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EFFECT OF HARVEST TIMING ON THE QUALITY OF *Vigna unguiculata* SEEDS

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Abstract

Harvest timing affects both physical and physiological characteristics of cowpea (*Vigna unguiculata*) seeds. This study aimed to evaluate the influence of harvest periods on the physical and physiological quality of seeds. It also assessed the initial development of *Vigna unguiculata* cv. Corujinha seedlings. Experiment I took place in the field, using a randomized block design during twelve harvest periods (57, 65, 71, 78, 85, 92, 99, 106, 113, 120, 127 DAS, and a single harvest) with four replications. Experiment II was carried out in the laboratory using a completely randomized design. It analyzed germination (%) and the germination speed index (GSI). After each harvest, evaluations included the number of seeds per pod, pod and seed biometry and moisture content, germination percentage, GSI, seedling length and dry mass, and fungal incidence in seeds. Biometric analysis revealed significant differences. The best pod length was at 120 DAS and 127 DAS, width at 57 DAS and between 85 and 113 DAS, and thickness at 92 DAS. The longest seed length was observed between 92 and 120 DAS, the width between 85 and 113 DAS, and the maximum thickness between 85 and 99 DAS. Single-harvest samples showed higher fungal incidence. They also recorded lower germination and seedling growth values. The maximum dry mass for pods and seeds occurred between 71 and 99 DAS, and in the single harvest. Staggered harvesting reduces seed exposure to field conditions. This enhances seed quality and initial seedling development.

Keywords: Cowpea. Environmental conditions. Pathogenic fungi. Physiological maturity.

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1. Introduction

In recent years, cowpea (*Vigna unguiculata* (L.) Walp.) cultivation has expanded across several regions of Brazil, attracting both small and large-scale farmers. It is predominantly grown in the semi-arid Northeast due to its broad edaphoclimatic adaptability and high productivity within a single agricultural cycle, which contributes to food security for farmers and local communities (Oliveira and Morais 2019).

Cowpea production is highest in West Africa, with additional cultivation in Southeast Asia, the Mediterranean Basin, Latin America, and the United States (Herniter et al. 2020). In Brazil, production is concentrated in the Northeast and North regions, with expanding cultivation in the Central-West (Sales Júnior et al. 2020). Despite the crop's success in major producing regions, information on the importance of harvest timing for seed quality remains limited.

Harvest timing plays a crucial role in cowpea (*Vigna unguiculata*) seed production because it influences the seeds' physical and physiological qualities. Maintaining seed quality during and after harvest may significantly increase crop yield (Asiedu et al. 2021). Seed quality is determined by multiple factors, such as physiological (germination and vigor) and sanitary conditions, physical purity, and genetic integrity (Werner et al. 2020). Seeds should be harvested when they reach physiological maturity, because the shorter the time they remain in the field from this stage to harvesting, the less their quality will be compromised (Araújo et al. 2020). Minimizing the period of seed exposure to climatic fluctuations, pest attacks, and diseases through staggered harvesting may be beneficial.

Single harvesting can reduce seed physiological and sanitary quality due to prolonged field exposure after physiological maturity, as harvesting begins only when all pods are dry. Because cowpea has indeterminate growth, plants may bear mature pods, green pods, and flowers simultaneously, causing early-maturing seeds to remain exposed to adverse conditions and rapidly lose quality (Rocha et al. 2021; Toledo and Ceccon 2023). Delayed harvesting, therefore, accelerates seed deterioration by increasing exposure to unfavorable environments and pathogens. However, the information on the effects of staggered harvesting on cowpea seed production remains limited.

This study aimed to evaluate the effects of different harvest periods on seed physical and physiological quality and the early development of *Vigna unguiculata* cv. Corujinha seedlings.

2. Material and Methods

Experiment I

Experimental location

The field experiment was conducted at the Chã-de-Jardim experimental farm, Center for Agricultural Sciences, Universidade Federal da Paraíba (UFPB), Areia, PB, Brazil, at the Brejo Paraibano Microregion (6° 57' 26" S and 35° 45' 30" W, altitude of 574.62 m). The climate is classified as *As* (Köppen), which is hot and humid, with an average annual rainfall of 1,200 mm. Weather conditions (January to March) were recorded throughout the experimental period of 180 days after sowing (DAS).

Experimental design and condition

Cowpea seeds of the Corujinha variety were sown in a randomized block design with 12 harvest times (57, 65, 71, 78, 85, 92, 99, 106, 113, 120, 127 DAS, and a single harvest) and four replications. Each plot consisted of 40 plants spaced 0.8 m between rows and 0.4 m between plants. All plants were considered useful.

The soil in the experimental area was classified as Regolithic Neosol with a sandy-loam texture. Soil samples were collected from the 0 to 20cm layer prior to the experiment's establishment. Chemical analyses showed pH (H_2O) 6.01, organic matter (OM) 2.39 dag/kg, phosphorus (P) 72.12 mg/dm³, potassium (K) 85.08 mg/dm³, sodium (Na) 0.18 cmolc/dm³, H + Al 2.02 cmolc/dm³, aluminum (Al) 0.00 cmolc/dm³, calcium (Ca) 2.60 cmolc/dm³, magnesium (Mg) 2.03 cmolc/dm³, base saturation (BS) 5.02 cmolc/dm³, and cation exchange capacity (CEC) 7.04 cmolc/dm³.

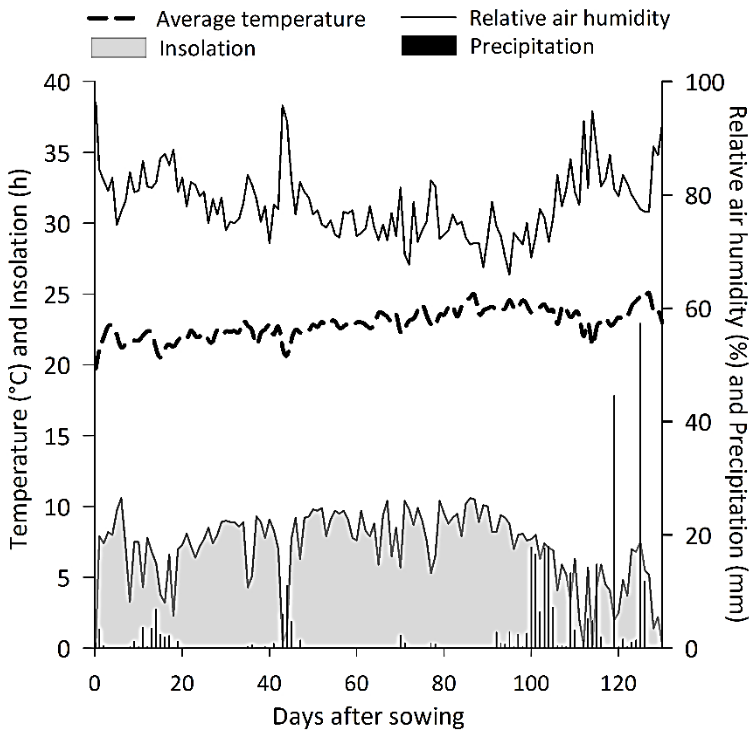

Figure 1. Meteorological conditions during the experimental period for cowpea grown under field conditions.

Soil preparation involved clearing the area with hoes and digging planting holes, with three seeds sown per hole. Thinning was performed after emergence, leaving one plant per hole. Plants were periodically monitored for flowering and pod formation. Fertilization consisted of 5.0 t/ha of cattle manure and 20 kg/ha of P_2O_5 (single superphosphate) at planting. Topdressing included 20 kg/ha of K_2O and 40 kg/ha of N (urea) (Cavalcante et al. 2008), split into two equal applications at 20 DAS and 35 DAS. Planting was carried out using cowpea seeds of the Corujinha cultivar.

Weeds were controlled manually with hoes. During dry periods, plants were drip-irrigated (drip tape with 1.8 L/h) every two days.

Evaluated characteristics

Staggered harvesting started at 57 DAS and was conducted at seven-day intervals until the final harvest. An additional treatment consisted of a single harvest at the end of the experimental period. Harvested pods were manually threshed in the UFPB Seed Analysis Laboratory to obtain seeds for testing.

Pod and seed biometry were evaluated after each harvest. Four replications of 25 pods and 25 seeds were measured using a digital caliper. Length, width, and thickness were recorded in millimeters (mm), except for pod length, which was measured in centimeters (cm).

The number of seeds per pod was determined by manually counting seeds from 40 pods after each harvest. Pod and seed moisture content was determined by the oven-drying method at 105 °C for 24 hours (Brasil, 2009). For each harvest, four replications of 25 seeds and four replications of five pods were analyzed, and results were expressed as percentages (%). Pod and seed dry mass were determined, along with moisture content, at all harvest times, with results expressed in grams (g).

The incidence of pathogenic fungi (%) in seeds from single and staggered harvests (127 DAS) was evaluated using the blotter test (Brasil 2009). A total of 200 seeds were distributed into 10 replications of 20 seeds each. Seeds were placed in Petri dishes (Ø 15 cm) under aseptic conditions on a double layer of sterilized filter paper moistened with distilled water. The dishes were incubated in a biochemical oxygen demand (B.O.D.) chamber for eight days at 25 ± 2 °C under a 12-hour light photoperiod. Fungal incidence was assessed using an optical microscope and stereoscope, with identification based on specialized literature (Seifert et al. 2011). Results were expressed as the percentage of fungal occurrence.

Experiment II

Experimental design

The experiment was conducted under laboratory conditions using a completely randomized design. Germination (%) and germination speed index (GSI) were determined using paper towel rolls (Germitest). Paper rolls were moistened with distilled water at 2.5 times their dry weight. Seeds were placed over two sheets of paper, covered by a third sheet, and rolled. For each harvest time, four replications of 25 seeds were prepared. The rolls were placed in plastic bags and incubated in a Biochemical Oxygen Demand (B.O.D.) germinator at a constant temperature of 25 °C (Brasil 2009).

Evaluated characteristics

Germination was evaluated based on normal seedlings (with root and hypocotyl), with results expressed as percentages. germination and GSI tests were conducted simultaneously from the first count and calculated according to Maguire (1962).

Seedling length and dry mass were determined at the end of the germination test. Normal seedlings from each replication were measured with a graduated ruler, and results were expressed in centimeters per seedling. The same seedlings were then dried in Kraft paper bags in an oven at 80 °C for 24 hours and weighed on an analytical balance (0.001 g precision).

Statistical analysis

The statistical analysis was performed using the R statistical software (R Development Core Team 2024). The results were first subjected to the Shapiro-Wilk test for normality of residuals and Bartlett's test for homogeneity of variances. Results showing normal distribution and homoscedasticity were then analyzed using analysis of variance, and treatment means were compared with the Scott-Knott test ($p < 0.05$). Fungal incidence values were initially transformed using the square root of (y + 1).

3. Results

Pod and seed biometry

The results indicate significant differences in pod biometry. The smallest pod lengths were observed at 120 DAS, 127 DAS, and the single harvest. As for pod width, the best values were recorded at 57 DAS and between 85 and 113 DAS. The greatest thickness was achieved at 92 DAS (Figure 2A). Seed biometry also presented statistical differences. The longest seeds were obtained between 92 and 120 DAS, the widest seeds emerged between 85 and 113 DAS, and the maximum thickness was observed from 85 to 99 DAS (Figure 2B).

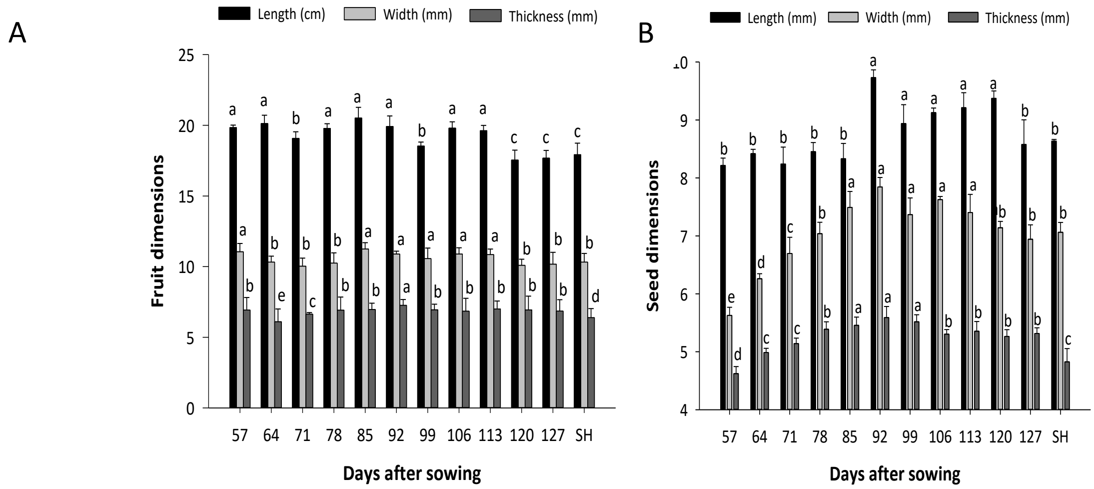

Figure 2. Length, width, and thickness of cowpea pods (A) and seeds (B) based on harvest timing.

Number of seeds per pod, moisture content, and fungal incidence

The number of seeds per pod did not show significant differences among harvest times. Regarding moisture content, the best values for pods were recorded at 57 DAS, 64 DAS, 71 DAS, 127 DAS, and the single harvest. The best values for seeds were observed at 57 DAS and the single harvest (Figure 3A). Regarding fungal incidence, the single harvest showed, on average, a higher presence of pathogens compared to the staggered harvest for all identified fungal genera (Figure 3B).

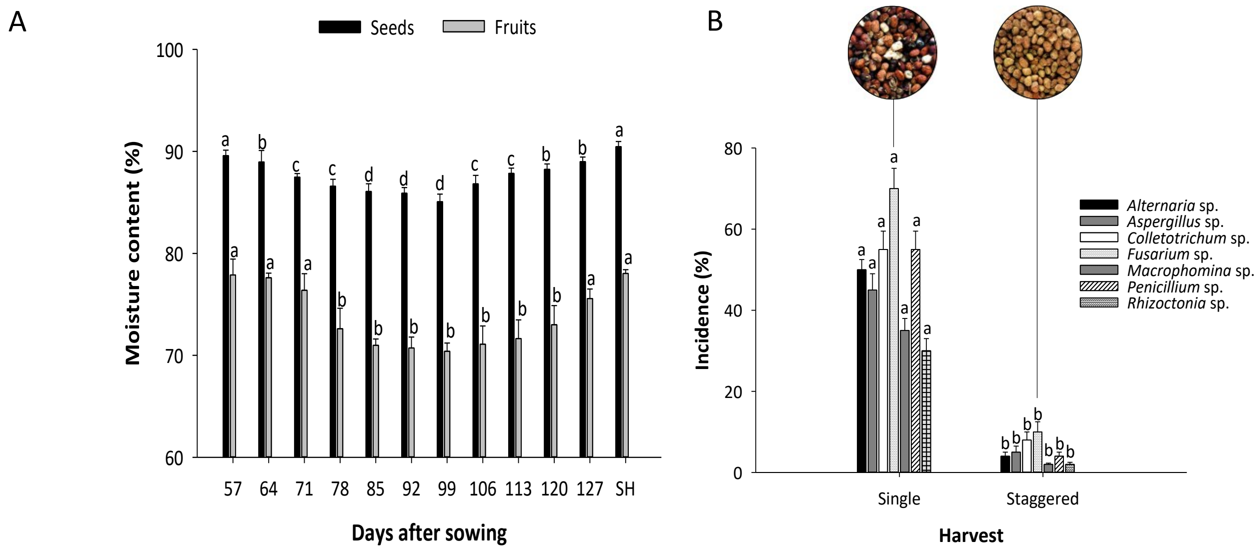

Figure 3. Moisture content of pods and seeds (A) and fungal incidence (B) in cowpea seeds based on harvest timing.

Dry mass of pods and seeds

The highest dry mass values for pods were observed between 71 and 99 DAS and during the single harvest. The maximum dry mass values for seeds were recorded between 85 and 99 DAS and during the single harvest (Figure 4).

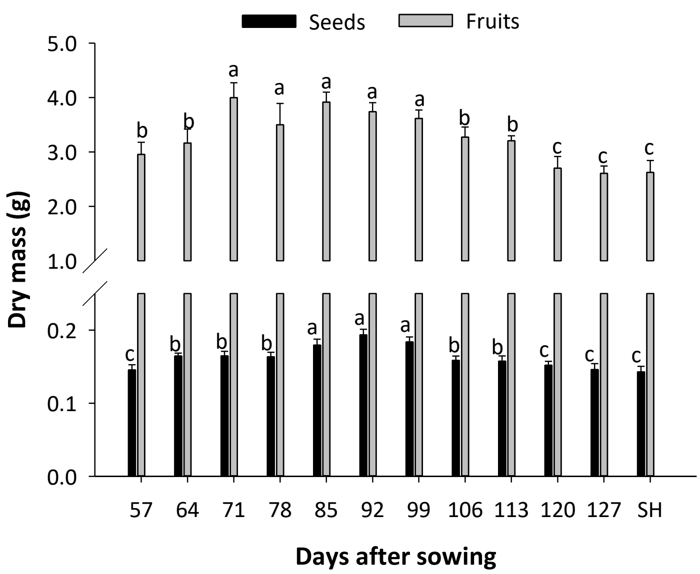

Figure 4. Dry mass of cowpea seeds and pods based on harvest timing.

Germination and germination speed index (GSI)

The lowest germination percentages and GSI were observed only in the single harvest (Figure 5).

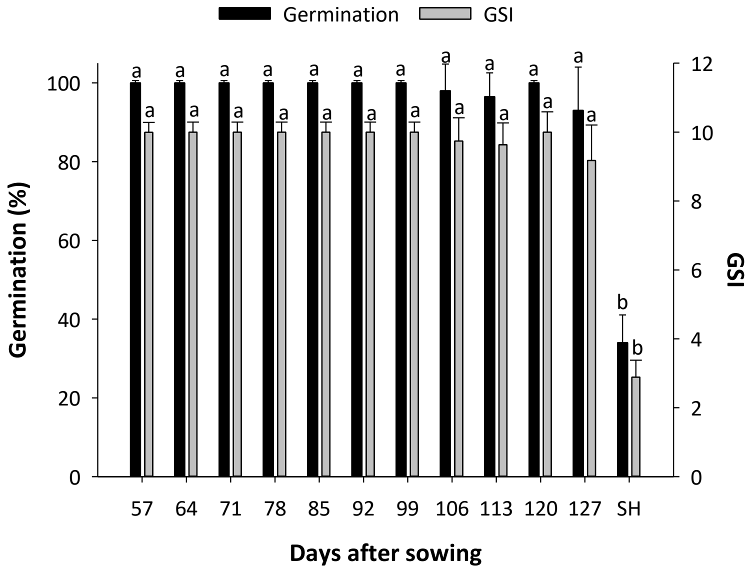

Figure 5. Germination and germination speed index of cowpea seeds based on harvest timing.

Seedling length and dry mass

The shortest root and shoot lengths, as well as their respective dry masses, were observed in seedlings from the single harvest (Figure 6A and B). Notably, the sixth harvest (92 DAS) showed the best performance for shoot length, while the best root length was observed between 78 and 99 DAS (Figure 6A). The highest values for shoot dry mass were recorded between 71 and 99 DAS, and the maximum root mass was obtained only in the seventh harvest (99 DAS) (Figure 6B).

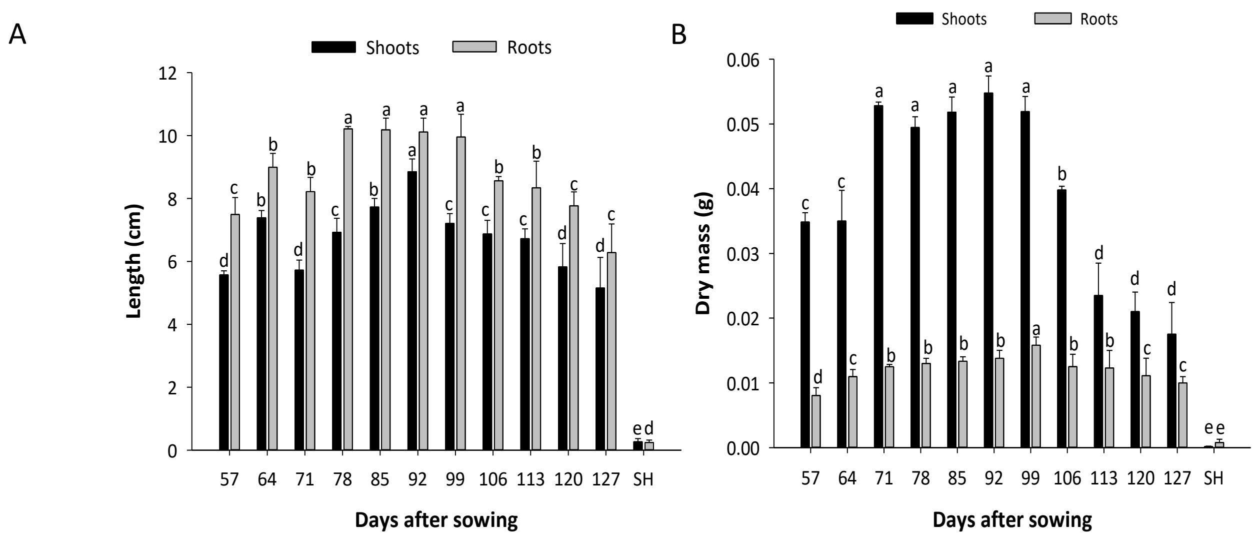

Figure 6. Length (A) and dry mass (B) of roots and shoots of cowpea seedlings based on harvest timing.

4. Discussion

Pod and seed biometry

Regardless of the harvest season, pod lengths varied between 17.55 and 20.52 cm. The pods were only above the commercial standard of 20 cm 64 DAS (20.13 cm) and 85 DAS (20.53 cm), as evidenced by Oliveira et al. (2015). Overall, pod biometry may vary due to temperature fluctuations in the field, which influence bean pod lengths (Barros et al. 2021).

Regarding seed biometry, the smallest lengths were observed at 57-85 DAS, 127 DAS, and during the single harvest. Seed width was lowest only at 57 DAS, and thickness was smallest at 64 and 127 DAS. However, the sixth harvest (92 DAS) was one of the best times for seed length and width. This scenario agrees with Oliveira and Morais (2019), who also found that the sixth week was optimal for these dimensions in their study on cowpea seed maturation and physiological quality.

The average seed thickness of 5.62 mm, reported by Oba et al. (2019) for cowpea, is similar to that observed in single and staggered harvests in this study. This similarity may be related to a reduction in dry mass loss due to respiration, allowing seeds to reach the genetically programmed dry matter content. The low seed width and thickness values observed in the first harvest (57 DAS) might be associated with a primary investment by plants in vegetative growth over reproductive development. This is consistent with the indeterminate growth habit of some cowpea cultivars, such as Corujinha (Herniter et al. 2020; Rocha et al. 2021).

Number of seeds per pod, moisture content, and fungal incidence

The average number of seeds per pod was 14, regardless of harvest time. This factor exhibits high variability and may be influenced by climatic factors, such as temperature and precipitation (Acosta-Quezada et al. 2022). However, low variation was observed across harvest times, which may be due to appropriate nutritional management, water supply, and the genetics of the Corujinha cultivar.

A high moisture content was observed in the early cowpea pod harvests, which was also reported by Cruz et al. (2019) for the same species. Botelho et al. (2010) noted similar behavior for *Phaseolus vulgaris* L., as they recorded a high moisture content in the first harvest, followed by a gradual decrease.

A reduction in moisture content was observed in seeds, adversely affecting yields, whereas pod moisture content showed variability. This may be attributed to the genetic characteristics of the cultivar (uniform pod formation) and the intrinsic influence of moisture content on the physiological maturity of pods and seeds (Marcos Filho 2015). Changes in the moisture content of pods and seeds are commonly used to characterize their maturation stage.

Data show that pods have lower moisture values than their seeds, which is a common physiological order during maturity, starting with pod drying, followed by the seeds. Additionally, healthy pods ensure better quality by protecting against biotic and abiotic factors that cause seed dry mass losses. Chang et al. (2020) described that potential pod infections by fungi of the *Fusarium* and *Colletotrichum* genera lead to pod and seed rot due to mycelial growth. Regarding abiotic factors, temperature imposes significant limitations on legumes, as higher temperatures (> 35 °C) may reduce seed mass due to faster growth rates and shorter seed filling durations (Barros et al. 2021; Bhardwaj et al. 2023). This underscores the importance of harvesting immediately after physiological maturity to minimize seed exposure to adverse conditions that degrade seed quality.

Regarding fungal incidence, the single harvest exhibited an average fungal presence above 60% for *Fusarium* sp. This fungus may reduce germination percentages and seedling development, as well as accelerate seed deterioration in *Vigna* spp. (Silva et al. 2021; Isalar and Ogbujia 2021). Isalar and Ogbujia (2021) reported that *Fusarium* species were responsible for the greatest reduction in average root and shoot lengths among eight inoculated organisms in cowpea plants.

Most identified fungi are present in residues from infected plants, which may affect and penetrate pods due to precipitation impacts on the soil. Therefore, staggered harvesting offers a viable alternative to ensure seed quality, as it may reduce fungi accumulation, which is often influenced by abiotic factors. Fotev and Kazakova (2020) describe that some fungi, such as *Fusarium* spp., *Mucor mucedo*, *Penicillium* spp., *Rhizopus* spp., *Aspergillus niger*, and moderately *Alternaria* spp., *Botrytis cinerea*, and *Sclerotinia sclerotiorum*, are present in low quantities in *Vigna* spp. seeds. This further emphasizes the importance of staggered harvesting to reduce the incidence of these and other fungi that may impair the quality of *Vigna unguiculata* seeds, as this method facilitates harvesting immediately after physiological maturity is reached.

Dry mass of pods and seeds

The highest dry mass values in pods may be related to the uptake of photosynthates from vegetative parts, regardless of harvest timing, due to the cultivar's indeterminate growth habit. Ayres et al. (2021) state that pods may reach their maximum length while seed development remains minimal. Later, the seed increases in dry mass and size.

The seed dry mass findings corroborate those of Oliveira and Morais (2019), who evaluated the maturation process and physiological quality of seeds in cowpea cultivars. They also observed the maximum dry mass accumulation at the seventh harvest (99 DAS). The first four harvests (57-78 DAS) yielded slow dry mass accumulation in cowpea seeds, followed by a stable increase from the fifth to the seventh harvest (85-99 DAS). This corroborates Oliveira and Morais (2019), who recorded maximum accumulation at the sixth harvest (92 DAS).

These variations in high and low dry mass may be attributed to an initial slow accumulation phase, followed by a period of rapid and steady accumulation until a maximum value is reached and maintained, with respiration potentially causing minor losses (Marcos Filho 2015). Additionally, the reduction in pod and seed dry mass after the seventh harvest (99 DAS) may be linked to exposure to adverse field conditions, such as pathogens.

Germination and germination speed index (GSI)

The low dry mass values observed in the single harvest may be attributed to the extended seed exposure to adverse field conditions, which diminishes their germinative potential. Elevated temperatures and humidity typical of the cultivation region may increase the incidence of pathogenic fungi, resulting in irreversible damage to seed quality. Vitti et al. (2022) noted that temperature is crucial for sporulation, conidial germination, and the development of pathogenic fungi; humidity is also significant. *Xerophytic* fungi, such as *Aspergillus* spp., prefer dry environments where spores germinate at low relative humidity (< 55%).

The higher germination rates result from staggered harvesting, possibly because it ensures seed health, thereby increasing the likelihood of successful seedling establishment (Moreno et al. 2022). High vigor and germination rates are achieved at physiological maturity (Marcos Filho 2015). In this context, staggered harvesting allows seeds to be collected immediately upon reaching maturity, resulting in higher germination rates and speed. Conversely, harvesting delays after physiological maturity may lead to quality losses.

Seedling length and dry mass

The dry mass and length of seedlings showed the poorest performance with single-harvest seeds, likely due to the prolonged exposure of seeds to adverse climatic and pathogenic conditions in the field. This extended exposure may decrease seed vigor, resulting in seedlings with shorter lengths and lower biomass. Additionally, fungal pathogens may compromise seed quality, reducing seedling dry mass. High-quality seeds typically produce heavier seedlings due to higher accumulation of dry mass (Marcos Filho 2015).

The superior performance of staggered harvesting may be attributed to its ability to maintain seed health and nutritional reserves by immediately collecting seeds after physiological maturity is reached. This method ensures seedling development with higher efficiency in dry mass production (Marcos Filho 2015; Cruz et al. 2019).

The data highlight how harvest timing impacts the quality of *Vigna unguiculata* seeds. Particularly after the seventh harvest (99 DAS), both seedling length and dry mass exhibited a similar decreasing trend, as observed in pod and seed dry mass. This finding suggests that this may be the optimal time to conclude harvest for seed production. Furthermore, the scarcity of information on this issue underscores the importance of this study in advancing seed production of this species, which holds significant socioeconomic value nationally and globally.

5. Conclusions

Harvest timing significantly influences the physical and physiological quality of seeds, and seeds from single harvests exhibit less vigor.

Staggered harvesting ensures better seed health, favors seedling development, and reduces the incidence of microorganisms with pathogenic potential.

Authors' Contributions: CRUZ, J.M.F.L.: Conception and design, acquisition of data, analysis and interpretation of data, drafting the manuscript, final approval; ALVES, E.U.: Analysis and interpretation of data, drafting the manuscript, final approval; SILVA, L.D.R.: Analysis and interpretation of data, drafting the manuscript, final approval; FARIAS, O.R.: Drafting the manuscript, final approval; SANTOS, J.P.O.: Drafting the manuscript, final approval; OLIVEIRA, A.P.: Conception and design, acquisition of data, analysis and interpretation of data, drafting the manuscript, final approval.

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Ethics Approval: Not applicable.

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