SHELTER EFFECT ON BUDDING AND SOME MORPHOMETRIC PARAMETERS OF SEEDLINGS OF PEPPER [*Piper nigrum* L. (Piperaceae)] IN NURSERY

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https://doi.org/10.35410/IJAEB.2024.5888

ABSTRACT
Cuttings are a vegetative propagation technique used by small farmers to improve crop yield and income. It promotes the conservation of the genetic heritage of plants. This study investigated the influence of shelter on sprouting and seedling growth of the Lompung cultivar of pepper (Piper nigrum L.) in nursery. This plant characterized by large leaves has been used for the production of tunnel and nursery shelter seedlings. The tests were conducted for three months (November 2016 - January 2017). The device used was a completely random block. The parameters studied included the timing and germination rate of the cuttings, the number of leaves, the leaf area, the length and the dry root biomass of the plants. ANOVA 1 and the Ducan test were used to analyze the results obtained. Statistical analysis of the data showed that the performance of the plants obtained under tunnels was higher than that of plants grown under nursery shelter. These include collet diameter, number of leaves and leaf area, length, and root biomass. On the other hand, the germination time was not influenced by the cropping systems. This study has shown that cuttings of pepper tunnels are favorable to vegetative multiplication.

Keywords: Black Pepper, Piper Nigrum, Tunnel, Nursery Shelter.

1. INTRODUCTION
Pepper is the fruit of the pepper tree (Piper nigrum L.), known as black pepper. Pepper is considered as the "queen of spices" because of its use in the spice industry and its value on the international market [1; 2]. It is native to the tropical and subtropical regions of India, more precisely on the west coast of Malabar in the state of Kerala [3; 4]. But, the pepper migrated to other tropical regions during the triangular trade with the navigators [5]. It was introduced in Ivory Caost during the colonization period. Given its importance as a spice and its multidimensional use in Asian medical systems [6]. In particular in the treatment of diseases (hypolipidemia, diabetes, lithogenesis, inflammation) and its use as an antioxidant, antimutagenic and anticarcinogenic agent [7; 8]. Pepper has been the subject of several studies. The work carried out on the pepper plant has most often been devoted to the mechanisms of action of the chemical components of the fruit in the human body or that of experimental mice [9; 10; 11; 12]. Research into the agronomy of the plant is sparse. However, they have mainly focused on modern vegetative propagation techniques such as somatic embryogenesis or organogenesis [13]. The results are convincing but are not accessible to small farmers. Thus, they resort to cuttings of twigs because the pepper seeds quickly lose their germinative power during conservation [1; 3]. In addition, the vines obtained by these seeds germination are generally spindly, come into production late and may not contain all the agronomic and / or chemical characteristics of the mother plants. To obtain seeds at low cost, small farmers can

resort to cuttings. This technique makes it possible to produce plants in high quantities, more vigorous and combining the morphological, qualitative and quantitative characteristics of the mother plants. It is in this context that this work was undertaken with the objective of determining the influence of the shelter on the cutting germination and the pepper plantlets growth produced in the nursery.

1.1 Study area location

The study was carried out on an experimental plot in Azaguié - Ahoua in the south-east, 27 km from the city of Abidjan (Côte d'Ivoire). The site is located between 5 ° 38'21 '' N and 4 ° 3.'20 '' with an altitude of 69 m. The humid tropical climate is characterized by four seasons with two rainy seasons and two dry seasons, each with a longer one. Annual rainfall averages 1,400 mm of rain with a temperature varying between 23 and 28 ° C. The long dry season does not exceed more than three months.

1.2. Vegetal material

The vegetal material consisted of orthotropic cuttings taken from pepper plants at least one year old.

1.3. Shelters construction

The experimental system consists of two shelters, namely the tunnel shelter and the nursery shelter. For the 10 m long, 6 m wide and 1.90 m high shade house, bamboo wood was used as a frame. The woven palm leaves (Elaeis guineensis) were used to cover the roof and to protect the perimeter of the shelter to isolate it from the outside. As for the tunnel shelter, a shade house was built as before and under this shade house, a tunnel 4 m long, 1 m wide and 0.9 m high was built. To make this tunnel, flexible wooden hoops were used to support the structure. These arches were placed at two meter intervals and anchored in the ground. A translucent plastic film was stretched over the hoops and the sides were kept on the ground with a clod of earth. These two shelters provided the germination environments for the pepper cuttings. The cuttings were sown in Arab soil taken from the first few centimeters of the undergrowth of the farm. To do this, the black polyethylene bags 8 cm in diameter and 15 cm in height were used as culture pots. The substrate was disinfected using a granulated carbofuran (1G) insecticide at a rate of 10 g per pot. The pots were carefully watered 24 hours before the cuttings were grown to moisten and disinfect the substrate.

1.4. Cuttings pepper preparation and seedling

The orthotropic boughs were collected early in the morning around 6 a.m. when the plant's metabolism was not very active. They were cut into cuttings with two knots and an internodal segment of about 10 cm. These cuttings were stripped and then wrapped in tissue paper previously soaked in water. They were placed in a transparent plastic packaging in order to avoid their dehydration during transport to the experimentation place. Under the nursery shelter, the cuttings were taken out and rinsed thoroughly with tap water and then they were soaked for 2 min in an antifungal solution (Maneb 80 WP) prepared at 75 g per 15 L of water. Before sowing, the lower end of the cutting was cut just below the knot using a pruner and it was buried in the substrate on 2/3 of its length with an inclination of 45 ° relative to the horizontal. These cuttings were sown in the substrate contained in black polyethylene bags 8 cm in diameter and 15 cm in height. The substrate was disinfected using a granulated carbofuran (1G) insecticide at a rate of

10 g per pot. The pots were carefully watered 24 hours before the cuttings were grown to humidify and destroy the larvae and telluric insects. The pots were arranged in double rows of eight for each experimental unit. The data being collected from all the plants, each plant constituted a repetition in each experimental unit, either 16 repetitions for an evaluated parameter.

1.5. Nursery upkeep

After sowing, the watering rate was 2 times a day for the cuttings placed under shade house. It was reduced to one watering a day after the budding of the cuttings. For those placed in a tunnel, watering is done once a day and was then carried out once every two days after germination. Every week, an antifungal treatment was carried out using a solution of Banko plus (1G) obtained by putting 40 mL of the fungal solution in water for a final volume of 15 L. After budding of the cuttings , fertilization was carried out every two weeks with a foliar fertilizer prepared as above, using 50 mL of the DI-Grow green stock solution. To control weed control, regular manual weeding was carried out. The plastic film was partially lifted to provide ventilation for the tunnel.

1.6.1. Time and germination rate determination

The germination time is the period which has elapsed between the date of sowing the cuttings and the appearance of the first open leaf. To assess this parameter, a daily visit to the nursery was made to determine the date of appearance of the first open leaf. The budding rate is the number of germinated cuttings out of the number of seeded cuttings. It is expressed as a percentage.

1.6.2. Collet diameter determination

The collet diameter was determined using an electronic caliper and expressed in millimeters. Germination systems were determined on each of the plants, either 32 plants in each experimental unit. The average was calculated by adding the values determined by plants to the total number of plants.

1.6.3. Determination of the average number of leaves per plant

Three months after sowing the cuttings, a direct count of the number of leaves opened on each plant was carried out in each of the two cultivation systems. The average number of leaves (NL) per plant was calculated by the following formula:

$$NL = \frac{\sum(\text{ leaves per plant})}{(\text{Total number of plants})}$$

1.6.4. Leaf area determination

The study of this parameter was evaluated on the first three basal leaves of the pepper plantlets of three-month-old plants. Measurements were taken on all of the plants in each batch. The length and width were determined using an electronic caliper. The length was determined from the sheath to the apex. The leaf area per plant (LA) was determined by the classic formula of [15].

Al = Lmax x lmax x 3 x 0.75

Lmax: Maximum length; lmax: Maximum width; 3: Number of well-developed leaves per plant; 0.75: Coefficient

The total leaf area (LA) is determined by the following formula:

$$LA = \frac{\sum(Al)}{\text{Total number of plants}}$$

The leaf area is expressed in cm2.

1.6.5. Determination of the average length of roots

Root length was determined on the four longest roots of the lot plants. In fact, three months after the nurseries were completed, the pots were split, then watered in order to soften the substrate. Roots stripped of their substrate and then washed with tap water. They were detached from the stems with pruning shears. The length of the roots was determined using an electronic caliper like Mastercraft. The average root length (LA) under the shelters was calculated by the following formula:

$$LA = \frac{\sum(\text{Length of roots per plant})}{\text{Total number of plants}}$$

1.6.7. Dry root biomass

All the roots collected from the plants cultivated in each culture system were placed in an oven regulated at 45 ° C. Root biomass was assessed daily using an Adventurer Pro Model AV313 type electronic balance until mass stabilization was achieved. The average root biomass of plants (BR) was determined by the following formula:

$$BR = \frac{\sum(\text{root mass per plant})}{\text{Total number of plants}}$$

1.7. Experimental appliance and data analysis

The experimental system consists of completely random blocks with a main factor comprising two modalities (tunnel and nursery shelter). The data obtained was subjected to analysis of variance (ANOVA) using Statistica version 7.1 software. The analysis of the variance to a classification criterion was made at the probability threshold ($P \leq 0.05$) to classify the means.

When there is a significant difference, the homogeneous groups of individuals are determined by the Duncan method. This method is based on the comparison of all pairs of means.

2. RESULTS

2.1. Evolution of temperature and humidity during the experiment

Analysis of Figure 1 showed that the temperature in the tunnel was higher than that recorded in the shade. It was generally 0.24 to 2 ° C higher, sometimes 4 ° C depending on the period, with an average of 25.69 ± 1.07 under the tunnel and 23.69 ± 1.83 under the shade house. Analysis of the data showed a significant difference between the temperatures in the tunnel and those in the shade ($P = 0$). The humidity rate fluctuated within each system where the variation was between

1 and 5 % depending on the systems and the weeks. Depending on the systems, the average humidity in the tunnel (88.03 ± 2.08) is slightly higher compared to that under the shade which is 87.03 ± 2.94, a difference of 1 % in humidity. The results of the statistical analysis showed that there is no significant difference between the humidity of these two shelter systems ($P = 0.155$).

2.2. Time of germination of pepper plant cuttings placed under the various shelters
Table I analysis showed that the cutting germination started in the second week after sowing. The cuttings budding sown under the nursery shelter started on the 16th day after their planting. After 50 % germination rate on the 21st after sowing, all the cuttings broke out on the thirtieth day after sowing. For those placed in a tunnel, germination started from the 17th and ended on the 27th with 100 % germination rate with a 50 % germination rate after 20 days of sowing. The statistical data analysis has shown that there is no significant difference ($P = 0.75$) between the time of germination in the tunnel and those germinated in the shade house. However, it is noted that germination in tunnels was grouped with an average of 21.93 days compared to 22.49 days for cuttings in shade houses with more spread germination.

2.3. Diameter at the collet
Table I reading shows that the plants produced under tunnel have a larger stem circumference than those produced under nursery shelter. The average diameter is 3.91mm and 3.36 mm for the seedlings produced in tunnel and shade house respectively. Statistical analysis showed a significant difference between the circumferences of the stems of the seedlings produced in the tunnel and those produced in the shade house with $P \leq 0.05$.

2.4. Average number of leaves emitted by pepper plantlets under different shelters
The data in Table I showed that the number of sheets emitted was higher in the tunnel. The average number of leaves developed per seedling under tunnel is 5.8 leaves compared to 4.93 for seedlings under nursery shelter. Analysis showed that leaf production was strongly influenced by the cover with $P = 0.0054$.

2.5. Leaf area
The seedlings produced in the tunnel had a larger leaf area than that produced in the shade house. In fact, the data presented in Table I have shown that the average leaf area (77.81 cm2) of seedlings produced in tunnels is significantly higher than the average of those produced in shade houses with a rate of 58.34. Table I analysis also showed a very significant difference with $P = 0.036$.

2.6. Average root length of seedlings in the nursery
The table I analysis has shown that the plants cultivated in a tunnel produced long roots (11.33 cm) compared to those produced in a nursery shelter. The maximum length under the shade was 7.81 cm. Statistical analysis showed a significant difference at the shelter level ($P \leq 0.05$)

2.7. Root biomass
The dry biomass of the roots of the plants in the tunnel and in the shade house was 0.86 g and 0.44 g respectively (Table I). The difference in average showed that production in the tunnel

increased twice as much as that in the nursery shelter. The data analysis showed that the shelter significantly influenced root production with P = 0.00129.

3. DISCUSSION

The success of the cuttings depends on the organogenesis which is the first stage of the development of a plant. This organogenesis is regulated by several factors including hormonal, trophic and climatic factors. Pepper nurseries were conducted in systems with slightly different hygrothermal data. This small difference could be explained by the timing of the data collection. Indeed, the experiment took place from mid-November to mid-January. However, this period is characterized by high humidity, often accompanied by fog in the morning, when the data was collected. But during the hot hours of the day, the humidity decreases as the temperature rises.

The germination time was almost identical in the two production systems (tunnel and shade house). This lack of significant effect on the budding of cuttings could be due to the intrinsic characteristics of the plant. Indeed, the cuttings were taken from the stems of plants of the same age therefore of the same phenological stage which would contain similar levels of phytohormones. However, these growth regulators are involved in the establishment of organs. In addition, these plants belong to the same variety. The results of this work corroborate those reported by Verheij [16], who suggested that the germination of cuttings is under the action of physiological and genetic factors.

Concerning the agrophysiological parameters evaluated on the plants, it was noted that the values recorded in the tunnel were higher than those of the plants in the shade house. This situation could be explained by the fact that the tunnel constitutes a favorable shelter for the development of pepper plants. In fact, temperature, humidity and tunnel lighting positively influenced the vegetative growth of the seedlings. The temperature in the tunnel underwent a small fluctuation with an average around 25.69 ° C. This temperature would be close to the optimal pepper growth temperature as [17] noted it in the mango tree. In the latter, the number of leaves increased with temperature. This would result from the activation of mitoses in meristematic cells and their elongation. The same would be true of the diametral and root growth observed in the pepper plant. The seedlings cultivated under-tunnel benefited from a rise in temperature of 2 ° C sometimes 4 ° C more compared to the seedlings produced under-shade. Generally speaking, the rise in temperature is accompanied by a significant demand for leaf water, which results in a high absorption of water and nutrients. The increase in hydromineral nutrition [18] favored the establishment of dry matter. The beneficial effect of seedling growth in tunnels would be due to the combined effect of light and temperature well channeled and uniformly distributed under this shelter [19]. These same effects have been observed in certain plants such as the Cantaloupe melon [20; 21]. The average relative humidity (88.03 ± 2.08) high in the tunnel but with a gradual increase favored the water supply of the plants whose substrate, provided with water, and favored the organs growth. On the other hand, under shade, even if the average humidity was high, the strong weekly variation experienced could temporarily induce a water deficit generally detrimental to growth [22]. This water deficit was induced by the transpiration of plants following the drop in humidity during the hot hours of the day under the shade house. To this end, Gérard [23] has shown that root elongation is a decreasing linear function of water potential. The elongation of the roots observed in the plants germinated in a tunnel could be favored by chemotaxis because the mineral elements were drained to the bottom during watering.

Light, through the activation of photosynthesis, has contributed to the synthesis of carbohydrates essential for plant production. Being channeled in a tunnel, it allowed the establishment and growth of all the organs of the plants unlike those placed under the shade house where the light was diffuse [24]. In addition, the work carried out by Lambert & Bakes [25] has shown that the reduction in illuminance strongly affects the number and leaf area as well as root production. This would explain why the establishment of the leaves and roots was done at a higher rate in the seedlings sown under tunnel. Indeed, the work of certain authors [26; 27]. has shown that the reduction of lighting and water lead to a decrease in root elongation as well as the production of secondary roots.

4. CONCLUSION

All the cuttings of the pepper plant (Piper nigrum L.) seeded broke regardless of the shelter used. The seedlings produced in the tunnel had a good vegetative development compared to those produced in the shade house despite the germination time which was almost identical in the two cropping systems. Under tunnel, the values of the diameter at the collar, the number and the surface of the leaves, the length and the biomass of the roots were higher than those of the plants raised under shade house. However, the agronomic parameters of the nurseries transferred to the field may guide the choice of shelter.

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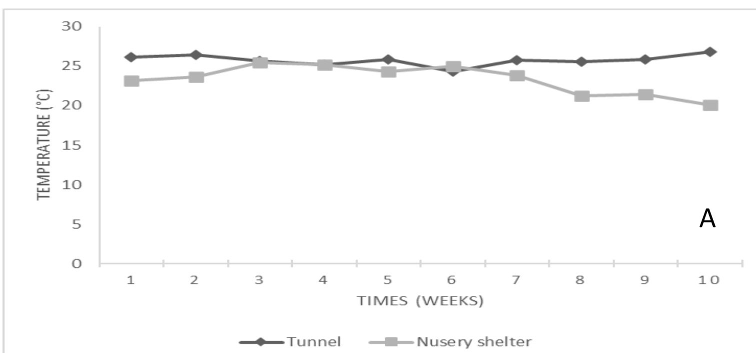
Table I. Statistical data of climatic parameters and growth

Parameters	Shelters		Statistics	
	Tunnel	Nursery shelters	F	P
Temperature (ºC)	25.69±1.07a	23.69±1,83b	27.05	0
Hygrometry (%)	88.03±2.08a	87.03±2,94a	2.08	0.155
Germination time (D)	21.93±3.95a	22.46±5,37a	0.09	0.759
Diameter at the collet (mm)	3.91±0.56a	3.36±0,42b	9.09	0.0054
Leaves nombre	5.8±1.01a	4.93±1,09b	5.03	0.032
Leaf eara (cm2)	77.81±31.14a	58.34±14,55b	4.81	0.036
Root length (cm)	11.33±3.85a	7.2±2,09b	13.24	0.0019
Root biomass (g)	0.86±0.41a	0.44±0,163b	12.77	0.0013



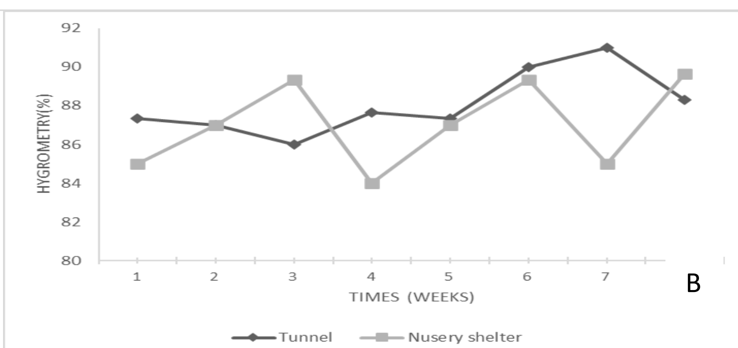


Figure 1. Evolution of temperature and humidity in tunnel and nursery shelter
A. Evolution of the temperature; B. Evolution of humidity