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Effect of different concentrations of ethylmethanesulfonate (ems) on seeds and seedlings of balsa (*Ochroma pyramidale* Cav. Ex Lam.), teak (*Tectona grandis* L.f.) and melina (*Gmelina arborea* Roxb.) at nursery stage

Efecto de diferentes concentraciones de etilmetanosulfonato (ems) en semillas y plántulas de balsa (*Ochroma pyramidale* Cav. Ex Lam.), teca (*Tectona grandis* L.f.) y melina (*Gmelina arborea* Roxb.) en etapa de vivero

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Abstract

The absence of genetic improvement in forest species of commercial interest such as balsa (*Ochroma pyramidale* Cav. ex Lam.), teak (*Tectona grandis* L.f) and melina (*Gmelina arborea Roxb*.), widely cultivated in Ecuadorian territory, can be attributed in large part to the scarce implementation of innovative techniques such as mutagenesis induction, which can generate a series of morphological changes and physiological benefits to improve agronomic performance. In the absence of mutagenic protocols of proven efficacy in these species, the present research sought to evaluate the effect of the application of different concentrations of the mutagen ethylmethanesulfonate (EMS) on the growth of balsa, teak and melina seedlings in the nursery stage, which has been successfully tested in different plant species, by means of three independent trials. The experimental part was carried out in the greenhouses of the company Plantabal S.A., located at Km 4.5 of the Quevedo - Valencia road, province of Los Ríos. A completely randomized design was used in each trial, with four treatments (T1: 0.0% EMS, T2: 0.3% EMS, T3: 0.6% EMS, T4: 1.0% EMS) and five replications.

Key words: Forest species, genetic improvement, mutagenic, EMS, morphological changes.

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Abstract

Resumen

La ausencia de mejoramiento genético en especies forestales de interés comercial como la balsa (*Ochroma pyramidale* Cav. ex Lam.), la teca (*Tectona grandis* L.f) y la melina (*Gmelina arborea Roxb*.), ampliamente cultivadas en territorio ecuatoriano, se puede atribuir en gran parte a la escaza implementación de técnicas innovadoras como la inducción de mutagénesis, misma que puede llegar a generar una serie de cambios morfológicos y beneficios fisiológicos que permitan mejorar el comportamiento agronómico. Ante la ausencia de protocolos mutagénicos de eficacia comprobada en dichas especies, la presente investigación buscó evaluar mediante tres ensayos independientes el efecto de la aplicación de distintas concentraciones del mutágeno etilmetanosulfonato (EMS) sobre el crecimiento de plántulas de balsa, teca y melina en etapa de vivero, el cual ha sido probado con éxito en distintas especies vegetales. La parte experimental se llevó a cabo en los invernaderos de la empresa Plantabal S.A., ubicada en el Km 4.5 de la vía Quevedo – Valencia, provincia de Los Ríos. Para ello, en cada ensayo se empleó un diseño completamente al azar, con cuatro tratamientos (T1: 0.0% EMS, T2: 0.3% EMS, T3: 0.6% EMS, T4: 1.0% EMS) y cinco repeticiones.

Palabras clave: Especies forestales, mejoramiento genético, mutagénico, EMS, cambios morfológicos

Introduction

Ecuador is home to approximately 5000 tree species, which are scattered over a preferentially forested area of about 14.4 million hectares, i.e. more than 50% of the national territory Machmudah et al. (2020). The diversity is so high that in certain areas of the rainforest, more than 200 trees per hectare have been recorded. The National Forestry Directorate has identified 362 species, of which 91 are commercial, and of which native species such as balsa (Ochroma pyramidale Cav. ex Lam.) and exotic species such as teak (Tectona grandis L.f.) and melina (Gmelina arborea Roxb.) could be highlighted. (Warner et al. 2016, p. 34).

According to the natural distribution in Ecuador, balsa predominates in tropical zones in the western and eastern regions and in the foothills of the Andes Mountains Koirala et al. (2021). Teak plantations are present in coastal provinces such as: Los Ríos, Guayas, Esmeraldas, Manabí, and El Oro Ramirez-Gomez et al. (2015). Melina is mostly established in provinces such as Santo Domingo de los Tsáchilas, Esmeraldas, Los Ríos, northwestern Pichincha, part of the provinces of El Oro and Guayas, and certain Amazonian provinces, where most of the planted area is concentrated (Carvalho-Sobrinho et al. 2016, p. 98).

In view of the above, the importance of these species as a source of economic, social and environmental sustenance throughout the national territory is evident Machmudah et al. (2020); Sears et al. (2018). However, in general terms, it is important to highlight that in many cases, these, as well as other species of interest, have not been efficiently exploited despite being an important part of one of the productive sectors with the greatest potential for development and growth Ferraz et al. (2013). This is due to the existence of a series of factors that affect their production, among which we could highlight the lack of quality of the seedlings obtained during the nursery stage, making it impossible later on to develop vigorous and profitable crops from the perspective of the producer (Ghosh et al. 2021, p. 54).

This biological limitation leads to joint efforts to generate alternatives that allow directing actions that enhance and promote the planting of these crops Panyamang et al. (2018), which could become possible if genetic improvement programs are projected, which contribute to increase their yields and resistance to adverse factors Warner et al. (2016) through the establishment of different alternatives and protocols to obtain genetic material with the best possible characteristics (Ghosh et al. 2021, p. 30), so the use of mutagenic agents through induced mutagenesis could be a way to achieve these goals. The use of mutagens in agriculture, such as ethyl methane sulfonate (EMS), has played a key role in this regard Benjavad et al. (2012) and has been reflected in a number of studies on different plant organisms, including microalgae.

For this reason, the present study sought to evaluate the effect of different doses of EMS on seeds and seedlings of balsa, teak and melina during the nursery stage, in order to determine its effectiveness in the production of plants with elite characteristics, which will allow greater productivity and a better benefit/cost ratio for the producer in the future.

Materials and methods

The research was carried out at the Plantabal S.A. industrial complex, whose facilities are located at kilometer 4.5 of the Quevedo-Valencia road, whose geographical coordinates are 0° 59' 19" south latitude and 79° 26' 28" longitude, at 60 meters above sea level and under the following climatic conditions: average temperature of 26.5 ºC, relative humidity of 80% and an annual rainfall of 2162 mm. The experiment consisted of three independent trials, which were carried out under a completely randomized design (CRD), consisting of four treatments and five replications, giving a total of 20 experimental units per forest species (Table 1). To determine the difference between averages, Tukey's multiple range test was used at 95% probability. Data tabulation was carried out in Excel 2016 and statistical analysis was performed using the free software Infostat version 2019. Additionally, the correlation between germination percentage and EMS concentrations was established.

Table 1. *Characteristics of the treatments.*

Trat	Species+EMS concentration	Repetitions	EU/Seeds	No Seed/Treat
Balsa (*Ochroma pyramidale* Cav. ex Lam.)				
1	Balsa + 0.0 % EMS	5		1000
	Balsa + 0.3 % EMS	5		1000
	Balsa + 0.6 % EMS	5		1000
	Balsa + 1.0 % EMS	5		1000
Teak (*Tectona grandis* L.f.)				
1	Teak + 0.0 % EMS	5		1000
	Teak + 0.3 % EMS	5		1000
	Teak + 0.6 % EMS	5		1000

	Teak + 1.0 % EMS	5	1000
	Melina (*Gmelina arborea* Roxb.)		
1	Melina + 0.0 % EMS	5	1000
	Melina + 0.3 % EMS	5	1000
	Melina + 0.6 % EMS	5	1000
	Melina + 1.0 % EMS	5	1000
Total			12000

Trat: Treatments**; TUE:** Experimental Unit Size

Prior to sowing the balsa, teak and melina seeds, a water test was carried out, in which only the fertile seeds were selected and those floating (empty) seeds were discarded. Once homogenized, ethylmethanesulfonate (EMS) was applied to the seeds. For this, it was necessary to mix the mutagen with water to expose the seeds for a period of 8 hours, using the following concentrations: 0.0%, 0.3%, 0.6% and 1.0%. Subsequently, 200 units per replicate were sown, giving a total of 1000 seeds per treatment. Finally, data collection concerning the variables under study was carried out by direct observation.

The number of germinated plants per experimental unit was recorded 15 days after sowing. To establish the data in percentages, it was necessary to use the following formulation (Warner, Jamroenprucksa, and Puangchit 2017)

$$PG = NSG/NSSx100$$

Where:
PG: Germination percentage
NSG: Number of germinated seeds
NSS: Number of seeds sown
Seedling mortality was determined 45 days after planting, counting the total number of dead seedlings up to that date in each experimental unit, using the following formula:

$$PM = \frac{NPM}{NPT} * 100$$

Where:
PM: Mortality percentage
NPM: Number of dead plants
NPT: Number of total plants

Plant height was recorded at 30 and 45 days after planting (dps) for balsa and at 30 and 60 dps for teak and melina. This parameter was considered from the soil surface to the terminal apex of the plant. A sample of 20 plants per experimental unit was randomly selected and measured with a ruler graduated in centimeters.

Stem diameter was recorded at 30 and 45 days after planting (dps) for balsa and at 30 and 60 dps for teak and melina. This parameter was measured at a height of 1 cm above the soil. A sample of 20 plants was selected at random per experimental unit and measured with a caliper.

Plant growth was determined by the difference achieved between the height records at 30 days after planting (dps) and 45 or 60 dps depending on the species. Stem thickening data were obtained from the difference between the values recorded for diameter at 30 days after planting (dps) and that reached at 45 or 60 dps, depending on the species. To determine the degree of relationship between germination percentage and ethylmethanesulfonate (EMS) concentrations, a coefficient of determination called R2 was used.

Result

According to the analysis of variance, significant statistical differences were found between treatments in each of the three trials ($p \leq 0.05$). In the case of the balsa, it was determined that the statistically different treatments were T1 and T2, which obtained 75.90 and 67.90% germination respectively, followed by T3 with 58.70 and finally T4 with 49.80%. Regarding the germination percentage of teak seeds, the treatment that was statistically different was T2 with 67.81%, while T1, T3 and T4 registered similar values with 49.86, 49.78 and 46.43%, respectively. Finally, in the case of melina, it was found that T4 registered a mean different from that obtained by the other treatments with a germination percentage of 89.10%, followed by T2 and T3 with 86.65 and 85.79%, respectively, while T4 was distantly located with 53.86% (Table 2).

Table 2.*Seed germination percentage of balsa (Ochroma pyramidale Cav. ex Lam.), teak (Tectona grandis L.f.) and melina (Gmelina arborea Roxb.) in response to the application of different doses of Ethylmethanesulfonate (EMS).*

Treatments	**Germination percentage**		
	Raft	**Teak**	**Melina**
T1: 0.0% EMS (control)	75.90 a	50.14 b	89.10 a
T2: 0.3% EMS	67.90 a	67.81 a	86.65 b
T3: 0.6% EMS	58.70 b	50.22 b	85.79 b
T4: 1.0% EMS	49.80 c	53.57 b	53.86 c
Average	63.08	55.43	78.85
C.V. (%)	7.29	8.60	1.08

Means in each column with equal letters do not differ statistically (Tukey p≤0.05).

This agrees with the results obtained, who induced phenotypic variations in Phaseolus vulgaris L. cv. 'DOR 364' and observed that, with the increase of EMS concentration, the germination percentage of treated seeds decreased. This trend was observed both in evaluations at 7 and 14 days of the study, so that when the concentration of EMS applied was from 20 to 60%, the number of germinated seeds decreased by 81.30 to 99.19% at 7 days, and from 36.29 to 96.77% at 14 days, respectively.

As described, the germination percentage has been a decisive variable in determining the optimum concentration of EMS to establish mutant populations. As the concentration of EMS increases, the germination percentage is drastically affected, even causing dilation of the germination stage, in many cases resulting in the death of the embryo.

With respect to the mortality variable, statistical differences were found in each of the trials (balsa, teak and melina) (p≤0.05). In the case of balsa, the treatments that had a greater distinction in relation to the averages obtained by the others were T1 and T2 with values of 24.10 and 32.10% respectively, followed by T3 with 41.30 and T4 with 50.20%. Regarding the teak trial, T2 (32.19%) was statistically different from the rest of the treatments (T1, T3 and T4). Finally, in the case of melina, T1 was the most distinct treatment with 10.90%, followed by T2 and T3 with 13.35 and 14.21%, while T4 was even more distant with a mortality rate of 46.14% (Table 3).

Table 3. *Percentage mortality of balsa (Ochroma pyramidale Cav. ex Lam.), teak (Tectona grandis L.f.) and melina (Gmelina arborea Roxb.) seedlings in response to the application of different doses of Ethylmethanesulfonate (EMS).*

Treatments	**Mortality rate**		
	Raft	**Teak**	**Melina**
T1: 0.0% EMS (control)	24.10 c	49.86 a	10.90 c
T2: 0.3% EMS	32.10 c	32.19 b	13.35 b
T3: 0.6% EMS	41.30 b	49.78 a	14.21 b
T4: 1.0% EMS	50.20 a	46.43 a	46.14 a
Average	36.93	44.57	21.15
C.V. (%)	12.45	10.69	4.02

Means in each column with equal letters do not differ statistically (Tukey (P>0.05).

In retrospect to the previous variable, it could be indicated that the percentage of mortality was also inversely proportional to the levels of EMS, both in balsa and melina seeds, maintaining positive records in teak seeds exposed to a concentration of 0.3% EMS; however, at higher levels of the mutagen, mortality gradually increased.

Seventy percent of bell pepper (Caspicum annuum) plants survived at relatively low concentrations between 0.75 and 0.50 % EMS, with the opposite naturally occurring with increasing dosage.

Taking into account the analysis of variance, it was possible to determine that in the variable height at 30 days there were significant differences between treatments in only one of the three trials carried out. Both teak and melina registered statistically similar values (P>0.05), while in the case of the balsa, treatments T1, T2 and T3 with values of 2.30, 2.25 and 1.97 cm, respectively, were statistically different (P≤0.05) compared to T4, which reached a value of 1.21 cm.

Table 4. *Plant height at 30 days in seedlings of balsa (Ochroma pyramidale Cav. ex Lam.), teak (Tectona grandis L.f.) and melina (Gmelina arborea Roxb.) in response to the application of different doses of Ethylmethanesulfonate (EMS).*

Treatments	**Plant height (30 days)**		
	Raft	**Teak**	**Melina**
T1: 0.0% EMS (control)	2.30 a	5.52 a	23.92 a
T2: 0.3% EMS	2.25 a	5.98 a	22.83 a
T3: 0.6% EMS	1.97 a	5.68 a	23.01 a
T4: 1.0% EMS	1.21 b	5.07 a	19.95 a
Average	1.93	5.56	22.43
C.V. (%)	20.63	9.90	11.15

Averages in each column with equal letters do not differ statistically (Tukey (P>0.05). Regarding the second data collection of this variable, which in the case of the trial developed in balsa was at 45 days and in both teak and melina at 60 days. It was found that there were no statistically different differences in balsa and melina (P>0.05). The opposite was the case with teak seedlings, where T2 (17.13 cm) obtained a statistically different average (P≤0.05) than T1 (14.15 cm) and T4 (12.70 cm) (Table 5).

Table 5. *Plant height at 45 days in balsa (Ochroma pyramidale Cav. ex Lam.) seedlings and at 60 days in teak (Tectona grandis L.f.) and melina (Gmelina arborea Roxb.) seedlings in response to the application of different doses of Ethylmethanesulfonate (EMS).*

Treatments	**Plant height (45-60 days)**		
	Raft	**Teak**	**Melina**
T1: 0.0% EMS (control)	10.50 a	14.15 b	40.50 a
T2: 0.3% EMS	11.13 a	17.13 a	41.80 a
T3: 0.6% EMS	10.62 a	14.60 ab	44.35 a
T4: 1.0% EMS	8.58 a	12.70 b	39.45 a
Average	10.21	14.64	41.53
C.V. (%)	48.06	11.18	10.16

Means in each column with equal letters do not differ statistically (Tukey (P>0.05).

As for the variable stem diameter and according to the analysis of variance, it was determined that 30 days after planting, there were no significant statistical differences between the treatments that make up the teak and melina trials (P>0.05). On the contrary, it happened in the balsa seedling trial, where the treatment that showed statistical differences was T4 with 2.26 mm (P≤0.05).

Table 6. *Stem diameter at 30 days in seedlings of balsa (Ochroma pyramidale Cav. ex Lam.), teak (Tectona grandis L.f.) and melina (Gmelina arborea Roxb.) in response to the application of different doses of Ethylmethanesulfonate (EMS).*

Treatments	**Stem diameter (30 days)**		
	Raft	**Teak**	**Melina**
T1: 0.0% EMS (control)	2.97 a	1.90 a	1.90 a
T2: 0.3% EMS	3.16 a	1.80 a	2.00 a
T3: 0.6% EMS	2.85 a	1.70 a	1.80 a
T4: 1.0% EMS	2.26 b	1.60 a	1.65 a
Average	2.81	1.75	1.84
C.V. (%)	8.21	9.90	10.32

Averages in each column with equal letters do not differ statistically (Tukey (P>0.05). The trend was maintained during the second data collection, where, as in the 30-day sampling, the tests carried out on teak and melina showed no statistically significant differences between treatments (P>0.05). While in the case of balsa, it could be evidenced that the statistically different treatment was T2 with a record of 11.92 mm (P≤0.05), followed by T1, T3 and T4 with 3.91, 3.69 and 3.22 mm respectively.

Table 7. *Stem diameter at 45 days in balsa (Ochroma pyramidale Cav. ex Lam.) seedlings and at 60 days in teak (Tectona grandis L.f.) and melina (Gmelina arborea Roxb.) seedlings in response to the application of different doses of Ethylmethanesulfonate (EMS).*

Treatments	**Stem diameter (45-60 days)**		
	Raft	**Teak**	**Melina**
T1: 0.0% EMS (control)	3.91 ab	2.47 a	2.70 a
T2: 0.3% EMS	11.92 a	2.50 a	2.50 a
T3: 0.6% EMS	3.69 bc	2.60 a	2.85 a
T4: 1.0% EMS	3.22 c	2.55 a	2.45 a
Average	5.69	2.53	2.63
C.V. (%)	7.15	11.97	10.86

Means in each column with equal letters do not differ statistically (Tukey (P>0.05). EMS has punctual effects showing genetic lesions induced according to the concentrations and the time that the seeds of a given species are exposed. Such is the case that, in the values obtained for this variable, a not so defined relationship was shown in teak and melina seedlings, where no decrease was evidenced with higher levels of EMS, but this did occur in the case of balsa, where although 0.3% EMS notably surpassed the averages obtained in the other treatments, in treatments such as T3 (0.6% EMS) and T4 (1.0% EMS), shorter diameters were shown in relation to the control.

Regarding plant growth, it was possible to determine that in the trial developed with balsa and melina seedlings, there were no statistical differences between the treatments (P>0.05). The opposite occurred in the trial developed with teak seedlings, where T2 was statistically different with a record of 11.15 cm (P≤0.05), followed by T1 with 8.92 and T3 with 8.63 cm, and finally, T4 with 7.63 cm (Table 8).

Table 8. *Growth of balsa (Ochroma pyramidale Cav. ex Lam.), teak (Tectona grandis L.f.) and melina (Gmelina arborea Roxb.) plants in response to the application of different doses of Ethylmethanesulfonate (EMS).*

Treatments	**Plant growth**		
	Raft	**Teak**	**Melina**
T1: 0.0% EMS (control)	8.21 a	8.63 ab	16.59 a
T2: 0.3% EMS	8.88 a	11.15 a	18.98 a
T3: 0.6% EMS	8.64 a	8.92 ab	21.34 a
T4: 1.0% EMS	7.37 a	7.63 b	19.50 a
Average	8.27	9.08	19.10
C.V. (%)	60.59	20.91	26.22

Means in each column with equal letters do not differ statistically (Tukey (P>0.05).

Finally, with regard to the variable stem thickening and taking into account the ANOVA, it was found that there were no significant statistical differences in the treatments present in each of the trials evaluated (balsa, teak and melina) (P>0.05). This would indicate that the influence of the different doses of EMS was null in each of the case studies (Table 9).

Table 9. *Stem thickening of balsa (Ochroma pyramidale Cav. ex Lam.), teak (Tectona grandis L.f.) and melina (Gmelina arborea Roxb.) plants in response to the application of different doses of Ethylmethanesulfonate (EMS).*

Treatments	**Stem thickening**		
	Raft	**Teak**	**Melina**
T1: 0.0% EMS (control)	9.40 a	0.57 ab	0.80 a
T2: 0.3% EMS	10.92 a	0.70 a	0.50 a
T3: 0.6% EMS	8.40 a	0.90 a	1.05 a
T4: 1.0% EMS	9.60 a	0.95 a	0.80 a
Average	9.58	0.78	0.79
C.V. (%)	35.10	46.02	45.18

Means in each column with equal letters do not differ statistically (Tukey (P>0.05). In any case, it is important to point out that the mutagenic effect is caused randomly on the plants and the value of the mutations in different variables such as stem thickening will depend on the changes produced in the morphology and physiology of the plants and that these have a favorable impact on yield and quality in the long term.

In the trial developed with balsa seeds, a strong negative correlation was obtained between the germination percentage variable and the EMS concentrations used with a correlation coefficient of -0.99. This would suggest that increasing the concentration of EMS applied to the seeds prior to sowing has a negative influence on seed germination (Figure 1).

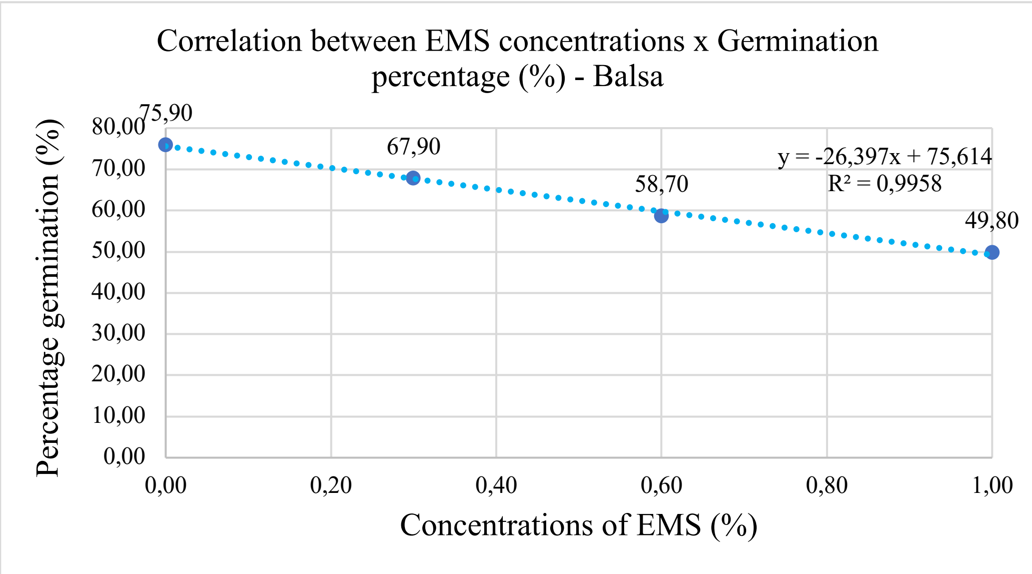

Figure 1. Correlation between EMS concentrations and germination percentage (%) - Balsa

As for the test carried out on teak seeds, a slight negative correlation was recorded between the germination percentage variable and the concentrations of EMS used, with a correlation coefficient of -0.12. This would indicate that a high concentration of EMS would not necessarily have a negative effect on the germination percentage of the seeds of this species (Figure 2).

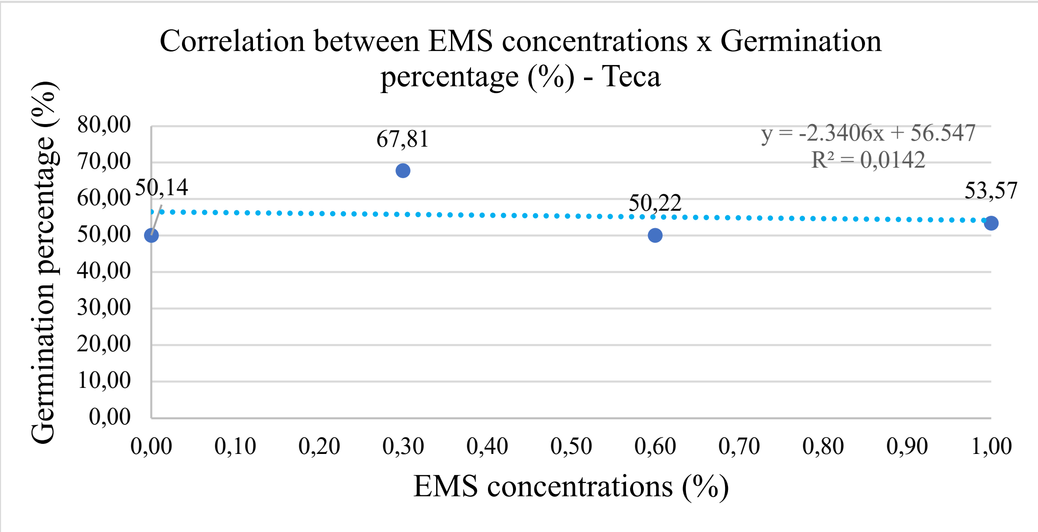

Figure 2. Correlation between EMS concentrations and germination percentage (%) - Teak.

Finally, in the test carried out on melina seeds, as in the balsa seeds, a strong correlation was found between the germination percentage variable and the concentrations of EMS used, with a correlation coefficient of -0.86. This suggests that the higher the concentration of EMS, the lower the germination percentage of melina seeds (Figure 3).

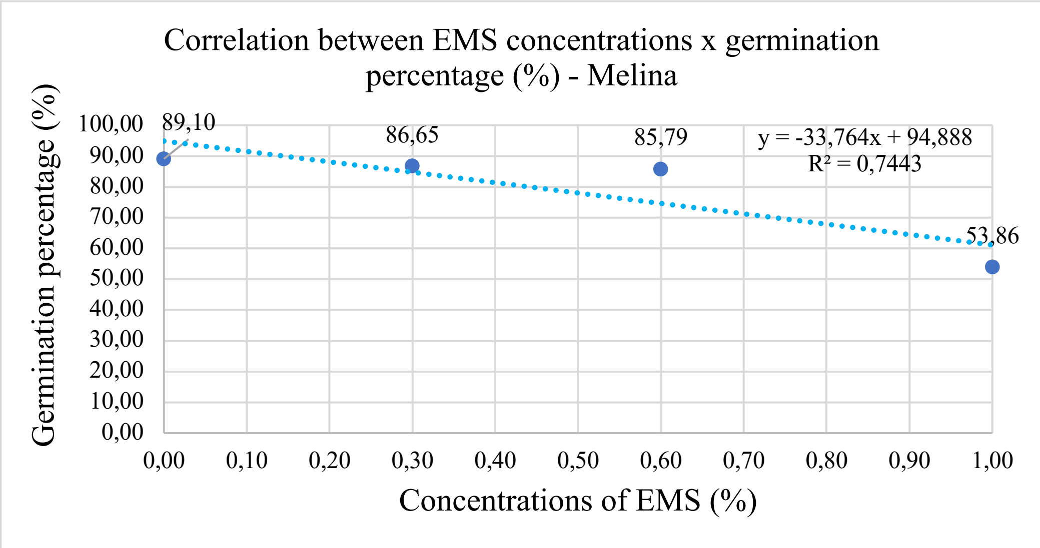

Figure 23. Correlation between EMS concentrations and germination percentage (%) - Melina.

Conclusions

The absence of EMS was associated with a higher germination percentage in balsa and melina seeds. Except in the case of teak seeds, whose best record was obtained with a concentration of 0.3% of the mutagen EMS. The higher the concentration of EMS used, the higher the mortality in balsa, teak and melina seedlings. Regardless of the concentration of EMS used, its influence did not generate a positive phenotypic effect on teak and melina seedlings, precisely in the increase of variables such as height and stem diameter. While in small concentrations such as 0.3% EMS could notably improve these characteristics in balsa seedlings.

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