SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2026v47n2p625

Ethylene on the physiological quality of sunflower seeds

Etileno na qualidade fisiológica de sementes de girassol

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Highlights

Ethylene modulates hormonal balance and metabolic processes.
Ethephon concentrations around 250 mg L^{-1} enhance germination and vigor.
Concentrations above 400 mg L^{-1} lead to progressive physiological collapse.

Abstract

Ethylene plays a fundamental role in the regulation of seed germination, modulating hormonal balance and metabolic processes associated with germination speed, uniformity, and early seedling performance. In this study, the effects of different ethephon concentrations on the physiological quality and vigor of sunflower (*Helianthus annuus* L.) seeds, cv. BRS 323, were evaluated. The experiment was conducted in a Seed Analysis Laboratory at a State University in Minas Gerais, Brazil, using a completely randomized design with five ethephon concentrations (0, 200, 400, 600, and 800 mg L^{-1}) and four replicates of 50 seeds. Water content, radicle protrusion, first germination count, emergence speed index, germination, shoot length, and root length were evaluated. The physiological variables were significantly influenced by ethephon ($p \leq 0.01$), showing quadratic behavior, with coefficients of determination above 91%. Intermediate concentrations promoted a simultaneous increase in germination, vigor, and early seedling growth, with maximum-efficiency points estimated between 251 and 281 mg L^{-1}, depending on the variable. From the respective maximum-efficiency points estimated by the quadratic equations, increasing ethephon concentrations progressively reduced seed physiological quality. The concentration of 600 mg L^{-1} represented the critical dissociation point between vigor and germination capacity, as evidenced

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Received: Jun. 07, 2026 Approved: Out. 05, 2026

by a 24-percentage-point difference between the first count and final germination, indicating a loss of germination uniformity and efficiency despite high final germination. The concentration of 800 mg L^{-1}, in turn, resulted in complete absence of germination. Multivariate canonical variate analysis showed that the response to ethephon occurred along a single physiological gradient, with the first canonical variate explaining 99.56% of the total observed variance. It is concluded that ethephon concentrations close to the estimated maximum-efficiency points enhance the germination and vigor of sunflower seeds cv. BRS 323, whereas concentrations above these points progressively reduce seed physiological quality.
Key words: Dose-response curve. Ethephon. *Helianthus annuus.* Plant growth regulator. Seed vigor.

Resumo

O etileno desempenha papel fundamental na regulação da germinação de sementes, modulando o balanço hormonal e os processos metabólicos associados à velocidade, uniformidade e desempenho inicial das plântulas. Neste estudo, avaliou-se o efeito de diferentes concentrações de etefon sobre a qualidade fisiológica e o vigor de sementes de girassol (*Helianthus annuus* L.) cv. BRS 323. O experimento foi conduzido em Laboratório de Análise de Sementes em uma Universidade Estadual de Minas Gerais, em delineamento inteiramente casualizado, com cinco concentrações de etefon (0, 200, 400, 600 e 800 mg L^{-1}) e quatro repetições de 50 sementes. Foram avaliados o teor de água, a protrusão radicular, a primeira contagem de germinação, o índice de velocidade de germinação, a germinação, o comprimento de coleóptilo e o comprimento de raiz. As variáveis fisiológicas foram significativamente influenciadas pelas concentrações de etefon ($p < 0,01$), com comportamento quadrático e coeficientes de determinação superiores a 91%. As concentrações intermediárias promoveram incremento simultâneo da germinação, do vigor e do crescimento inicial das plântulas, com pontos de máxima eficiência estimados entre 251 e 281 mg L^{-1}, dependendo da variável. A partir dos respectivos pontos de máxima eficiência estimados pelas equações quadráticas, o incremento das concentrações de etefon reduziu progressivamente a qualidade fisiológica das sementes. A concentração de 600 mg L^{-1} representou o ponto crítico de dissociação entre vigor e capacidade germinativa, evidenciado pela diferença de 24 pontos percentuais entre a primeira contagem e a germinação final, indicando perda de uniformidade e eficiência germinativa mesmo na presença de elevada germinação final. Já a concentração de 800 mg L^{-1} resultou em ausência total de germinação. A análise multivariada por variáveis canônicas demonstrou que a resposta ao etefon ocorreu ao longo de um único gradiente fisiológico, com a primeira variável canônica explicando 99,56% da variância total observada. Conclui-se que concentrações de etefon próximas aos pontos de máxima eficiência estimados potencializam a germinação e o vigor de sementes de girassol cv. BRS 323, enquanto concentrações superiores a esses pontos reduzem progressivamente a qualidade fisiológica das sementes.
Palavras-chave: Curva dose-resposta; Etefon; *Helianthus annuus*; Regulador vegetal; Vigor de sementes.

Introduction

Sunflower (*Helianthus annuus*) has gained increasing relevance in Brazilian agribusiness, particularly as a strategic alternative in crop rotation and succession systems following soybean cultivation in the Midwest region. In addition to its agronomic adaptability, the species has high economic value due to the quality of its achenes, which are rich in unsaturated fatty acids, especially oleic and linoleic acids, and are widely used by the food, cosmetics, and biofuel industries (Malunjkar et al., 2024). In this context, obtaining seeds with high physiological quality is essential to ensure rapid and uniform emergence, a condition directly associated with stand establishment and crop productivity (Sghaier et al., 2023).

Germination represents one of the most sensitive phases of the plant life cycle and depends on the interaction between environmental factors and hormonal mechanisms that regulate the resumption of embryonic metabolism. Among the phytohormones involved in this process, ethylene plays a central role in overcoming dormancy and promoting radicle protrusion, acting in an integrated manner with abscisic acid (ABA) and gibberellins (Bewley et al., 2013; Marcos Filho, 2015). Studies have shown that ethylene synthesis progressively increases during imbibition, reaching its peak close to radicle emergence, a stage associated with the weakening of seed coat tissues and the activation of genes related to germination (Corbineau et al., 2014; Xia et al., 2018).

In sunflower seeds, physiological and molecular evidence reinforces the importance of ethylene signaling in the regulation of germination. The activation of ethylene-responsive genes, such as ERF1, is significantly higher in non-dormant embryos, indicating that this hormone acts early in the metabolic reorganization required for the onset of germination (Xia et al., 2018). In addition, ethylene exerts a direct antagonistic effect on ABA, favoring a shift in the hormonal balance toward the resumption of embryonic growth (Beaudoin et al., 2000; Corbineau, 2024).

Ethephon (2-chloroethylphosphonic acid) stands out as an important exogenous source of ethylene and is widely used in physiological studies because of its ability to release the hormone in a controlled manner (Abeles et al., 1992). However, the response of seeds to ethephon depends on the concentration applied. Moderate doses can stimulate germination and vigor through metabolic activation and enhanced hormonal signaling, whereas high concentrations tend to cause phytotoxic effects associated with oxidative stress and reduced germination performance (Kępczyński & Kępczyńska, 1997; Corbineau, 2024) Despite advances in the understanding of ethylene action, information on the response of non-dormant commercial sunflower seed lots to ethephon remains limited, particularly under standardized seed-testing conditions.

Given this gap, this study aimed to evaluate the effects of different ethephon concentrations on the physiological quality and vigor of sunflower seeds, seeking to characterize the dose-response curve to exogenous ethylene and identify concentrations associated with stimulatory or inhibitory effects on germination and early seedling growth.

Material and Methods

The experiment was conducted at the Seed Analysis Laboratory of the Department of Agricultural Sciences of a State University in Minas Gerais, Brazil, between November and December 2024. Sunflower seeds (*Helianthus annuus* L.), cv. BRS 323, were used from a lot with an initial germination rate of 90%, as determined in preliminary tests conducted before the experiment was established.

The experimental design was completely randomized, with five treatments corresponding to the ethephon concentrations (0, 200, 400, 600, and 800 mg L^{-1}) and four replicates of 50 seeds, totaling 20 experimental units. The control treatment (0 mg L^{-1}) consisted exclusively of distilled water. The concentrations were defined based on the physiological response ranges described in the seed literature (Nascimento, 2004; Corbineau, 2024), to encompass biologically active and potentially inhibitory concentrations and allow characterization of the dose-response curve.

The solutions were prepared from the commercial product Ethrel® (active ingredient: 2-chloroethylphosphonic acid, ethephon 240 g L^{-1}), diluted in distilled water at proportions of 0.8, 1.6, 2.5, and 3.3 mL L^{-1}, corresponding to concentrations of 200, 400, 600, and 800 mg L^{-1}, respectively. The solutions were prepared immediately before test installation and used to moisten Germitest® paper sheets at a volume equivalent to 2.5 times the weight of the dry paper. The seeds remained in continuous contact with their respective solutions throughout the evaluation period.

Seed water content was determined by the oven method at 105 ± 3 °C for 24 h, according to the Rules for Seed Testing (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2009), using four replicates of 100 seeds, with the results expressed as a percentage.

For the germination test, the paper rolls containing the seeds were placed in a digital germinator set at a constant temperature of 25 °C, according to the Rules for Seed Testing. Evaluations were performed at four and nine days after sowing by counting the number of normal seedlings, with the results expressed as a percentage. Seedlings were considered normal when they showed complete, developed, proportional, and healthy essential structures, according to the criteria established by the Rules for Seed Testing (MAPA, 2009).

Radicle protrusion was evaluated 48 h after test installation by counting the seeds that showed radicle emission with a minimum length of 2 mm, with the results expressed as a percentage (Rego et al., 2023). This procedure is based on the principle that seeds with higher vigor show faster emission of the primary root (Pereira et al., 2012). The first germination count was performed on the fourth day after test installation by counting the normal seedlings. The emergence speed index (ESI) was determined through daily evaluations and calculated at the end of the test according to the formula proposed by Maguire (1962). On the ninth day after sowing, 20 normal seedlings from each replicate were randomly selected and sectioned at the collar region, separating the shoot from the root system. The lengths of the shoots and primary roots were individually measured

using a millimeter ruler, and the results were expressed in centimeters.

Data were first evaluated for the assumptions of normality of residuals and homogeneity of variances using the Shapiro–Wilk and Bartlett tests, respectively. Once the assumptions were met, analysis of variance (ANOVA) was performed. When a significant effect of ethephon concentration was detected by the F test ($p \leq 0.05$), regression analysis was performed. The models were selected based on the significance of the coefficients by Student's t test ($p \leq 0.05$), the coefficient of determination (R^2), and their biological adequacy to the behavior of the evaluated variables.

In addition, Pearson correlation coefficients (r) were estimated among the evaluated physiological variables to quantify the intensity and direction of the linear associations among the traits in response to ethephon concentrations. The significance of the coefficients was evaluated by Student's t test at 5% probability.

The correlation matrix was used as the basis for multivariate canonical variate analysis to jointly evaluate the physiological responses to ethephon concentrations and identify patterns of similarity among treatments. The number of canonical variates interpreted was defined based on the proportion of accumulated total variance. Statistical analyses were performed using R software, version 4.3.1 (R Core Team [R], 2023), with the aid of the *stats* and *ggplot2* packages for conducting the analyses and graphically representing the results.

Results and Discussion

The average seed water content was 8.3%, a value suitable for orthodox seeds of oilseed species such as sunflower, whose high lipid concentration is associated with lower hygroscopicity compared with starchy species (Bewley et al., 2013; Marcos Filho, 2015). At this moisture level, the seeds maintain metabolic stability and physiological integrity, reducing interference associated with the initial water status and ensuring greater reliability of the responses observed as a function of ethephon concentrations.

Analysis of variance revealed a significant effect ($p \leq 0.01$) of ethephon concentration on all evaluated variables, with coefficients of variation ranging from 3.30 to 5.48%, indicating high experimental precision (Table 1). The quadratic fit observed for all traits demonstrates a strong relationship between the applied concentrations and the physiological responses of the seeds, characterizing a biphasic pattern typical of plant growth regulator action, in which moderate concentrations promote metabolic stimulation, while high concentrations trigger progressive inhibitory effects (Corbineau, 2024).

Table 2
Mean squares of the analysis of variance for germination (G), emergence speed index (ESI), radicle protrusion (RP), first germination count (FC), shoot length (SL), and root length (RL) of seedlings from sunflower seeds subjected to different ethephon concentrations

Source of variation	DF	G	ESI	RP	FC	SL	RL
Treatments	4	7071.200**	59.219**	2091.200**	6400.700**	22.407**	26.951**
Replicates	3	11.400^{ns}	5.972^{ns}	20.467^{ns}	4.800^{ns}	0.034^{ns}	0.010^{ns}
Residual	12	11.067	1.76	9.467	4.967	0.05	0.032
CV (%)	**–**	**4.44**	**4.94**	**3.7**	**3.3**	**5.48**	**3.86**

** significant at 1% probability by the F test; ns = not significant; CV = coefficient of variation.

Germination showed a significant quadratic response to ethephon concentration, with maximum efficiency estimated at 252 mg L^{-1} (Figure 1A). According to the fitted model, germination increased up to this estimated concentration and, from that point onward, progressively decreased with increasing ethephon concentrations, culminating in complete absence of germination at 800 mg L^{-1} (Figure 1A). The emergence speed index showed a similar behavior, with maximum efficiency estimated at 281 mg L^{-1} (Figure 1B). Beyond this point, increasing ethephon concentrations resulted in a progressive reduction in the ESI, evidencing a quadratic response similar to that observed for germination.

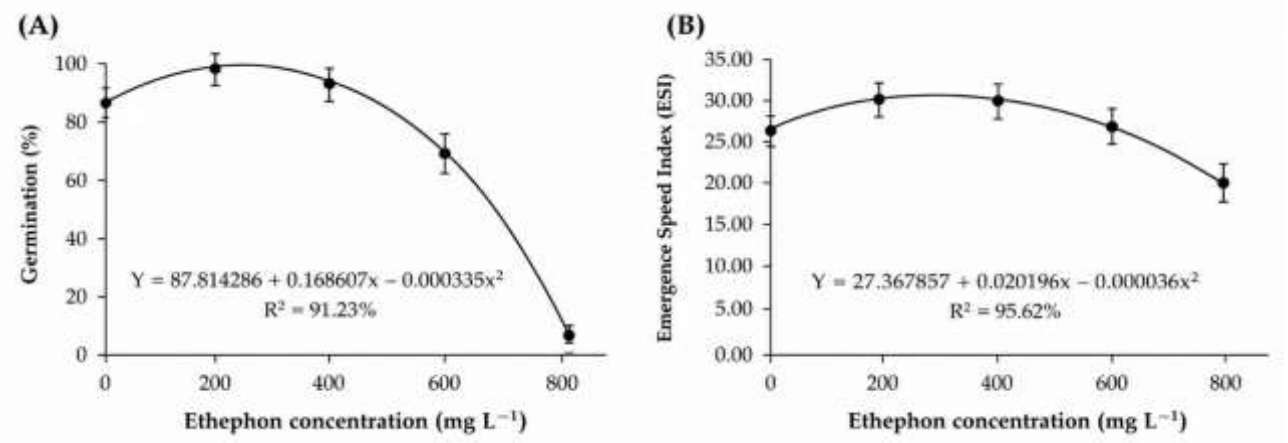


Figure 1. Germination (A) and emergence speed index (B) of sunflower seeds subjected to different ethylene concentrations.

This behavior demonstrates that ethylene acts directly on the modulation of the hormonal balance associated with germination. By attenuating the inhibitory signaling mediated by ABA, ethylene favors the activation of germination metabolism and intensifies the events that culminate in radicle protrusion (Beaudoin et al., 2000; Corbineau, 2024). In non-dormant sunflower seeds, such as those of the cultivar BRS 323 used in this study, this effect was evidenced by the quadratic responses observed, with maximum efficiencies estimated at 252 and 281 mg L^{-1} for germination and the emergence speed index, respectively. Beyond these estimated concentrations, increasing ethephon concentrations resulted in a progressive reduction in the respective physiological responses.

In contrast, the complete absence of germination observed at 800 mg L^{-1} (Figure 1A) evidences complete inhibition of the germination process. Excessive ethylene concentrations can intensify the formation of reactive oxygen species (ROS), compromising cell membrane integrity and disrupting metabolic processes essential for germination (Corbineau et al., 2024). In addition, changes in osmotic dynamics and cell expansion may physically prevent radicle protrusion, even in metabolically active seeds.

Radicle protrusion also showed a quadratic response, with maximum efficiency estimated at 277 mg L^{-1} (Figure 2), corresponding to the concentration estimated by the model for the maximum percentage of seeds showing radicle protrusion. Beyond this point, increasing ethephon concentration resulted in a progressive reduction in this variable (Figure 2). This result reinforces the role of ethylene in coordinating the events associated with the weakening of the restraining tissues and embryonic expansion during germination (Corbineau et al., 2014). The weakening of these tissues involves changes in their mechanical properties, reducing the resistance exerted on the embryo and favoring its expansion and, consequently, radicle protrusion. In sunflower seeds, ethylene-responsive genes, such as ERF1, show higher expression in non-dormant embryos, indicating intense ethylene signaling activity during germination sensu stricto (Xia et al., 2018).

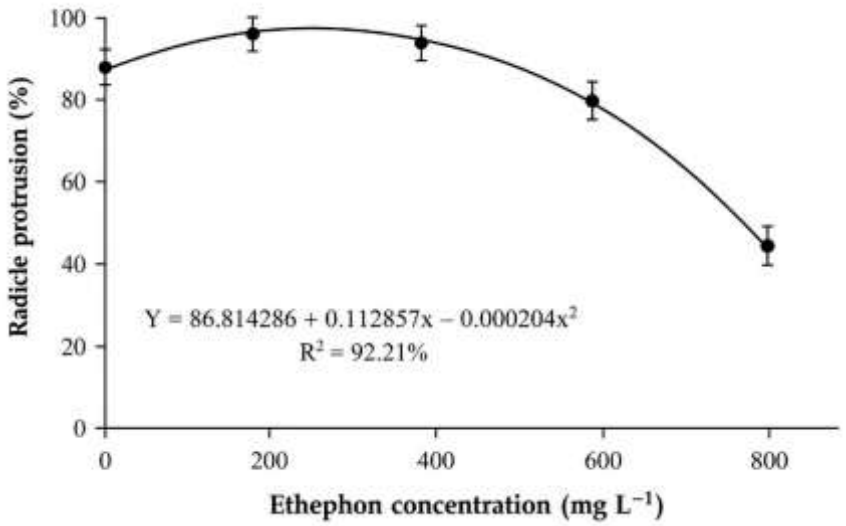


Figure 2. Radicle protrusion of sunflower seeds subjected to different ethylene concentrations.

The first germination count showed a quadratic response to ethephon concentration, with maximum efficiency estimated at 251 mg L^{-1} (Figure 3). Beyond this estimated concentration, a progressive reduction in the first count was observed with increasing ethephon concentrations. The comparison between final germination and the first count at 600 mg L^{-1} shows that vigor was compromised before the complete loss of germination capacity. Although some seeds still retained germination capacity, there was a marked reduction in performance at the first count, indicating greater sensitivity of vigor to increasing ethephon concentrations (Marcos Filho, 2015).

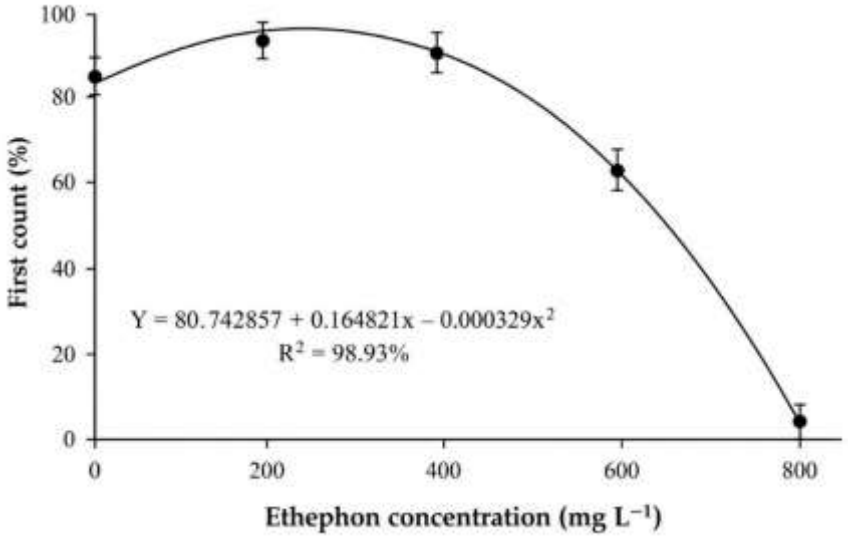


Figure 3. First germination count of sunflower seeds subjected to different ethylene concentrations.

Shoot and root lengths fitted the quadratic model, with maximum efficiencies estimated at approximately 268 and 260 mg L^{-1}, respectively (Figure 4A and 4B). According to the fitted equations, these concentrations corresponded to estimated maximum lengths of approximately 6.12 cm for the coleoptile and 6.77 cm for the root. Beyond the respective maximum-efficiency points, increasing ethephon concentration resulted in a progressive reduction in growth, reflecting impairment of tissue elongation associated with excess ethylene (Corbineau, 2024).

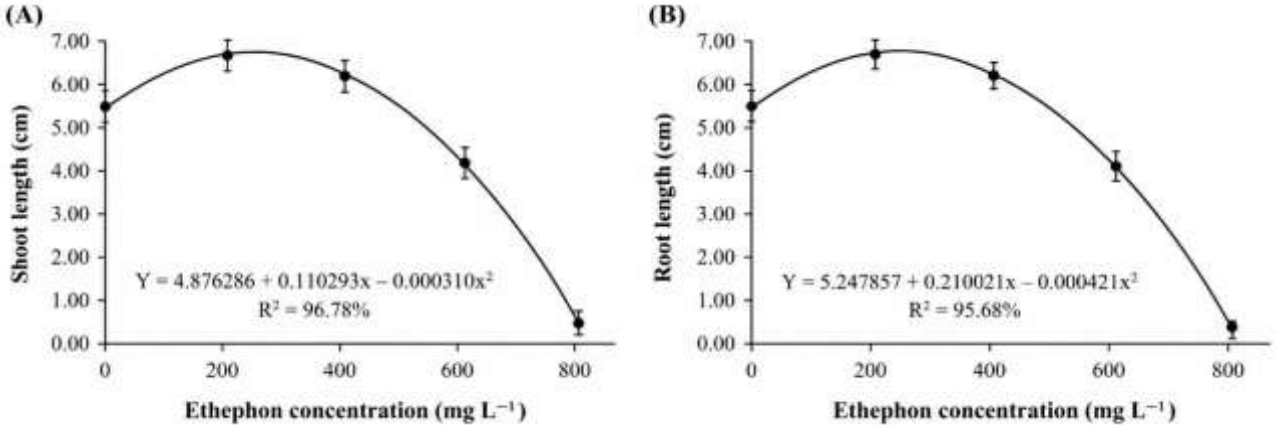


Figure 4. Shoot length (A) and root length (B) of seedlings from sunflower seeds subjected to different ethephon concentrations.

The high coefficients of determination obtained for the fitted equations indicate a strong fit of the data to the models, allowing the responses of the variables to be explained as a function of ethephon concentration. This behavior was corroborated by the multivariate canonical variate analysis (Figure 5). The first canonical variate explained 99.56% of the total variance among treatments, whereas the second accounted for only 0.42%, demonstrating that the response of the seeds to ethephon occurred predominantly along a single physiological gradient. Considering the fitted equations, the maximum-efficiency concentrations estimated for the physiological variables were close to one another, ranging from 251 to 281 mg L^{-1}, depending on the trait evaluated. Beyond these estimated concentrations, increasing ethephon concentration resulted in a progressive reduction in the physiological responses. This result shows that ethephon acted in an integrated manner on germination, vigor, and early growth rather than on isolated variables.

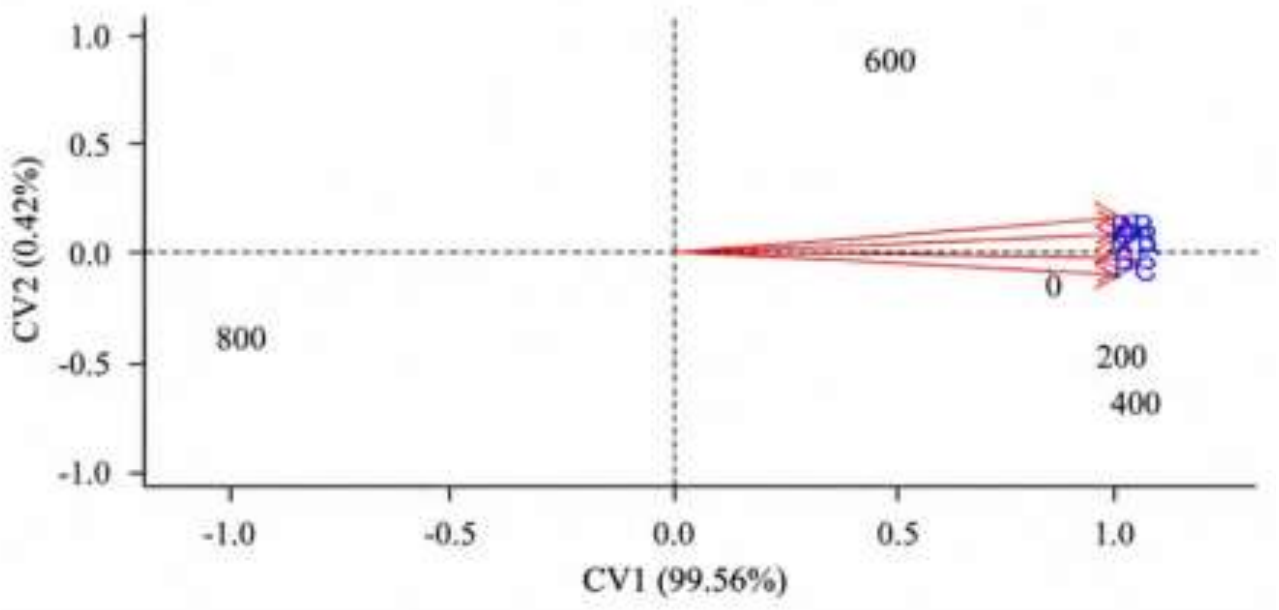


Figure 5. Scatter plot of the scores on the first two canonical variates (CV1 and CV2) as a function of ethephon concentrations (0, 200, 400, 600, and 800 mg L^{-1}).

The analysis of the normalized response curves (Figure 6) provided an integrated view of the physiological behavior of the seeds in response to increasing ethephon concentrations. Standardization of the variables relative to the maximum observed value made it possible to identify a high degree of convergence among germination, vigor, and early growth, as all traits displayed their highest relative values at concentrations ranging from 251 to 277 mg L^{-1}. In this range, higher germination, greater germination speed, and greater seedling growth were observed simultaneously, indicating that moderate ethephon concentrations enhance different metabolic processes involved in germination and early seedling establishment.

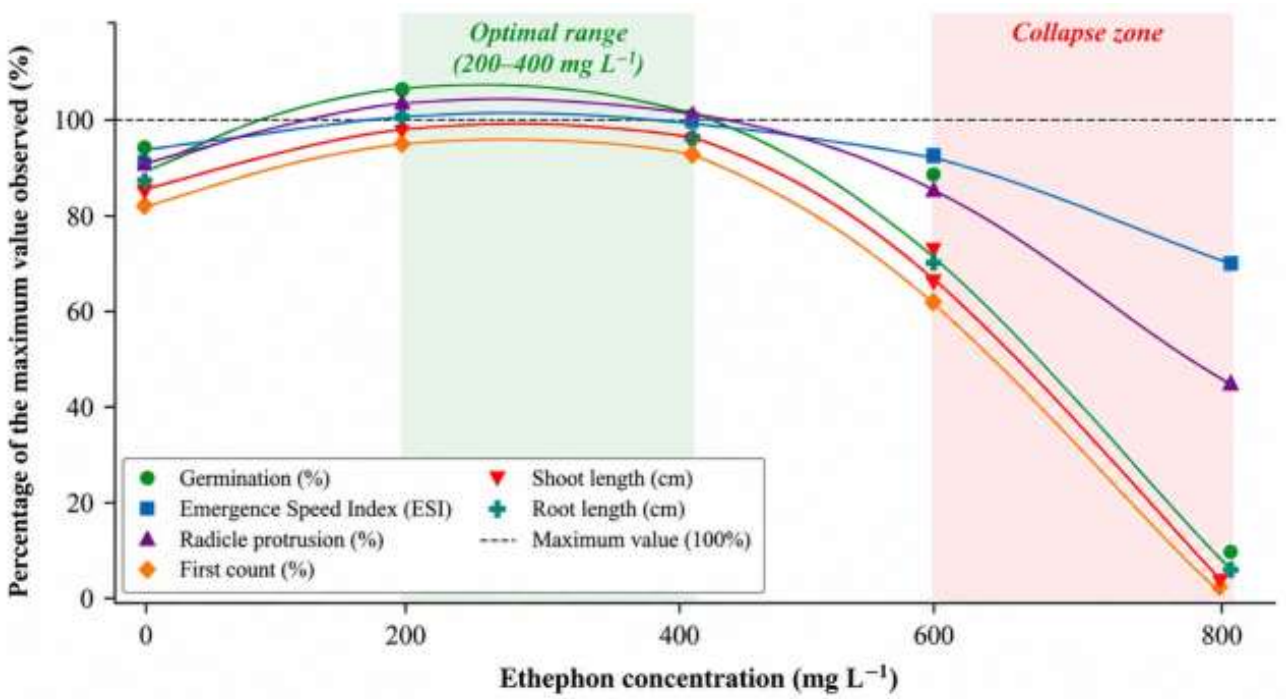


Figure 6. Normalized response curves of the physiological variables of sunflower seeds (*Helianthus annuus* L.) cv. BRS 323 subjected to different ethephon concentrations. Values expressed as a percentage of the maximum value observed for each variable.

However, the responses of the variables to increasing concentrations did not occur uniformly. The emergence speed index (ESI) and radicle protrusion showed greater stability in response to increasing concentrations, maintaining relatively high values even at 600 mg L^{-1}. In contrast, the first count, shoot length, and root length showed a marked reduction at this concentration, reaching values close to zero at 800 mg L^{-1}. This behavior evidences differences in the sensitivity of physiological responses to excess ethephon, with processes related to seedling growth and vigor being compromised more rapidly than the early events associated with metabolic activation and radicle emission.

The partial maintenance of ESI and radicle protrusion at high concentrations suggests that the initial activation of germination metabolism still occurs even under conditions of ethephon excess. However, subsequent seedling development becomes severely limited, particularly in processes dependent on cell expansion and differentiation. This pattern may be associated with increased oxidative stress and impairment of membrane and cell wall integrity under high ethylene concentrations, primarily affecting actively growing tissues (Corbineau, 2024). Another relevant aspect was that, in the control treatment (0 mg L^{-1}), the normalized values of the variables remained below their respective maximum

values observed between 251 and 277 mg L^{-1}, as shown in Figure 6. This behavior indicates that intermediate ethephon concentrations produced greater responses than those observed in untreated seeds for the evaluated traits, reinforcing its positive effect on physiological quality when applied at adequate concentrations.

The analysis of the dissociation between the first count and final germination revealed important changes in the physiological response of the seeds to increasing ethephon concentrations (Figure 7). The relationship between these variables made it possible to identify not only changes in final germination but also changes in seed performance at the first count. At concentrations of 200 and 400 mg L^{-1}, the points remained close to the line of equality between the variables (FC = G) (Figure 7A), indicating a small difference between final germination and the first count. At these concentrations, the seeds showed high final germination associated with high first-count values, indicating better physiological performance.

In contrast, the concentration of 600 mg L^{-1} led to a marked displacement of the point from the line of equality, reflecting a significant increase in the difference between final germination and the first count (Figure 7A). This dissociation was 24 percentage points (Figure 7B), evidencing greater impairment of vigor relative to final germination. Although final germination remained high, there was a substantial reduction in the first count, indicating a delay in the germination process. This behavior suggests that high ethephon concentrations initially compromise processes associated with vigor before completely inhibiting germination.

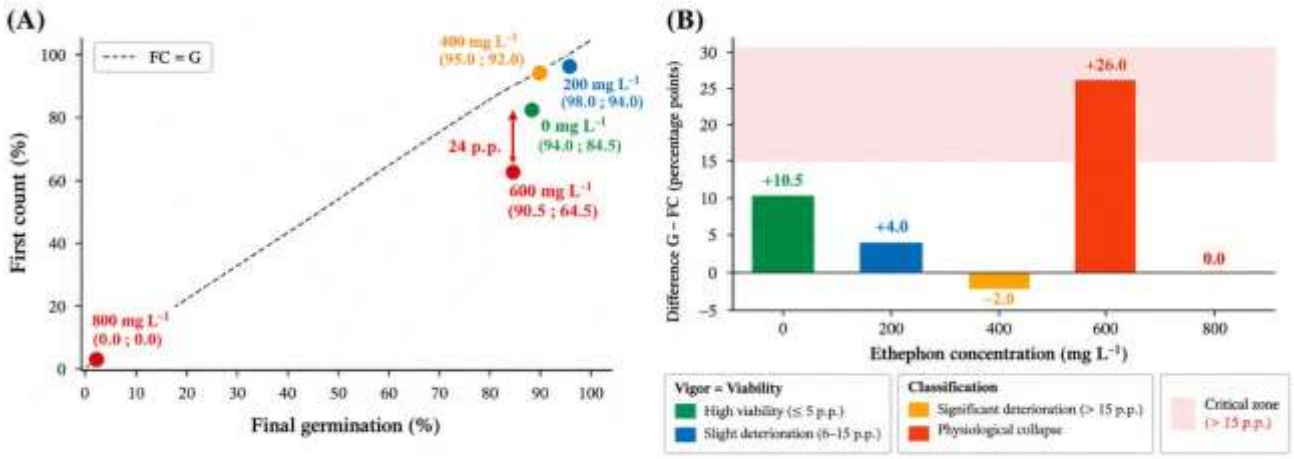


Figure 7. Dissociation between vigor and viability of sunflower seeds (*Helianthus annuus* L.) cv. BRS 323 subjected to different ethephon concentrations.
(A) Scatter plot between first germination count (vigor indicator) and final germination by concentration. The dashed line represents the condition of equality between the two variables (FC = G). (B) Difference, in percentage points, between final germination and first count by ethephon concentration.

From a physiological point of view, this pattern indicates that the initial activation of germination metabolism still occurs under high ethephon concentrations, but the continuation of processes related to cell expansion and seedling establishment becomes limited. Under field conditions, this dissociation between vigor and germination capacity may result in non-uniform emergence, lower stand establishment, and greater exposure of seedlings to biotic and abiotic soil stresses (Sghaier et al., 2023).

The concentration of 800 mg L^{-1} caused a simultaneous reduction in vigor and germination capacity, positioning the treatment close to the origin of the scatter plot (Figure 7A) and resulting in a null difference between the variables (Figure 7B). However, this apparent "equality" does not represent physiological balance but rather a complete absence of germination response. This result shows that the concentration of 800 mg L^{-1} ethephon exceeded the seeds' tolerance limit under the evaluated conditions, resulting in complete inhibition of germination.

Taken together, the results demonstrate that ethephon exerts a biphasic effect on the physiological quality of sunflower seeds. The estimated maximum-efficiency concentrations ranged from 251 to 281 mg L^{-1}, depending on the variable evaluated, and, beyond these points, increasing ethephon concentrations resulted in a progressive reduction in the physiological responses. The convergence of the estimated maximum-efficiency concentrations, associated with the integrated response of the evaluated physiological variables, demonstrates that ethylene acts in a coordinated manner on multiple processes involved in germination and early seedling establishment. These results broaden the understanding of ethylene physiology in sunflower seeds and provide an experimental basis for studies aimed at improving seed physiological quality.

Conclusions

The estimated maximum-efficiency concentrations indicated that ethephon enhances the germination performance and vigor of sunflower seeds at concentrations close to 250 mg L^{-1}, with a progressive reduction in physiological quality beyond the respective maximum-efficiency points.

Acknowledgments

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) – Finance Code 001, the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig), and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and the granting of study fellowships, as well as the home institution and collaborators for making this research possible.

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