

**EFFECT OF SHADE
DENSITY ON SEEDLINGS
OF *Cinchona pubescens*
VAHL IN MARANURA
NURSERY THE
CONVENTION-CUSCO**

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Abstract: The work has been carried out at the “Beatriz Baja” Agrarian Experimental Center of Maranura of the “Signos de Fe” Higher Technological Institute in Quillabamba. The objective was to evaluate the effect of shade in the nursery phase on *Cinchona pubescens* Vahl, with 4 Treatments and 4 repetitions. The seed comes from the mountains of the Apurímac, Echarate and Mantaro River Valleys (VRAEM) with 20% germination; The substrate for the seedling and bagging bed was land from the secondary forest of the place without disturbing the biota. As shade regulator in the nursery was the Raschel mesh, T2- 65%, T3-130%, T4-85% and T1- coffee plant in production (control). The model used was a Complete Randomized Design and correlation. The variables Length and diameter of growth of the seedlings reported a positive correlation $r: 0.887$, this has led to the Evaluation of the means of the growth of the Treatments with the Duncan test with a level of confidence at ($p: 0.05$). The result indicates the effect of the evaluated shade densities are not significant, which implies conducting the nursery under the shade of coffee (plant), is a viable option to promote the sustained propagation of the cinchona tree and friendly to the environment ambient.

Keywords: Propagation, seed, germination, substrate, shade, sustained.

INTRODUCTION

Natural forest species in Peru have been severely affected by the irrational extraction of wood, forest fires and informal mining. The *Cinchona* sp, known by the name of the quina tree, which is a legendary plant in our country, however, was on the verge of extinction for many decades due to the excessive extraction of the bark to control the malaria disease caused by *Plasmodium* sp., whose transmitting agent is the insect *Anopheles* sp. Two centuries have passed to remember

that plant that is on the national shield, and we see that it is on the verge of extinction, which is why, the institutions, individuals, are committed to recovering at least the three most emblematic species. The objective is to test the effect of shade on the development of *Cinchona* sp. in nursery.

The experiment lasted five months, one month in bed (nursery), after the bagging in which the Length and diameter have been measured every 30 days on four occasions.



Figure 1: Geographical location of the experiment: Lat. -12.9556, Long: -72.6508.

The *Cinchona*. *Calisaya* is native to Bolivia and southern Peru and is also cultivated in Java. The total alkaloid content was 3-7%, being the quinine more than 50% and it is used in the production of quinine and quinidine. (Indecopi 2018).

In the mid-19th century, explorers of the quinine bark *Cinchona officinalis* arrived in the southern Madre de Dios basin, and had violent encounters with the ancestors of the Harakbut and Ese Eja, guardians of the forest where this species was found. The genetic material has been stolen by the Europeans to plant in Southeast Asia, with which the wild cinchona collapsed, and has given rise to the extraction of rubber, cited by (Chirif 2021).

Deforestation has increased in recent years, in the buffer zone of the Manu National Park, which had a surface area of one million hectares in 2005, some 55,477 ha have been deforested. In the buffer zone of the Yasuní National Park, with an area of 656,000 ha, a deforestation rate of 40,000 ha/year has been registered (Chirif 2021).

The organography and sexual propagation system have been described by researchers. Zevallos, (1989) flowers in the form of terminal panicles of 20 to 25 cm in length, slightly pubescent, hermaphroditic, actinomorphic; gamosepalous calyx about 4 mm in length, cylindrical, with 5 small lobes; corolla white-red, with fused petals, 1.5 cm long. cited by (Aguirre, Cango, and Quizhpe 2021).

The length of the fruits can vary between 1.7 to 2.8 cm by 0.6 to 1.9 cm wide. (Gabriel et al. n.d.)

The seeds of *Cinchona* sp. they are plano-convex, winged, at the mature stage they contain a small amount of oily endosperm and a well-differentiated embryo, and the thread is located \pm centrally on the flat side (Huamán et al. 2019).

The shape of the seed varies slightly with the species, in some cases they are spindle-shaped, soft testa with a membranous surface and very fragile wings that break easily and end in small, simple, yellowish-brown trichomes and are light, Average weight. from 0.54 to 0.84 g. The analysis of the quality of the seed provides information related to its performance in the field, which is useful for the conservation of the species and, at the same time, to initiate afforestation and reforestation programs. (Gabriel et al. n.d.)

The seed production potential per axis carried out in Naque concludes 1837.58 ± 360 seeds and a weight of 6.94 g.; followed by Selva Alegre with 1795.57 ± 850.28 seed units, weighing 8.81 g. (Gabriel et al. 2019.)

There are research proposals on the use

of the substrate in sexual propagation in nurseries; An investigation indicates that Treatment (T4): 100% forest land favored the seed germination process of *C. officinalis*, followed by T3: 75% forest land + 25% sand and T2: 50% forest land and 50% area. It is not recommended to use pure sand as a substrate during the germination stage. (Zarate et al. 2022).

The preparation for post planting in the definitive field is carried out by peeling or bagging Aguirre et al. (2021) proposes the bagging in polyethylene bags with a volume of 684.4 cm³ (7.5x15.5 cm. of cylindrical section) containing a substrate collected from the forest, whose physical-chemical characteristics were; texture: sandy loam, pH: 4.2 ± 0.1 , electrical conductivity: 0.5 ± 0.0 dS/m, P: 8.5 ± 2.5 mg/kg, total N: 0.3 ± 0.0 %, Ca: 1.2 ± 0.0 meq/100 g, K: 82.7 ± 1.7 mg/kg, Na: 0.4 ± 0.0 meq/100 g, Mg: 0.3 ± 0.0 meq/100g.

The use of chemical inputs in the fertilization of seedlings in the nursery, peeling and bagging must be taken with greater care. An investigation concludes: The elements of the agroecosystem are susceptible to chemical and technogenic effects, it has been verified that the increase in the chemical load in the fruit nursery has caused the destruction of useful species of the microflora, the nature of the infection of the organs of the plant have changed, and there have been negative changes in the immunological state of the plants, consequently the priority role of biologization has been corroborated to guarantee the stability of the agroecosystem of the nurseries. (Egorov, Shadrina, and Kochyan 2020).

The sexual propagation of *Cinchona* sp. It requires taking into account some basic aspects, Tapia, (2013) the seeds need little light while they are germinating and a uniform humidity must be maintained, if the substrate is allowed to dry after the seeds have begun

to swell, they die. When the seeds germinate, they are prone to die from wilting (damping off), to counteract this, a delicate adjustment of light and ventilation must be used. (Tapia 2013).

Seed germination is related to viability, quality, humidity, shade, temperature and substrate. It is observed that most of the seeds germinate after 13 days in all soils, in Average 58.1% germinate in that time. (Rodríguez Barrutia, Barrutia Barreto, and Velásquez 2020).

Rodríguez Barrutia, et al. (2020), the type of soil has a significant influence on the germination percentage with $p < 0.05$; It also demonstrated that it can reproduce outside its habitat for reforestation purposes in areas where this emblematic plant of Peru has disappeared.

Seeds obtained from the mountains are heterogeneous, which is why it is necessary to create seed Production Centers. To reduce heterogeneity to ensure vigor and phytosanitary quality, Aguirre et al. (2021), groups the size of the five-month-old plants in the nursery into three groups according to their size: 1) from 2.0 to 5.0 cm, 2) from 5.1 to 8.0 cm and 3) greater than 8.1 cm.

The use of specific inputs favors germination. The use of potassium nitrate at a concentration of 1,000 ppm is 90.67 %, 88.66 % and 13,436 favors germination (Campos, Cerna, and Chico 2014).

Seed growers of the *Cinchona* sp tree must take into consideration the following: heights between 3.4 to 5.9 m, DAP between 5.0 and 12.4 cm and a good phytosanitary state. The production potential reached in Average between 318.92 to 1054.36 fruits/tree and between 464.88 to 1837.00. The experiences of the vegetative propagation of the *Cinchona* species have not always been successful (Jerez 2017; Vásquez et al. 2018; Villar et al. 2018 Sánchez et al. 2020)

and although it is an alternative, it has the advantage of multiplying the same genotype without segregation (Albán-Castillo et al. 2020) seeds/tree. (Moreno-Serrano et al. 2018).

The reproduction of the quina is carried out sexually and asexually by seed, cutting and graft. Seeds are sown in rich, sandy soil and kept until seedlings are 5 cm, then bagged or pricked in the bed until it reaches 9 inches, then moved to the final field (Hodge 1947).

At present, the task of reforestation with species of *Cinchona* sp. DIAR-AGRO RURA approves the action plan for Forest Repopulation with species of the genus *Cinchona* (2020-2022) to be applied in ten departments of our territory, two of them located on the western Andean slopes. (Albán-Castillo et al, 2020).

The plants are arboreal with an altitude of 6 to 12 m; A report indicates that the recommended planting distance is 1.3 x 1.3 m, resulting in approximately 5,929 plants per hectare (Loján, 2003). A distance is also recommended (Pappa, 2004; (Tapia 2013).

The yield in the final field is directly related to the quality of the plant. The smaller the size of the seedlings and without morphological alterations of the stem, the larger it will be; the survival percentage and the number of *Cinchona* sp. seedlings (Aguirre et al. 2021).

Botanists mention at least 12 species, however, three emblematic species are recommended by experts for reforestation: *C. calisaya* Wedd., *C. officinalis* L. and *C. pubescens* Vahl. (Albán-Castillo et al. 2020). In RD No. 067-2020.

Seedlings in the nursery and in the field are no stranger to the damage caused by pests and diseases Jäger, (2015) in the *Cinchona* sp. nursery, in Guatemala, India and Java, *cinchona* is susceptible to many pests and diseases, the seedlings are frequently affected by *Rhizoctonia solani* J. G

The most common diseases that affect *Cinchona* in the nursery are: *Armillaria mellea* Vahl ex. Fr., which causes root canker disease; *Fomes noxim* Corner, which causes brown root disease; *F. semitostm* Berk, and *F. lignosus* (Klotzsch) Bres, which causes white root disease; *Rosellinia arcuata* Fetch and *R. hunoides* (Berk, and Br.) Sacc, which causes gray root rot; *Ganoderma pseudoferreum* (Wakef.) v. Over, and Steinm., which causes red root rot; and *Sporedesmium* sp., *Fusarium* sp., and physiological root disease, which occurs in *cinchona* in moist soils (No 1950).

The practice of composting occurs from the nursery to the final planting, the friendly option with the environment is biological. Arbuscular mycorrhizal fungi (AMF) are ubiquitous in agroecosystems, but their role in agricultural yield is scarce (De Bauw et al. 2021).

MATERIALS AND METHODS

The nursery has been installed in “Beatriz Baja” of the C.E.A. Signs of Faith Higher Technological Institute of Quillabamba. The materials used were: seed, forest soil (in situ), polyethylene bags, kituchi, pick, shovel, metal sieve, wooden stake, shower-type sprinkler, ¼” corrugated iron, PVC tube, wood, camera, tape metric, notebook, pencil and computer. The Treatments were: Control with natural coffee shade (T1), tunnel with 65% agricultural Raschel mesh (T2), double shade with 130% agricultural Raschel mesh (T3), tunnel with 85% agricultural Raschel mesh (T4).



Figure 2: Seedling field (Secondary Forest).

The seed comes from the mountains of the district of Pichari La Convención in the amount of 2 kg. (dry seed), then it has been selected whose weight was 300 g. and counted 20,000 units, the same that have been stored.



Figure 03: Seedling.



Figure 4: Seedling evaluation.

The substrate for the seedling was 100% (Mull) secondary forest soil from the A horizon: (Ao, Aoo) without disturbing the biota. For the stockpiling, 50% of Mull + 50% of soil from the underlying horizon (A) was used.

The model used was the Completely Randomized Design, and correlation; the type of research is experimental, prospective, Lengthinal, analytical.

RESULTS AND DISCUSSION

The storage has been carried out in two beds of 1 m² each, at 10,000 seeds in each case, the germination percentage has been evaluated after 14 days: 14, 21, 28 and 35, counting 4,030 germinated seeds, corresponding at 20.15%. The Average temperature has been registered 31.8 oC. Later they have been bagged for final planting. The low percentage of germination refers to the quality of the seed and inadequate selection, because they have been collected from old trees in the mountains of the Pichari district of the VRAEM, therefore it is necessary to establish Quina seed centers.

Evaluation samples from each shoot have been randomly taken from the nursery; One month after the seedlings were installed, the length and diameter of the seedlings were measured every 30 days for 4 months. The measurement began on November 13, 2020 and ended on February 2, 2021 in the "Beatriz Baja" nursery of the C.E.A. of the Higher Technological Institute of Signs of Fe-Quillabamba.

Evaluation	1ra	2da	3ra	4ta
No.	T1	T2	T3	T4
1	6.3	7.54	7.62	6.2
2	7.98	8.82	11.4	8.4
3	10.56	11.26	17.66	10.8
4	13.3	19.8	22.32	16.3

Total	38.14	47.42	59	41.7
Average	9.53	11.86	14.75	10.42

Table 1: Height of seedlings in nursery in (cm).

The growth of the planulas, expresses the quality of the substrate, management and effect of the shade. (Graph 1)

Evaluation	Treatment			
	T1	T2	T3	T4
1	3.6	3.3	3.1	3
2	4.4	4.04	4.8	4.6
3	5.1	5.58	5.4	5
4	5.8	6.44	6.4	6
Total	18.9	19.36	19.7	18.6
Average	4.72	4.84	4.92	4.65

Table 2: Seedling diameter in (mm)

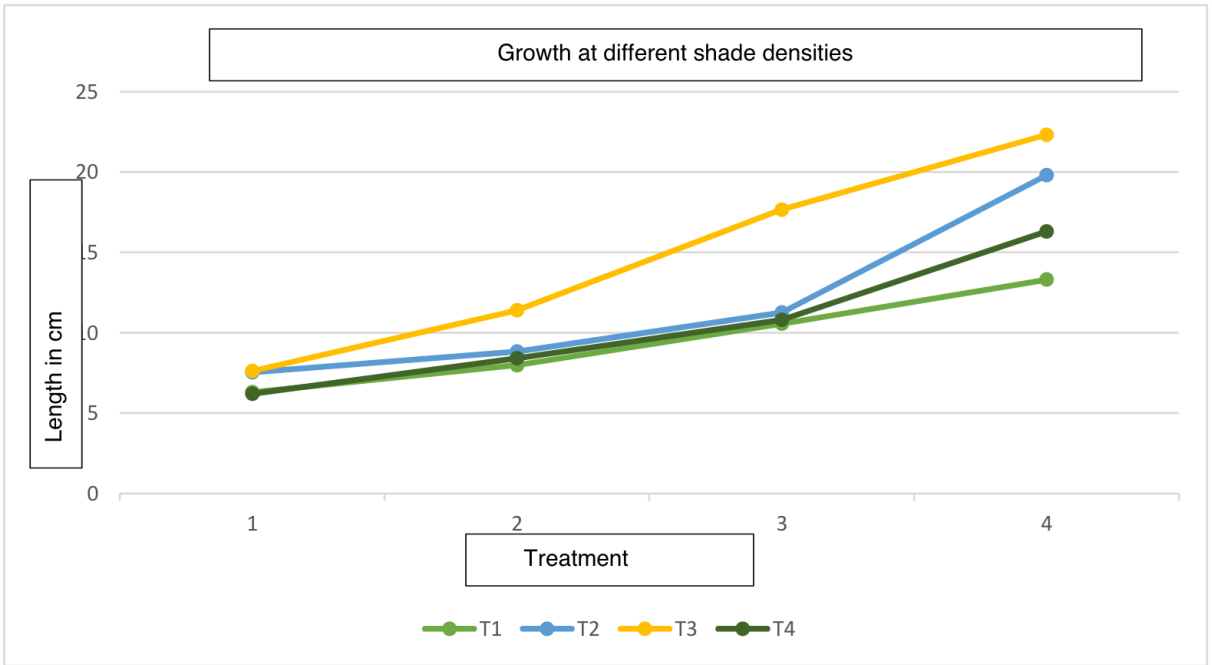
(Graph 2).

The homogeneous development of the diameter of the seedlings corroborates the adequate management and the low presence of *Fusarium* sp, which was not significant, likewise, it has not been fertilized.

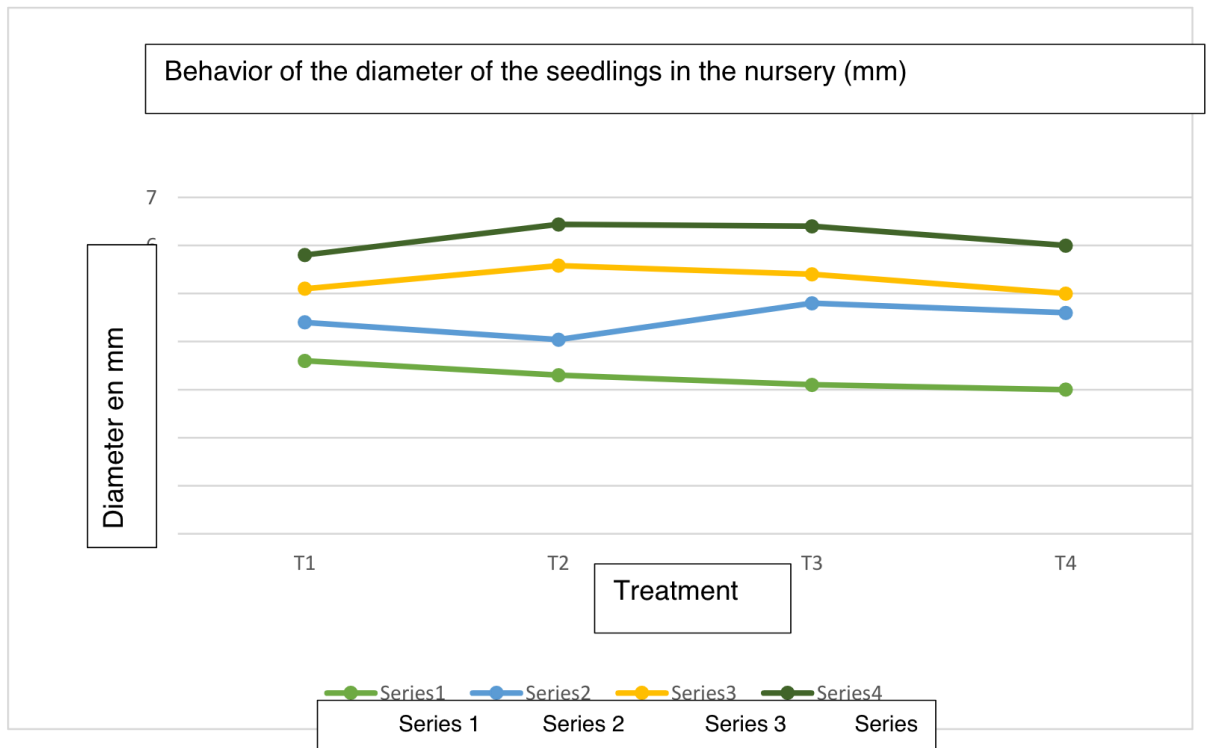
The evaluation of the growth in the treatments has been put into trial, for which the Duncan model (α : 0.05) has been used, said samples have been taken randomly in number of five individuals, whose results are the following: CME: 20.83, SCT: 62.52, SCE: 289.2, SCE:289.20, GLT:3, GLE:12.

Difference of means	VS	Critical range of Rp	Result
T3-T1	5.22	< 7.592	ns
T3-T4	4.33	< 7.364	Ns
T3-T2	2.89	< 7.022	Ns
T2-T1	2.33	< 7.364	Ns
T2-T4	1.44	< 7.22	Ns
T4-T1	0.89	< 7.022	Ns

Table 3: Duncan's contrast test



Graph 1: Growth behavior of seedlings in the nursery.



Graph 2: Evolution of the diameter of the seedlings in the nursery (mm).

	Length en (cm)	Diameter in (mm)
T1	9.53	4.72
T2	11.86	4.84
T3	14.75	4.92
T4	10.42	4.65

Table 4: Average growth of the Length and diameter of the seedlings in the nursery.

	Length (cm)	Diameter in (mm)
Min	9.53	Min 4.65
1st Qu.	10.2	1st Qu. 4.702
Average	11.14	Average 4.78
Average	11.64	Average 4.782
3rde Qu.	12.58	3rde Qu. 4.86
Max	14.75	Max 4.92

Table 5: Correlation coefficient (r=0.887)

The correlation coefficient determined with R for the variables length and diameter in the nursery is $r = 0.887$, positive, however, it is not perfect.

CONCLUSION

The seed comes from the mountains of the Pichari district of the Apurímac, Echarate and Mantaro River Valleys (VRAEM), by direct collection of old plants. The low percentage of germination expresses the poor quality and deficient selection, in addition to the provenance of two or more axes. The even development of the plants in the nursery indicates a good quality of forest substrate, and suitable installation conditions for *Cinchona pubescens* VAH. The growth and even development in length and diameter report a positive correlation ($r = 0.887$), which has given rise to the judgment to evaluate the length based on the difference of the means of the growth of the treatments (4x4), with the model of Duncan ($\alpha: 0.05$), in the following treatments with four repetitions each: (T:

1 “control” coffee shade, T: 2 Rachel mesh 65%, T: 3 double mesh 130%, and T4: 85%) table No.3. It has been proven that they are not significant, and it is concluded that the control with leftover coffee works the same as the rest with Raschel mesh shade, and it is a good indicator of the use of local inputs in the propagation of cinchona seedlings, and mitigate the contamination, it is also recommended to replicate under the same modality to validate or set aside this result as it is viable and sustainable.

REFERENCES

- Aguirre, Z., I. Cango, and Quizhpe, W. 2021. Revista cubana de ciencias forestales 9:17..
- Bowen, S. Crandall 1950. Cinchona Root Collar Rot in Perú and Bolivia. Washington D.C. "No. 855." (855). Estación Experimental Tingo María Perú.
- Castillo, A. J, Chilquillo, E., Castro, B. M., Arakaki, M., León, B. and Suni.M. 2020. "Cinchona l. 'quina tree': Repopulation and reforestation in Peru." Revista peruana de biología 27(3):423–26. doi: 10.15381/rpb.v27i3.18697.
- De bauw, P., Birindwa, D., Merckx, R., Boeraeve, M., Munyahali, W., Gerrit, P. Bolaji, T., and Honnay, O. 2021. "Improved genotypes and fertilizers, not fallow duration, increase cassava yields without compromising arbuscular mycorrhizal fungus richness or diversity." *mycorrhiza* 31(4):483–96. doi: 10.1007/s00572-021-01039-0.
- Campos, J., Cerna, L., and Chico, J. 2014. "Efecto del ácido giberélico , nitrato de potasio y agua de coco en la germinación de semillas de quina , *Cinchona pubescens* seed germination of *Cinchona pubescens*." *Rebiolest* 2(1):e20.
- Chirit, A.2021-2019.The defaunation of the tropical forests and its enviromental implicatio in Perú. Deforestation in times of climate change edited by Alberto Chirif, Iwgia.
- Egorov, E., Shadrina, Z.and Kochyan, G., 2020. "Increasing the technological and economic efficiency of nursery production based on processes biologization." bio web of conferences 25:01001. DOI: 10.1051/bioconf/20202501001.
- Gabriel, M, Frei, S.,Oswaldo, C., and Ortega, V. 2019. Estructura, composición florística y fisiología reproductiva de *Cinchona pubescens* L.Universidad de Loja Ecuador.
- Hodge, W. H. 1947. "The plant resources of Peru." Economic botany 1(2):119–36. DOI: 10.1007/bf02859223.
- Huamán, L., Albán, J., and Chilquillo, E., 2019. "Taxonomic aspects and advances in the knowledge of the current state of the quina tree (*Cinchona officinalis* L.) in the North of Perú." *Ecología aplicada* 18(2):145.
- Remuzgo,F.J.,Álvarez, M.J.,Salas, D.F., 2020 Indecopi. 2018. Caracterización taxonómicay fitoquímica de *Cinchona pubescens* y *Landenbergia* en el valle del Alto Huallaga Huánuco.Quina cinchona spp . 3:8.
- Jäger, H. 2015. "Biology and impacts of pacific island invasive species. 11. *Cinchona pubescens* (red quinine tree) (rubiaceae)." *Pacific science* 69(2):133–53. doi: 10.2984/69.2.1.
- Moreno, S, J, A., Pérez R, C., Eras G, V.H.; Minchala P, J., and Arévalo, Y. M. 2018. "Caracterización de fuentes semilleras de la especie *Cinchona officinalis* L. (rubiaceae), con fines de propagación in Vitro." *Tzhoeoen* 10(3):361–70. doi: 10.26495/https://doi.org/10.26495/rtzh1810.327327.
- Rodriguez B. Barreto, B. and T. D. M. Velásquez. 2020. "Germination of *Cinchona Officinalis* L. Seeds in Three Types of Soils in Cajamarca, Perú." *Revista Cubana de Ciencias Forestales* 8(1):75–87.
- Zarate, F, Huaccha, A., Barturén, L.,Quiñones, L. and Sánchez, T. 2022. "Efecto del sustrato en la germinación de *Cinchona Officinalis* L. (Rubiaceae)." 11.

VIRTUAL REFERENCE

<https://www.youtube.com/watch?v=RC0WL5EjBb4>

https://www.youtube.com/watch?v=seshkT_4Cb0

<https://www.youtube.com/watch?v=Ync1pZukJ1Y>

<https://www.youtube.com/watch?v=oK9JE-nEJ28>