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Germination Response and Early Growth of Tropical and Temperate Legumes to Four Plant Extracts: A Comparative Analysis of the Efficacy of *Moringa oleifera*, *Kalanchoe pinnata*, *Azadirachta indica and Ocimum gratissimum*

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Abstract

Plant-based biostimulants are a viable alternative for improving the physiological quality and germination of seeds, combating plant pathogens, and producing food in sufficient quantity and quality whilst reducing the use of fertilisers and plant protection products. This study aims to investigate the efficacy of four leaf extracts as biostimulants for the germination of legume seeds. The methodological approach involved cultivating the seeds in Petri dishes with the various leaf extracts. The results showed that the leaf extracts from *Kalanchoe pinnata* performed better than all the other extracts. The germination rates observed were 96 per cent for peas, 90 per cent for lentils and soya beans, and 80 per cent for groundnuts. It also promoted the elongation of the hypocotyl and epicotyl and an increase in the number of roots. *Ocimum gratissimum* induced the highest germination rate in soya beans, at 100 per cent, whilst leaf extracts of *Azadirachta indica* recorded the lowest performance rates: 90 per cent for peas, 40 per cent for lentils, 83 per cent for soya beans and 26 per cent for groundnuts. Leaf extracts of *Ocimum gratissimum* performed better than those of *Moringa oleifera*. Leaf extracts of *Kalanchoe pinnata* and *Ocimum gratissimum* can be used as biofertilisers to improve the initial vigour of legumes.

Keywords

Biostimulants, Initial Vigor, Phytopathogens, Leaf Extract

1. Introduction

Legumes play an important role in the human diet because they are rich in protein, vitamins, and minerals. The success of their cultivation depends on the seeds. Indeed, the early stages of a crop's life determine its full productive potential. For all plants, and for legumes in particular, rapid growth helps reduce vulnerability to pests and shortens the period of competition from weeds, which can be harmful to the plant [1]. Strong, uniform, and healthy emergence leads to better root systems, improved nutrient uptake, and increased resilience throughout the growing season. The speed at which a plant's roots reach nutrients is a key factor in the plant's successful development [2]. Biostimulants enhance plant growth by providing nutrients derived from natural sources or by helping plants access their nutrients. They promote plant growth and development throughout the crop's life cycle, from seed germination to plant maturity. They improve the efficiency of plant metabolism, leading to increased yields and better quality. For growers seeking better crop establishment, seed-treatment biostimulants offer an effective and scientifically proven way to improve germination and early vigor. Unlike traditional seed treatments focused on protection, plant-based biostimulants activate metabolic, physiological, and microbial processes that promote rapid and successful emergence. Among these natural biostimulants, Moringa leaf extracts have been extensively studied. Several studies have shown that treating seeds with Moringa leaves improves germination rates and plant vigor [3] [4]. Meanwhile, *Ocimum gratissimum* and *Azaradirachta indica* have been studied as biopesticides and biofungicides [5]. *Kalanchoe pinnata*, however, remains understudied. Data on these last three plants as biostimulants are very limited. A comparative approach is important to identify the most effective plant extracts. The objective of this study is to determine the efficacy of four leaf extracts as biostimulants for legume seed germination and to identify the most effective plant extract for improving plant germination parameters.

2. Materials and Methods

2.1. Geographic Location and Climate

The experiment was conducted in Daloa, in central-western Côte d'Ivoire, in the Haut Sassandra region, between longitudes 6˚24' and 6˚29' West and latitudes 6˚50' and 6˚55' North. This city is located approximately 410 km northwest of Abidjan, the country's economic capital, and 141 km from Yamoussoukro. It is bordered to the north by the city of Vavoua, to the south by the city of Issia, to the east by the city of Bouaflé, and to the west by the city of Duékoué. The area has a hot, humid tropical climate with two seasons: a dry season from November to February and a wet season from March to October. The average annual rainfall is 1242 mm. The average annual temperature is 26˚C.

2.2. Plant Material

The plant material consists of seeds from four different legume species, including two legumes from tropical regions specifically the groundnut: *Arachis hypogaea* (*Fabaceae*) and soya: *Glycine max* (*Fabaceae*) and two legumes from temperate regions, namely the lentil: *Lens culinaris* (*Fabaceae*) and the garden pea: *Pisum sativum* (*Fabaceae*). The groundnut and soya bean seeds were purchased from local producers, whilst the pea and lentil seeds were purchased from seed suppliers in Europe. Prior to use, a viability test was carried out to select seeds with good germination potential. The seeds were immersed in distilled water. Those that remained submerged were used in the experiment. The leaf extracts were derived from four different plants: *Moringa oleifera* (*Moringaceae*), *Kalanchoe pinnata* (*Crassulaceae*), *Azadirachta indica* (*Meliaceae*) and *Ocimum gratissimum* (*Lamiaceae*).

2.3. Methods

Preparation of Leaf Extracts and Determination of Germination Parameters

To obtain the leaf extracts, 25 g of fresh leaves from each species were blended with 250 ml of distilled water in a blender for 10 minutes. The resulting mixture was left to macerate for 12 hours. The solution was filtered through Whatman filter paper. The filtrate was made up to 250 ml with distilled water. The leaf extracts were prepared on the day of application and used immediately. Each application of extract was preceded by a fresh extraction to ensure the extracts' efficacy. Ten (10) seeds of each legume, groundnut, lentil, pea and soya bean, were placed in Petri dishes previously lined with filter paper. The leaf extracts were applied three times, with 5 ml administered at the start of the experiment and then every 5 days over a 15-day period. The seeds were then exposed to these leaf extracts after germination to observe the effects on hypocotyl and epicotyl elongation and the number of roots. The experiment began on 5 May 2026 and ended on 20 May 2026. Distilled water was used as the control. The Petri dishes were incubated in a germination chamber at 25˚C with a relative humidity of 80 per cent. The treatments were arranged according to a completely randomised design with three replicates. In total, 60 Petri dishes and 150 seeds of each legume were used for this experiment. The parameters studied were the length of the hypocotyl and epicotyl, the number of roots, the germination rate and the duration of germination. A legume seed is considered to have germinated as soon as the radicle appears.

The length of the hypocotyl and epicotyl was measured using a tape measure. The number of roots was determined by counting; the germination rate was calculated by dividing the number of seeds that had germinated by the total number of seeds sown. The parameters studied were hypocotyl length, epicotyl length, number of roots, germination rate, and germination time.

The statistical analysis was carried out using STATISTICA 8.0 software. All data were subjected to statistical analysis after verifying the homogeneity of variances. The data are expressed as means. A one-way analysis of variance (ANOVA) was carried out to compare the germination rates of the seeds of each legume treated with the different leaf extracts. Subsequently, the effect of the leaf extracts on the mean length of the hypocotyls and epicotyls, and the mean number of roots of these legumes, was assessed. Where significant differences were found, post-ANOVA comparisons were used. Thus, the Newman-Keuls method was employed to examine the effect of the different extracts relative to the control. The level of significance, in the event of differences between the means, was estimated using the Newman-Keuls test at a 5 per cent probability threshold.

3. Results

3.1. Effect of Plant Extracts on the Germination Rate of Peas (*Pisum sativum*)

The effect of plant extracts on pea germination shows that all extracts promoted an average germination rate of 92.4%. The germination rate ranged from 96% to 90% (Figure 1). The highest rate was obtained with Kalanchoe extracts, while rates of 90% were obtained with the control and plant extracts from *Azaradichta indica*. *Moringa oleifera* and *Ocimum gratissimum* extracts recorded germination rates of 93%. The contamination rate was zero for all extracts. No fungi appeared on the seeds until the end of the experiment.

3.2. Effect of Plant Extracts on the Germination Rate of Lentils (*Lens culinaris*)

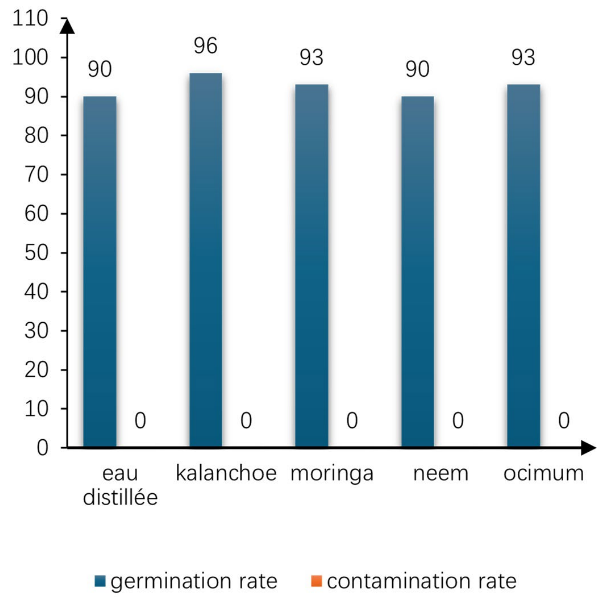

Figure 1. Germination and contamination rates of peas treated with leaf extract.

The germination rate of lentils ranged from 90% to 40%. Seeds grown with distilled water had a germination rate of 80%, while Kalanchoe leaf extracts yielded the highest germination rates of 90% (Figure 2), representing a 10% increase compared to the control. The lowest germination rate was obtained with neem leaf extracts; it was estimated at 40%, representing a 40% decrease compared to the control (distilled water). The germination rate with Ocimum extracts was 70%, and that recorded with moringa leaves was 60%, representing a reduction of 10% and 20%, respectively, compared to the control. The average germination rate was 75%. The contamination rate was zero for all plant extracts.

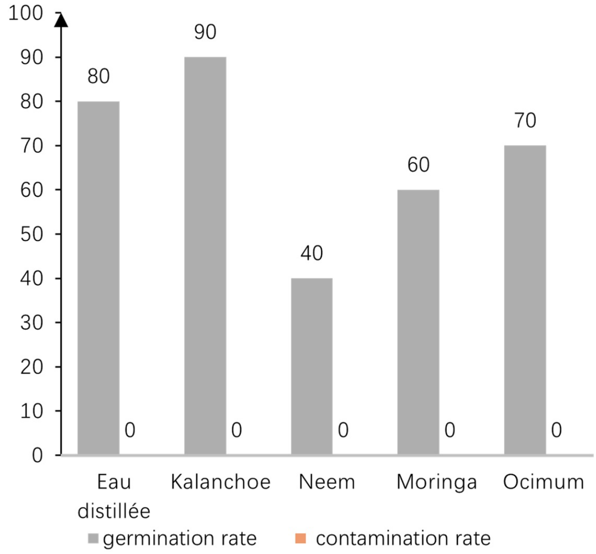

Figure 2. Germination and contamination rates of lentils treated with leaf extract.

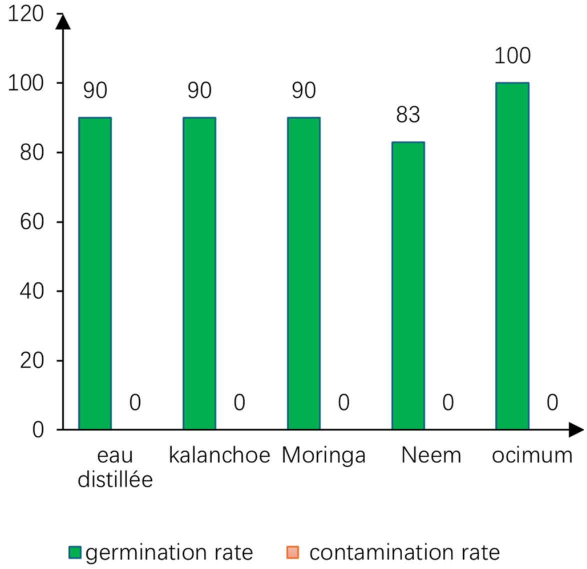

Figure 3. Germination and contamination of soybeans treated with leaf extract.

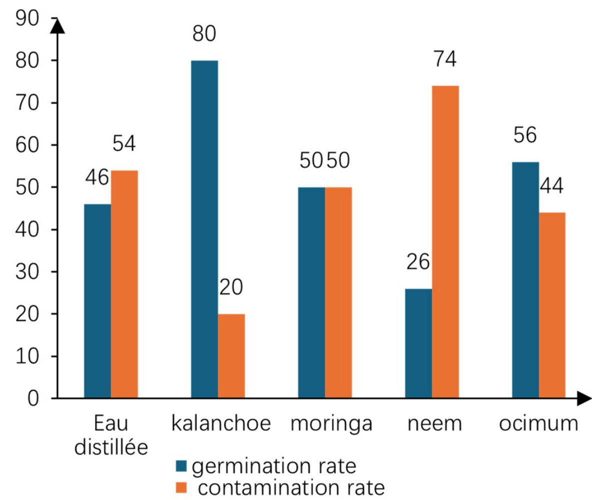

Figure 4. Germination and contamination of peanuts treated with leaf extract.

3.3. Effect of Plant Extracts on the Germination Rate of Soybeans (*Glycine max*)

The results show that germination rates were generally high across all treatments, although soybean germination rates varied depending on the extract applied (**Figure 3**). Specifically, for this plant, the germination rate ranged from 83% for neem extracts to 100% for ocimum leaf extracts. The seed germination rate for distilled water, Kalanchoe extracts, and moringa extracts was 90%. Ocimum leaf extracts recorded the highest rate of 100%, representing a 10% increase in germination compared to the control. Neem leaf extracts resulted in the lowest germination rate of 83%. Under the influence of neem leaf extracts, germination was reduced by 7%. The average germination rate was 90.6%. No fungal contamination was observed during the cultivation of soybean seeds with the various extracts.

3.4. Effect of Plant Extracts on the Germination Rate of Peanuts (*Arachis hypogaea*)

For peanuts, the average germination rate was 51.6%. Germination rates ranged from 80% for Kalanchoe extracts a 34% increase compared to the control to 26% for Neem extracts a 20% decrease compared to the control (**Figure 4**). The Ocimum and Moringa extracts resulted in germination rates of 56% and 50%, respectively, representing increases of 10% and 4% compared to the control. The highest contamination rates were recorded with Neem extracts, at 74%, while the lowest rates, estimated at 20%, were observed with Kalanchoe extracts. Ocimum and Moringa extracts showed contamination rates of 44% and 50%, respectively, representing a reduction of 4% and 10% compared to the control.

3.5. Effect of Kalanchoe, Moringa, Neem, and Ocimum Extracts on the Growth of Peas (*Pisum sativum*)

No significant difference was observed in germination time. The leaf extracts did not alter germination time. However, the leaf extracts did affect seedling germination. Specifically, the longest hypocotyl lengths were induced by Kalanchoe and Ocimum leaf extracts, at 5.23 and 6 cm, respectively. These extracts promoted longer hypocotyls compared to the control, which was distilled water. The shortest lengths were observed with neem leaves, at 2.54 cm. These were significantly shorter than the other lengths, including that of the control. Moringa leaf extracts yielded intermediate values of 3.94 cm, which were significantly longer than the control but also significantly shorter than those of Kalanchoe and Ocimum. Regarding epicotyls, extracts from Ocimum and kalanchoe leaves resulted in the longest lengths, namely 3.14 and 3.21, respectively.

Ocimum and Kalanchoe leaves resulted in the longest lengths, namely 3.14 and 3.21, respectively. These lengths were significantly different from those obtained with moringa and neem extracts, which yielded values of 1.36 and 1.2, respectively. The shortest lengths were obtained with the control, at 0.3. The Moringa and Neem extracts, as well as the control, showed significantly identical and the lowest root numbers. The highest number of roots was obtained with the Kalanchoe extracts (6.23). The Ocimum extracts promoted an intermediate number of roots that were significantly lower than that of Kalanchoe and higher than that of Moringa and Neem (**Table 1**).

Table 1. Effects of leaf extracts on pea germination.

Species and solution	Duration of first germination	Hypocotyl	Epicotyl	Number of roots
Green peas + distilled water	2 ± 0.0a	3.3 ± 1.08bc	0.3 ± 0.44c	2.9 ± 2.15c
Green peas + kalanchoe leaf extract	2 ± 0.0a	5.23 ± 2.43a	3.21 ± 2.75a	6.23 ± 3.56a
Green peas + moringa leaf extract	2 ± 0.0a	3.94 ± 1.42b	1.36 ± 1.04b	3.3 ± 1.76c
Green peas + azadirachta leaf extract	2 ± 2.2a	2.54 ± 0.63c	1.2 ± 0.76b	3.28 ± 1.99c
Green peas + ocimum leaf extract	3 ± 0.0a	6.0 ± 2.43a	3.14 ± 1.79a	4.7 ± 2.94b

Values with different letters (a, b, c, etc.) in the same column ($p < 0.05$) are significantly different. The values are the mean ± standard deviation, as determined by the ANOVA test.

3.6. Effect of Kalanchoe, Moringa, Neem, and Ocimum Extracts on Soybean (*Glycine max*) Growth

In the study of plant extracts on soybean germination, the germination time was statistically identical regardless of the extract used. The extracts had no effect on the speed of germination. However, hypocotyl length varied from 19.4 to 4.22 cm; the longest hypocotyl was observed with ocimum extracts, at 19.4 cm. Kalanchoe extracts resulted in hypocotyl lengths of 17.32 cm. These lengths were significantly different from and greater than that of the control, which was estimated at 9.24 cm. The shortest length, 4.22 cm, was observed with neem extracts. Neem extracts led to a reduction in length compared to the control.

Moringa extracts resulted in hypocotyl lengths of 7.85, which were statistically identical to the control. Distilled water, neem extract, moringa extract, and ocimum extract did not promote epicotyl formation. Only the kalanchoe extract resulted in the formation of an epicotyl 0.7 cm long. Root development was significantly influenced by the plant extracts. Specifically, the Kalanchoe and Ocimum extracts resulted in the longest roots. These lengths were statistically identical. The control group had an average of 14 roots, which was statistically higher than the numbers obtained with the moringa and neem extracts. The number of roots obtained with these two extracts was 11 and 9, respectively (**Table 2**).

Table 2. Effects of leaf extracts on soybean germination.

Species and solutions	Duration of first germination	Hypocotyl (cm)	Epicotyl (cm)	Number of roots
Soybeans + distilled water	2 ± 0.0a	9.29 ± 4.76c	0b	14 ± 7.21b
Soybeans + Kalanchoe leaf extract	2 ± 0.0a	17.32 ± 3.93b	0.7 ± 1.55a	18.53 ± 8.91a
Soybeans + Moringa leaf extract	2 ± 0.0a	7.85 ± 5.23c	0b	10.56 ± 6.46c
Soybeans + Neem leaf extract	2 ± 0.0a	4.22 ± 1.76d	0b	9 ± 3.9c
Soybeans + Ocimum leaf extract	2 ± 0.0a	19.4 ± 4.28a	0b	19.08 ± 6.9a

Values marked with different letters (a, b, c ...) within the same column are significantly different ($p < 0.05$). The values are the mean ± standard deviation according to the ANOVA test.

3.7. Effect of Kalanchoe, Moringa, Neem, and Ocimum Extracts on the Growth of Lentils (*Lens culinaris*)

The time to first germination varied depending on the plant extract. In fact, the control group recorded a germination time that was statistically identical to that of the Kalanchoe, Moringa, and Ocimum extracts. The longest germination time was observed with neem extracts. This time was estimated at 7 days and was shorter than the times observed with Kalanchoe, Ocimum, and Moringa extracts, as well as the control. Neem extracts have an inhibitory effect on lentil germination. Hypocotyl length is also influenced by the type of extract. The longest hypocotyls were observed with Kalanchoe extracts (4.63 cm), while the shortest were obtained with neem extracts (1.57 cm). Between these statistically different values lie the average lengths induced by the moringa and ocimum extracts and distilled water, which were statistically identical but statistically lower than the values obtained with the Kalanchoe extracts. Regarding epicotyls, Kalanchoe extracts also promoted the longest lengths, which were significantly greater than those of the control and the other extracts (4.4 cm). The control, neem extracts, and Ocimum extracts recorded statistically identical values, which were the lowest. Moringa extracts induced an intermediate length of 3.16 cm, which was significantly (**Table 3**).

Table 3. Effects of leaf extracts on lentil germination.

species and solutions	Duration of first germination	Hypocotyl (cm)	Epicotyl (cm)	Number of roots
lentil + distilled water	2 ± 0.0b	3 ± 1.3b	2.16 ± 0.9c	0.33 ± 0.77b
lentil + Kalanchoe leaf extract	2 ± 0.0b	4.63 ± 1.13a	4.4 ± 1.25a	2 ± 2.15a
lentil + Moringa leaf extract	3 ± 0.0b	2.87 ± 1.51b	3.16 ± 1.66b	1.71 ± 2.77a
lentil + Neem leaf extract	7 ± 0.0a	1.57 ± 1.63c	1.87 ± 1.24c	0 ± 0.0b
lentil + Ocimum leaf extract	3 ± 0.0b	2.77 ± 1.05b	1.82 ± 1.05c	0 ± 0.89b

Values marked with different letters (a, b, c ...) within the same column are significantly different ($p < 0.05$). The values are the mean ± standard deviation according to the ANOVA test.

3.8. Effect of Kalanchoe, Moringa, Neem, and Ocimum Extracts on Peanut (*Arachis hypogaea*) Growth

The time to first germination was not significantly affected by the plant extracts. However, hypocotyl length was affected by the leaf extracts. Kalanchoe leaf extracts produced the longest hypocotyls. These hypocotyls were significantly longer than those of the control and the other three leaf extracts. The number of roots was also influenced by the leaf extracts. The highest number of roots was observed with the Kalanchoe extracts. This extract increased the number of roots sixfold compared to the control. The neem and ocimum extracts yielded statistically similar numbers of roots, but these were lower than those obtained with the Kalanchoe extracts. The moringa extracts and distilled water yielded low numbers of roots that were statistically indistinguishable (Table 4).

Table 4. Effects of leaf extracts on peanut germination.

Species and solutions	Duration of first germination	Hypocotyl (cm)	Number of root
peanut + distilled water	2 ± 0.0a	2.92 ± 0.86b	3.27 ± 3.4c
peanut + Kalanchoe leaf extract	2 ± 0.0a	5 ± 2.45a	19.46 ± 10.5a
peanut + Moringa leaf extract	3 ± 0.0a	3.63 ± 1.03b	4.28 ± 5.9c
peanut + Neem leaf extract	3 ± 0.0a	3.53 ± 0.75b	12 ± 6.9b
peanut + Ocimum leaf extract	3 ± 0.0a	3.82 ± 1.7b	9.2 ± 6.03b

Values marked with different letters (a, b, c ...) within the same column are significantly different ($p < 0.05$). The values are the mean ± standard deviation according to the ANOVA test.

4. Discussion

The results obtained show that the germination rate varies depending on the species and the extract used. Pea seeds exhibited excellent germination capacity; germination rates were high and higher than the control for all extracts, with the exception of the neem leaf extracts, which produced a germination rate identical to that of the control. Neem therefore had neither a stimulating nor an inhibitory effect on pea germination. The concentrations of these three leaf extracts (moringa, kalanchoe, ocimum) were compatible with the metabolism of pea seeds. These extracts were not phytotoxic to these legumes. Overall, of the four legumes studied, Kalanchoe leaf extracts performed very well compared with the other three extracts, both in terms of germination rate and in terms of hypocotyl elongation, epicotyl elongation and root number. This is due to the presence of bioactive compounds such as flavonoids, phenolic compounds and organic acids, which are likely to stimulate seed metabolism by enhancing enzymatic activity and promoting the mobilisation of the nutrient reserves necessary for radicle growth [6].

Several studies have demonstrated the presence of flavonoids and phenolic compounds in the leaves of *Kalanchoe pinnata*, particularly quercetin, gallic acid and ferulic acids [7]. Aqueous extraction of the leaves would enable these bioactive compounds to be recovered [8] [9] and colleagues demonstrated the potential of products derived from leaf extraction to promote the germination of seeds from *Bryophyllum pinnatum*, a species related to Kalanchoe pinnata and belonging to the same family. The absence of contamination in peas, soybeans, and lentils resulted in high germination rates because there was no competition from pathogenic microorganisms. In the case of soybeans, the moringa and kalanchoe extracts did not affect germination. The increase in germination rates due to ocimum extracts indicates that these extracts promoted a physiological environment conducive to the growth of soybean seeds. Ocimum leaf extracts yielded promising results and ranked second in terms of growth-promoting potential after kalanchoe. In fact, during imbibition, the molecules produce reactive oxygen species (ROS), which, at low concentrations, act as signaling molecules in germination; however, their excessive accumulation can cause oxidative stress and damage cell membranes [10]. The antioxidant compounds present in Ocimum can neutralise this excess of ROS and promote cellular metabolism. The α-amylases then mobilise the nutrient reserves essential for root formation [11]. Previous studies, notably those in [12], have shown that Ocimum extracts can alter germination and root growth. Lentils appear to be more sensitive to plant extracts than peas and soya beans. This sensitivity could be explained by the small size of this legume, which may exhibit different imbibition kinetics due to its morphology. It is therefore possible that it absorbed the extracts more rapidly, thereby exposing the embryos to the bioactive compounds. This finding is consistent with that reported in [13] (2014). Indeed, biostimulants can stimulate germination at low doses and high concentrations; the same compounds may act as inhibitors due to their allelopathic and phytotoxic effects [14]. With regard to groundnuts, the germination rate was inversely proportional to the contamination level. It is the only legume that showed a contamination rate with all leaf extracts, including distilled water. This is because groundnuts are rich in lipids. Lipids provide a nutrient substrate for fungi such as *Aspergillus sp.*, *Penicillium spp.*, *Rhizopus spp. and Fusarium spp.* [15]. These fungi reduce the viability of the seeds and can cause them to rot, thereby reducing their germination capacity. Furthermore, when the seeds are placed in a damp environment, the seeds and fungal spores germinate simultaneously. If the microorganisms develop faster than the embryo, they utilise the seed's reserves and produce hydrolytic enzymes that degrade the membrane tissues [16]. Disinfecting groundnut seeds with sodium hypochlorite and reducing the volume of water supplied during cultivation could be a solution to limit groundnut contamination [17]. The poor performance of neem extracts compared with other extracts—in terms of both germination rate and the elongation of the hypocotyl, epicotyl and number of roots is due to the allelopathic properties of these secondary metabolites (azadirachtin, nimbin), which must have exerted a phytotoxic effect on the seeds, disrupting the mobilisation of reserves and the elongation of root tissues, hence the delayed germination observed in lentils [18] and the reduction in hypocotyl length observed in peas, soya beans and lentils. These results are corroborated by [19] and colleagues in 2018, who state that germination and seedling growth depend on the balance between several plant hormones. [20] showed that aqueous extracts of neem could exert both stimulatory and inhibitory effects on germination and seedling growth, but the inhibitory effect is pronounced at high concentrations. In this study, the results obtained with moringa extracts were lower than those for Kalanchoe and Ocimum leaf extracts, but significantly higher than those for the control and neem. These results contradict those of [21] in 2026, which stated that moringa extracts can improve aubergine germination by 92 per cent. Moringa leaf extracts are known as biofertilisers, and their efficacy is due to the concentration of the extracts, which varies depending on the crop species. Finally, the leaf extracts had no notable effect on the duration of germination and thus the germination process itself but rather on the formation of the epicotyl, hypocotyl and the number of roots.

5. Conclusion

The plant extracts used had varying effects on legume germination. However, the Kalanchoe extracts stood out from the other three extracts in terms of their performance, both in terms of germination rate and the elongation of epicotyls and hypocotyls, as well as the increase in the number of roots. This extract even reduced contamination in groundnuts. In contrast, *Azadirachta indica* produced the poorest results, with a reduction in the germination rate and in the lengths of the epicotyls and hypocotyls. The concentrations of this extract under the conditions of our experiment were not suitable for seed germination. *Ocimum gratissimum* exhibited significant biostimulant potential, whilst *Moringa oleifera* showed a weaker biostimulant effect than *Kalanchoe pinnata* and *Ocimum gratissimum*. Furthermore, the response to plant extracts is species-specific. Soya beans and peas exhibited very high germination potential, whilst the germination rate was average for lentils and relatively low for groundnuts. These results show that *Kalanchoe pinnata* and *Ocimum gratissimum* can be used as biostimulants, just like moringa. Their effectiveness depends on the plant species, the nature of the extract and its concentration.

Author Contributions

KONAN, A. E. designed the project, set up the experimental setup and wrote the article; Guinagui, N. B. et Silue took measurements of the various parameters studied and carried out the statistical analysis; Tuo, S. supplied the imported seeds and edited the article to improve the quality of the writing.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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