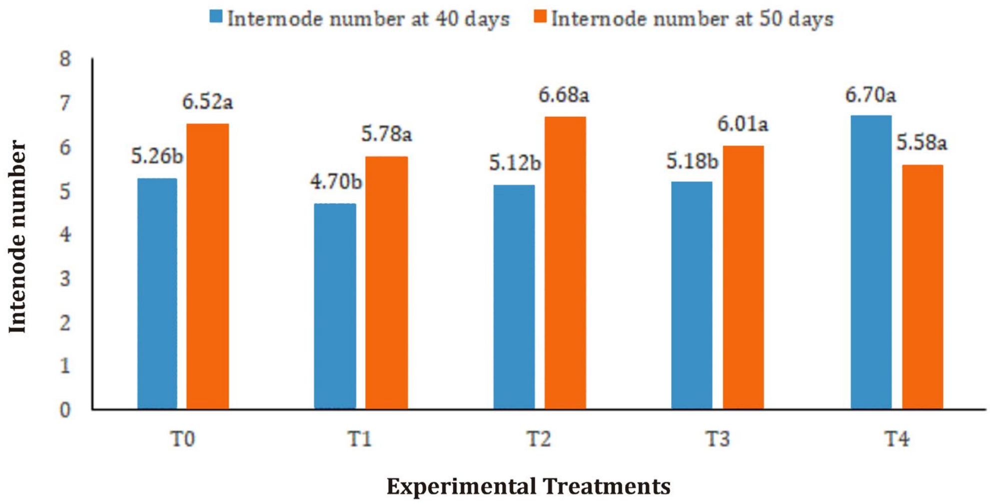

Figure 1. Internode number of *C. ternatea* at 40 and 50 Days according to Experimental Treatments.

Figura 1. Número de internudos de *C. ternatea* a los 40 y 50 días según los tratamientos experimentales.

Different letters in bars differ significantly for $p<0.05$.
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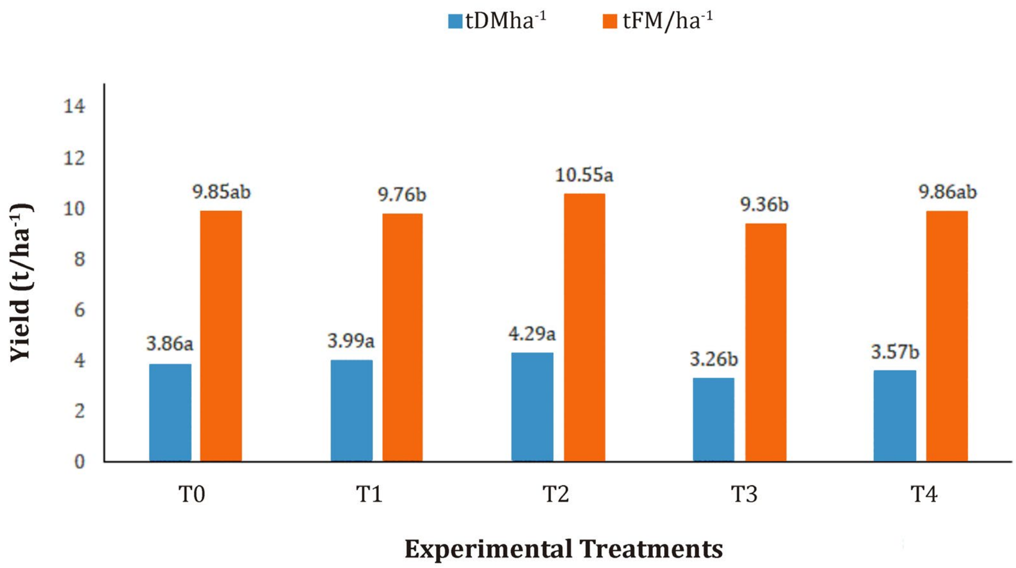

Figure 2. Fresh (FM) and Dry Matter (DM) of *C. ternatea* after 50 days of evaluation

Figura 2. Rendimiento de materia fresa y seca de *C. ternatea* después de 50 días de evaluación.

Alkaloids reached their highest value for T2 (watering every three days) with 14.2 g/kg^{-1} DM. Treatments T0 and T4 did not show differences between them. The lowest value was reached by T1 with 2 g/kg^{-1} DM (figure 3, page 7).

Different letters differ significantly for p<0.05.
Letras distintas en las barras difieren significativamente para p<0,05.

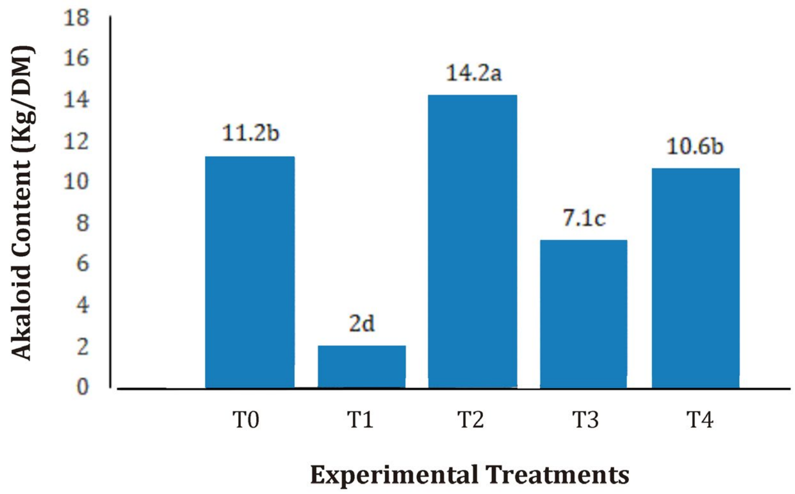

Figure 3. Alkaloid content of *C. ternatea* after 50 days of evaluation.
Figura 3. Alcaloides de *C. ternatea* después de 50 días de evaluación.

Discussion

Maximum plant height was achieved when *Clitoria ternatea* received irrigation every four or five days, with T4 reaching 48.50 cm at 50 days. These results differ from those reported by other authors (17), who observed plant heights ranging from 10.37 to 18.67 cm at 30 days and from 18.86 to 31.8 cm at 45 days under tropical conditions. Such discrepancies underscore the critical role of both irrigation management and environmental variables in modulating vegetative growth. Optimal water supply and climate conditions directly affect physiological processes, promoting stem elongation and overall development in *C. ternatea* (4, 13).

Leaf number increased as plants matured, with the highest value recorded in T3 (48 leaves at 50 days). According to previous studies (11), before 75 days, *C. ternatea* predominantly allocates photoassimilates to roots and young leaves, supporting the observed increase in leaf production and, consequently, biomass accumulation. This pattern also aligns with the trends noted for leaf area and internode development in this study.

Leaf area reached its maximum when irrigation occurred every three or four days and plant age exceeded 40 days, as similarly documented in previous studies (10). However, leaf area expansion and biomass production in *C. ternatea* depend on age, environmental factors, and agronomic practices. Irrigation management is a decisive factor in cell expansion, while excessive moisture induces root anoxia (17). Our findings highlight the necessity of balancing irrigation to optimize physiological performance and yield of *C. ternatea.*

Distinct values for leaf area in *C. ternatea* have been documented (18). An expanded leaf area enhances light interception and carbon assimilation (24, 25). Nevertheless, several agronomic and environmental factors-including irrigation regime, precipitation patterns, and organic fertilization-modulate the agronomic performance of *C. ternatea.*

In our investigation, optimal vegetative vigor and yield were measured when irrigation intervals ranged from three to four days. Environmental conditions and crop management practices, particularly irrigation frequency, affect organ differentiation and elongation (8, 24).

Yields exceeded 3.26 t DM ha^{-1} at 50 days, demonstrating the productive potential of *C. ternatea* under optimized irrigation. Literature indicates that, under adequate irrigation, this species may achieve up to 30 t ha^{-1} $year^{-1}$; however, reduced rainfall and limited soil moisture substantially decrease productivity (10). The highest yields in this study resulted from irrigation every three days, underscoring the importance of precise water management. Productivity in *C. ternatea* varies with seasonality, genotype, agronomic practices, and environmental variables like humidity and soil properties (9). These findings highlight the necessity of conducting field trials under diverse irrigation regimes to validate and potentially improve productivity.

Yield results obtained in this study exceed those documented by various authors for *C. ternatea* under irrigated conditions. Previous reports indicate yields ranging from 1.6 to 2.5 t ha^{-1}, as well as 1.3 t ha^{-1} at 60 days, and 0.86 t ha^{-1} of dry matter in grass-legume associations involving three rows of signal grass and two rows of *C. ternatea* (12, 25, 26). Several factors may explain these discrepancies. Low planting densities and full sunlight exposure enhance photosynthesis. This, along with an expanded root system, promotes higher growth rates and biomass production under adequate soil moisture (8, 15).

Alkaloid concentrations measured align with those reported for other legume species and tree crops. Literature cites values between 0.28 and 11 g kg^{-1}, noting that secondary metabolite content depends on plant age, defense mechanisms, nutrient status, and edaphoclimatic factors (27). In this study, alkaloid levels ranged from 12 to 14 g kg^{-1}, with the highest value recorded in plants irrigated every three days. These findings suggest that, under optimal agronomic management, *C. ternatea* can accumulate substantial quantities of alkaloids, enhancing its functional and nutritional value.

Environmental contexts strongly affect the synthesis of secondary metabolites. Climate and soil properties modulate the presence and concentration of bioactive compounds. Stressful deficiencies or excesses in edaphoclimatic factors often upregulate the biosynthesis of compounds like alkaloids. For instance, increased wind speed accelerates evaporation of essential oils, while higher transpiration rates facilitate the upward movement of tropane alkaloids from roots to canopies (5, 6). This research corroborates these observations and emphasizes the importance of integrated environmental and agronomic management for optimizing yield and quality in *C. ternatea* (16).

Conclusions

Reducing irrigation frequency affects the agronomic performance of *Clitoria ternatea* under protected conditions. In fact, most agronomic variables-including plant height, leaf number, leaf area, and biomass yield-reached their highest values when irrigation occurred every three or four days. These findings indicate that watering within this interval optimizes vegetative growth and productivity by avoiding drought and excessive soil moisture.

Given that daily irrigation didn't enhance growth parameters compared to less frequent regimes under protected conditions, future research should assess whether such intensive watering is justified under field conditions. Alkaloid stability across all irrigation treatments further suggests that secondary metabolite accumulation in *C. ternatea* remains largely unaffected by variations in watering frequency within the tested range.

The evidence presented here supports the strategic management of irrigation schedules according to crop developmental stage and physiological indicators. This approach enables producers to optimize yield and resource use efficiency without compromising quality.

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