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EFFECTS OF BOTANICAL AND COMMERCIAL SEED TREATMENTS ON GERMINATION AND EARLY SEEDLING DEVELOPMENT IN MAIZE, SUNFLOWER AND OILSEED RAPE

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Abstract: This study evaluated the effects of selected biological and commercial seed treatment products on seed germination and early radicle development in maize (*Zea mays*), oilseed rape (*Brassica napus*) and sunflower (*Helianthus annuus*) under controlled laboratory conditions. Treatments included a plant-based decoction applied at different concentrations, commercial formulations containing methyl anthranilate (Repel Aves) and terpenoid compounds (Requiem Prime) as well as a vacuum-assisted application method with untreated seeds used as controls. Across all crops and treatments, final germination percentages remained high and comparable to the control, generally ranging between 92.5 and 100%, indicating no adverse effects on seed viability at the tested concentrations. In maize, vacuum-assisted application of the plant-based decoction did not reduce final germination (76-100%), but significantly inhibited early radicle elongation in a concentration-dependent manner ($p < 0.001$). In oilseed rape, treatments with Repel Aves and Requiem Prime maintained stable germination levels (≥94%) with only minor and transient delays observed at early evaluation stages. Sunflower seeds showed high germination across all variants, reaching 90-100% by day 5, with no significant effects on radicle growth. These results indicate that the tested biological products and commercial formulations are compatible with seed germination under short-term laboratory conditions. Early radicle growth proved more sensitive than germination to treatment effects, highlighting the importance of including additional growth parameters when evaluating alternative seed treatment strategies.

Keywords: *seed germination, maize, sunflower, oilseed rape, botanical decoction, seed treatment.*

INTRODUCTION

Seed treatment is widely recognized as a key component of modern crop production, providing protection during the most vulnerable stages of plant development and contributing to improved stand establishment. By reducing losses caused by seed- and soil-borne pathogens as well as early-season pests, seed treatments play a critical role in securing yield stability and crop uniformity (Munkvold et al., 2014; Lamichhane et al., 2020). In major field crops such as maize, wheat, oilseed rape and soybean, seed treatment represents an essential and often irreplaceable tool for early crop protection, being applied on a large proportion of the cultivated area worldwide (Lamichhane et al., 2022; Vojvodić & Bažok, 2021). Beyond plant protection, appropriate seed treatment strategies may also positively influence germination, emergence, and early seedling vigor, thereby supporting crop performance under diverse agro-environmental conditions (Sharma et al., 2015).

Despite their agronomic importance, chemical insecto-fungicidal seed treatments are increasingly shaped by regulatory and environmental constraints, particularly within the

European Union. The restriction of certain neonicotinoid active substances for outdoor use has been driven by risk assessments highlighting potential impacts on non-target organisms (Bonmatin et al., 2015; Wood & Goulson, 2017; Vojvodić & Bažok, 2021). Nevertheless, chemical seed treatments continue to play a crucial role in crop protection systems, especially in regions and cropping systems where alternative control measures remain limited or insufficient. Under these conditions, there is growing interest in complementary and alternative seed treatment approaches that can support existing protection strategies, improve sustainability, and reduce dependence on a narrow range of active substances while maintaining effective control of early-season pests and pathogens (Douglas & Tooker, 2015; Lamichhane et al., 2020).

In this context, biological and botanical seed treatments have attracted increasing attention as environmentally friendly alternatives to synthetic chemicals. Biological seed treatments, including microbial inoculants and biostimulants, have been shown to enhance germination, seedling vigor and stress tolerance, while potentially reducing reliance on conventional pesticides (Lamichhane et al., 2022; Cardarelli et al., 2022). Similarly, plant-based products and botanical extracts applied as seed treatments have demonstrated promising insecto-fungicidal activity against seed-borne pathogens, often characterized by low residue levels and good compatibility with organic and integrated pest management systems (Șuteu et al., 2020; Mongiano et al., 2021). Many of these approaches are also aligned with circular-economy principles, as they may be derived from renewable or low-impact raw materials and help reduce chemical inputs in agriculture (Wazeer et al., 2024).

The magnitude and direction of biological seed treatments are strongly dependent on formulation, concentration, and application method. Inappropriate dosing, poor compatibility, or unsuitable application techniques may result in phytotoxic effects or reduced seed viability, emphasizing the need for careful laboratory evaluation prior to field implementation (Cardarelli et al., 2022; Carvalho et al., 2020). These considerations are particularly relevant when new biological or botanical formulations are proposed as alternatives to conventional seed treatments.

Seed treatment technologies currently range from simple soaking and priming methods to various coating techniques designed to deliver active substances to the seed surface. Conventional approaches, such as seed dressing and film coating, are widely used due to their simplicity and scalability (Halmer, 2006; Afzal et al., 2020). However, these methods may present limitations related to application uniformity and the penetration of active compounds into internal seed tissues, particularly when biological formulations are employed (Pedrini et al., 2020). Although industrial seed treatment systems generally offer improved dose accuracy and reduced mechanical damage compared with on-farm applications, challenges remain in ensuring efficient delivery of active substances without compromising seed viability (Reis et al., 2023). These limitations highlight the need for innovative application techniques capable of enhancing treatment efficacy while preserving seed quality.

Recently, vacuum-assisted seed treatment has been proposed as a promising approach to improve the penetration and internal distribution of treatment solutions within seeds. By removing air from seed coat pores before infiltration, vacuum application facilitates deeper uptake of active compounds compared with conventional soaking or priming methods (O'Keefe et al., 2025). Preliminary studies have demonstrated that vacuum-based approaches can be successfully applied to maize seeds, allowing optimization of technical parameters such as pressure and exposure time while maintaining high germination rates (Ipate et al., 2024). However, the application of vacuum-assisted techniques for biological insecto-fungicidal seed treatments remains insufficiently explored and requires further investigation

across different crop species.

The aim of this study was to evaluate, under controlled laboratory conditions, the effects of selected commercial and experimental biological insecto-fungicidal seed treatments on germination and early seedling development in maize, sunflower and oilseed rape. In addition, a vacuum-assisted seed treatment method was developed and preliminarily assessed in maize in order to evaluate its technical feasibility and its effects on seed germination and early root growth. This work represents a preliminary step toward the development of alternative biological seed treatment technologies with improved application efficiency and potential applicability to major field crops.

MATERIAL AND METHODS

Plant material

In vitro experiments were conducted using seeds of maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.) and oilseed rape (*Brassica napus* L.) with uniform morphology and without visible mechanical damage.

Tested products

The study evaluated both commercial and experimental biological seed treatment products. The experimental product consisted of a complex plant-based decoction applied at two concentrations (100 and 50%, v/v). In addition, two commercial products were tested: Repel Aves (Orange Saft), based on methyl anthranilate (30%), and Requiem Prime, a blend of terpenoids (α-terpinen: 59.7%; p-cymen: 22.4%; d-limonene: 17.9 %), used for its repellent, insecticidal and fungicidal effects, applied at various concentrations. Distilled water-treated seeds served as control.

Preparation of the plant-based decoction

The plant-based decoction was prepared using dried or fresh plant materials, including *Agropyron repens* L. (leaves and stems), *Calendula officinalis* L. (inflorescences), *Capsicum annuum* L. (fruit powder), *Ailanthus altissima* Mill. (fresh bark), and *Lavandula angustifolia* Mill. (leaves and inflorescences). A total of 120 g of plant biomass was boiled in 1000 mL of distilled water for 15 minutes. After cooling, the decoction was decanted, filtered and stored at 4°C until use.

Vacuum-based seed treatment of maize

Treatment solutions were prepared at concentrations of 25, 50, 75, and 100%. Maize seeds were treated using a vacuum-assisted immersion method. For each treatment, 1000 seeds were selected and divided into five lots of 200 seeds each. Seeds were placed in a 150 mL glass beaker containing 100 mL of the treatment solution and introduced into a vacuum chamber. A vacuum was applied for 6 minutes to remove air from the seed coat pores, followed by an additional 6-minute immersion at atmospheric pressure to facilitate solution uptake. The procedure was repeated for all treatment variants. Control seeds were treated with distilled water following the same protocol. After treatment, seeds were placed in ventilated Petri dishes on cellulose paper moistened with 5 mL of distilled water. Each replicate consisted of 25 seeds. Germination tests were conducted at 25°C for 4-7 days.

Seed surface sterilization for conventional treatments

Before conventional treatments, sunflower and oilseed rape seeds were surface-sterilized. Seeds were visually inspected and damaged seeds were discarded. Selected seeds were placed in 50 mL sterile conical tubes (25 seeds per tube) and sequentially treated with 70% ethanol for 3 minutes and 2% sodium hypochlorite for 3 minutes under continuous inversion. Seeds were rinsed five times with sterile deionized water and air-dried at room temperature for 24 hours.

Conventional seed treatment with commercial products

Treatment solutions were prepared at different concentrations according to species-specific protocols. Two commercial products were tested, Repel Aves (Orange Saft) and Requiem Prime (Bayer), which were applied at doses of 125, 250, 400, 500, 700 and 1000 ppm (product per dried seed). Seeds (20 per replicate, four replicates per treatment) were immersed in the treatment solutions for 30–45 minutes, then air-dried for 48 hours at 25°C. Treated seeds were placed in 9 cm Petri dishes lined with sterile filter paper moistened with 3 mL of sterile distilled water and incubated at 25°C for 4–7 days. Germination was recorded daily, and root length was measured at the end of the incubation period.

Conventional seed treatment with the plant-based decoction

Sunflower and oilseed rape seeds were treated with the plant-based decoction at two concentrations: 100 and 50% (v/v). Treatments were performed by conventional immersion, maintaining the seeds in contact with the solutions for 30 minutes, followed by air-drying for 24 hours at 25°C. Germination tests were conducted under the same conditions described above. After treatment and air-drying, seeds were subsequently arranged in Petri dishes for germination testing.

Experimental design and evaluation

All experiments were conducted under controlled laboratory conditions using untreated seeds as controls. Germination percentage was used as the primary parameter for evaluating seed viability following treatment, and germination was recorded daily during the incubation period. Early seedling development was assessed by measuring primary radicle length at the end of the incubation period. Germination and radicle length data were analyzed by one-way analysis of variance (ANOVA) to assess the effects of treatment and concentration within each crop species. When significant differences were detected, means were compared using Tukey's honestly significant difference (HSD) post hoc test at $p \leq 0.05$. In addition, linear regression analysis was performed on mean germination percentages to evaluate the relationship between treatment concentration and germination response.

RESULTS

Maize: effect of vacuum-applied plant-based decoction on seed germination and radicle elongation

The assessment of maize seed germination showed that vacuum-assisted treatment with the plant-based decoction at concentrations of 25, 50, 75 and 100% did not result in statistically significant differences compared with the control. One-way analysis of variance (ANOVA) confirmed the absence of significant treatment effects on final germination percentage (F(4.35)

= 0.64, $p = 0.64$) and Tukey's HSD post hoc test did not reveal significant differences between the control and any of the treated variants. Germination percentages remained high across all treatments, ranging from 76 to 100%, with substantial overlap among concentrations, indicating good seed viability irrespective of the decoction dose applied. Linear regression analysis of mean germination percentages suggested a negative trend between increasing decoction concentration and germination response ($R^2 = 0.8762$). However, this relationship did not translate into statistically significant differences among treatments, indicating that the observed trend was not biologically limiting under the experimental conditions tested. These results indicate that vacuum-assisted application of the plant-based decoction, at both low (25-50%) and high (75-100%) concentrations, did not negatively affect the final germination capacity of maize seeds (Figure 1a).

Measurements performed on day 5 of incubation revealed a significant effect of the plant-based decoction on radicle elongation in maize seedlings following vacuum-assisted treatment (Figure 1b). One-way ANOVA indicated highly significant differences among treatments ($F(4.35) = 20.6$, $p < 0.0001$). Tukey's HSD post hoc test showed that all decoction-treated variants (25, 50, 75 and 100%) exhibited significantly shorter radicles compared with the control. The 25% concentration resulted in intermediate radicle lengths, which were significantly lower than those of the control but higher than those observed at higher concentrations. Treatments with 50, 75 and 100% decoction caused a pronounced inhibition of radicle elongation, with no statistically significant differences among these higher doses, suggesting that the inhibitory effect reached a plateau at medium to high concentrations. These results indicate that vacuum-assisted application of the plant-based decoction exerted a clear inhibitory effect on early radicle growth in maize, even at moderate concentrations, despite the absence of negative effects on final germination percentage.

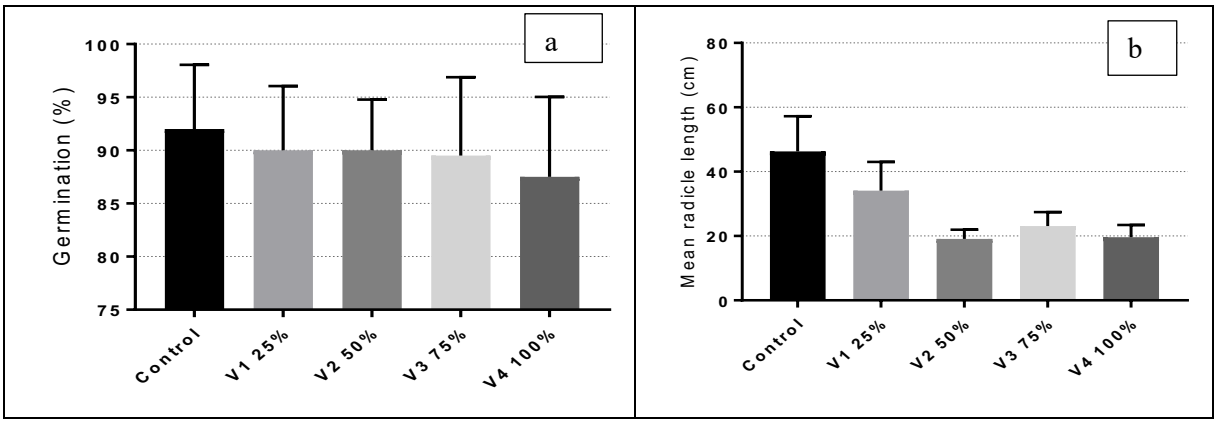

Figure 1. Effect of vacuum-applied plant-based decoction on maize seed germination (a) and radicle elongation (b)

Oilseed rape: effects of plant-based decoction and commercial treatments

Germination

Effect of conventional immersion treatment with the plant-based decoction on oilseed rape germination

Germination of oilseed rape seeds treated by conventional immersion with the plant-based decoction at two concentrations (undiluted, V1 and diluted 1:1, V2) was not negatively affected compared with the control (Figure 2). On day 3, germination reached 97.5% in the

control and 92.5% in both treated variants, with no statistically significant differences among treatments ($F(2.9) = 0.86$, $p = 0.457$). On day 4, germination increased to 97.5% in both the control and the undiluted decoction treatment, while the diluted variant reached 93.8%; however, these differences were not statistically significant ($F(2.9) = 0.79$, $p = 0.485$). By day 5, all variants exhibited high and comparable germination percentages (97.5% for the control, 98.8% for the undiluted decoction, and 96.3% for the 1:1 dilution), and ANOVA confirmed the absence of significant treatment effects ($F(2.9) = 1.00$, $p = 0.404$). Tukey's HSD post hoc test did not reveal significant differences between treatments at any evaluation time point. Taken together, these results indicate that application of the plant-based decoction, both in undiluted form and at a 1:1 dilution, did not adversely affect the germination capacity of oilseed rape seeds. Minor variations observed during early evaluation stages were transient and not statistically significant.

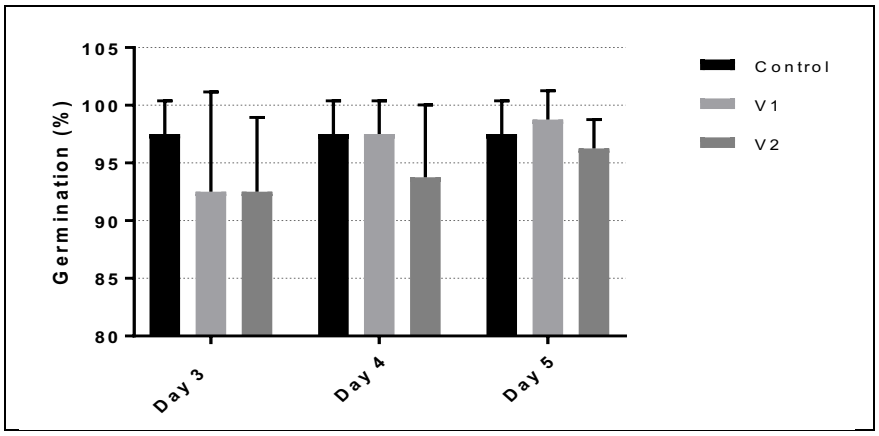

Figure 2. Germination percentage of oilseed rape seeds treated with a plant-based decoction at two concentrations

Effect of conventional immersion treatment with Repel Aves on oilseed rape germination

The germination of oilseed rape seeds treated by conventional immersion with the commercial product Repel Aves (methyl anthranilate 30%) was evaluated across a range of concentrations. Final germination percentages were not significantly affected by the treatments (Figure 3). On day 5 of incubation, germination values remained high across all variants, ranging from 92.5 to 100%, and were comparable to the untreated control (97.5%), indicating that Repel Aves did not impair final germination capacity within the tested concentration range.

One-way ANOVA conducted separately for each evaluation day revealed significant differences among treatments only on day 3 ($F(6.21) = 2.66$, $p = 0.044$). Tukey's HSD post hoc test identified a single significant difference between the control and the treatment with 400 µL Repel Aves ($p = 0.023$), where germination was approximately 13.8 percentage points lower than the control. However, no significant differences were detected on days 4 and 5 ($p > 0.05$), indicating that the observed reduction in germination at the intermediate concentration represented a temporary delay rather than a persistent inhibitory effect. By day 5, germination levels across all treatments had converged, and no significant differences remained.

Taken together, these results demonstrate that Repel Aves, applied at concentrations up to 1000 µL, did not adversely affect the final germination capacity of oilseed rape seeds. The only detectable effect was a transient delay in germination at an intermediate concentration, which diminished during the course of incubation.

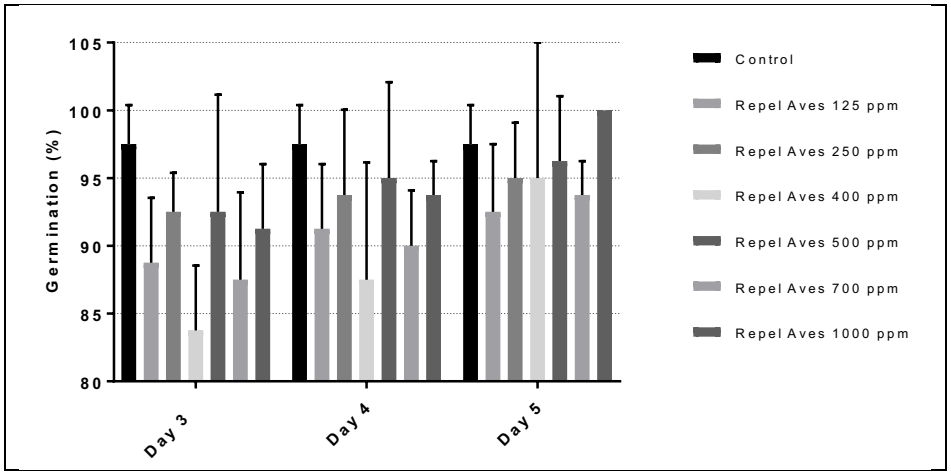

Figure 3. Germination percentage of oilseed rape seeds treated with Repel Aves (30% methyl anthranilate) at different concentrations

Effect of requiem prime treatment on oilseed rape seed germination

Germination percentages of oilseed rape seeds treated with Requiem Prime at concentrations ranging from 125 to 1000 ppm remained high across all tested variants, with values between 94 and 98%, and were comparable to those of the untreated control (97.5%) (Figure 4). A slight increase in mean germination was observed at the lowest concentration (125 ppm) reaching 98.3%; however, this increase was not statistically significant.

One-way analysis of variance (ANOVA) indicated a significant overall effect of treatment concentration on germination ($F = 2.53$, $p = 0.027$). Nevertheless, Tukey's HSD post hoc test did not reveal statistically significant differences between any pairs of treatments, including comparisons between treated variants and the control ($p > 0.05$). This discrepancy suggests that, although variability among treatments existed, individual treatment effects were not sufficiently distinct to produce significant pairwise differences. These results indicate that seed treatment with Requiem Prime did not adversely affect the germination capacity of oilseed rape seeds at any of the tested concentrations. The lowest dose (125 ppm) exhibited the most consistent germination values, while higher concentrations (≥250 ppm) resulted in only minor reductions and increased variability, without evidence of phytotoxic effects under the tested experimental conditions.

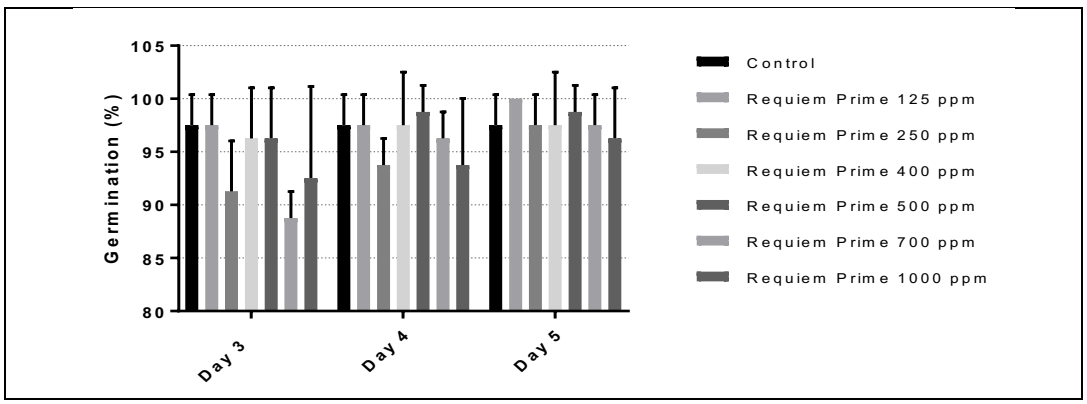

Figure 4. Germination of oilseed rape seeds treated with Requiem Prime at different concentrations

Radicle elongation

Effect of plant-based decoction treatment on radicle length in oilseed rape

Measurements of radicle length performed on day 5 after incubation showed that the application of the plant-based decoction, both in undiluted form and at a 1:1 dilution (50% decoction + 50% distilled water), did not result in statistically significant differences compared with the control (Figure 5). One-way analysis of variance (ANOVA) confirmed the absence of significant treatment effects on radicle length ($F(2,9) = 0.38$, $p = 0.695$ and Tukey's HSD post hoc test did not reveal significant differences between the control and the two decoction-treated variants. Mean radicle lengths ranged between 7.9 and 8.6 cm, with only minor variations among treatments that were not statistically supported. These findings indicate that the application of the plant-based decoction, whether undiluted or diluted 1:1, did not significantly influence radicle elongation in oilseed rape during the first five days of germination (Figure 9).

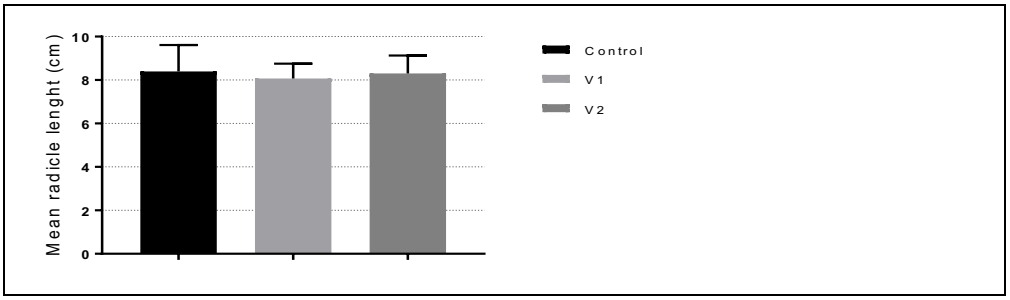

Figure 5. Radicle length of oilseed rape seeds treated with a plant-based decoction

Effect of Repel Aves treatment on radicle length in oilseed rape

Measurements of radicle length performed on day 5 of incubation showed that application of Repel Aves at concentrations ranging from 125 to 1000 ppm did not result in statistically significant differences compared with the control (Figure 6). One-way analysis of variance (ANOVA) confirmed the absence of a significant treatment effect on radicle length ($F(6.21) = 1.74$, $p = 0.156$), and Tukey's HSD post hoc test did not reveal significant differences between the control and any of the treated variants. Although some variation in mean radicle length was observed among treatments, particularly in variants V3 and V5, which showed lower mean values than the control, and in variant V6, which exhibited a higher mean value, these differences were not statistically supported. These results indicate that Repel Aves, applied to oilseed rape seeds across the tested concentration range, did not significantly influence radicle elongation during the first five days of germination.

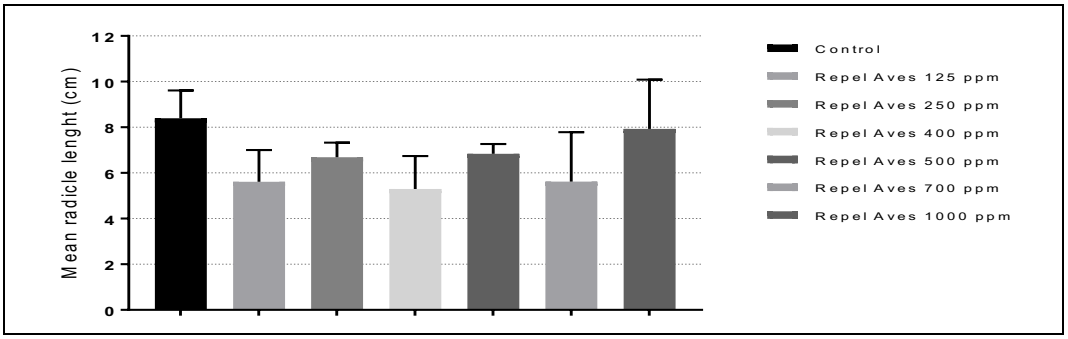

Figure 6. Effect of Repel Aves seed treatment on primary radicle length in oilseed rape

Effect of Requiem Prime treatment on radicle length in oilseed rape

The effect of Requiem Prime treatment on radicle length of oilseed rape seedlings was evaluated on day 5 after incubation (Figure 7). One-way analysis of variance (ANOVA) revealed statistically significant differences among treatments ($F = 3.00$, $p = 0.028$). Tukey's HSD post hoc test identified significant differences between the lowest concentration (125 ppm) and the treatments applied at 400 ($p = 0.017$) and 1000 ppm ($p = 0.035$). Compared with the 125 ppm treatment, intermediate and higher concentrations (400-1000 ppm) resulted in significantly greater mean radicle lengths. No statistically significant differences were observed among the higher concentrations themselves, indicating a comparable response across this concentration range. These results demonstrate that Requiem Prime did not exert inhibitory effects on radicle elongation in oilseed rape. On the contrary, radicle growth was enhanced at intermediate and higher concentrations relative to the lowest dose, suggesting a concentration-dependent response without evidence of phytotoxicity under the tested experimental conditions.

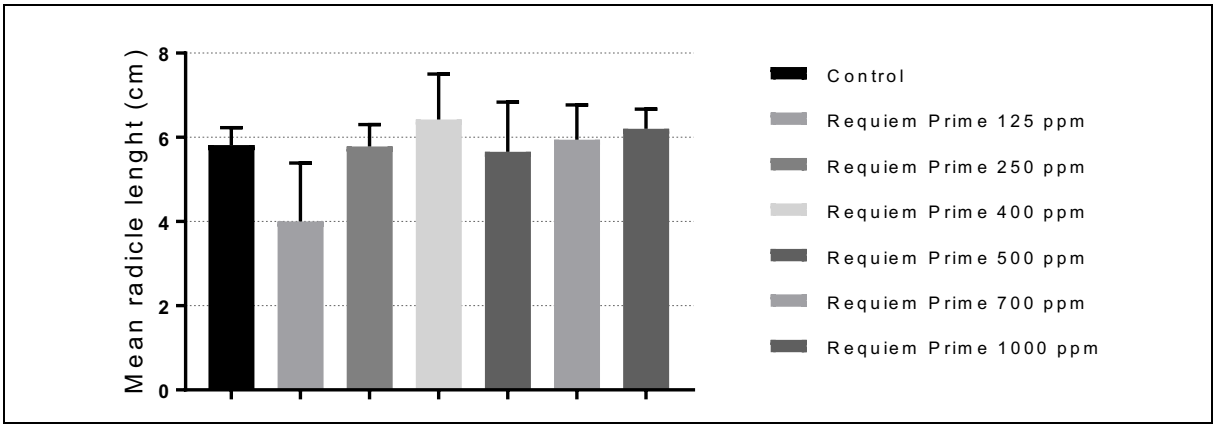

Figure 7. Primary radicle length of oilseed rape seedlings following Requiem Prime treatment

Sunflower: effects of plant-based decoction and commercial treatments

Germination

Effect of plant-based decoction treatment on sunflower seed germination

Germination of sunflower seeds treated with the plant-based decoction, applied in two variants (undiluted and diluted 1:1, i.e., 50% decoction + 50% distilled water), did not differ significantly from that of the control (Figure 8). One-way analysis of variance (ANOVA) revealed no statistically significant differences among treatments on day 3 ($F(2.9) = 0.47$, $p = 0.64$), day 4 ($F(2.9) = 0.65$, $p = 0.54$), or day 5 ($F(2.9) = 0.32$, $p = 0.73$). Tukey's HSD post hoc test confirmed the absence of significant differences between the control and the decoction-treated variants at all evaluation time points. Mean germination percentages remained high and comparable across treatments throughout the experimental period (Figure 13). These results indicate that the plant-based decoction, irrespective of concentration, did not adversely affect the germination capacity of sunflower seeds under the tested laboratory conditions.

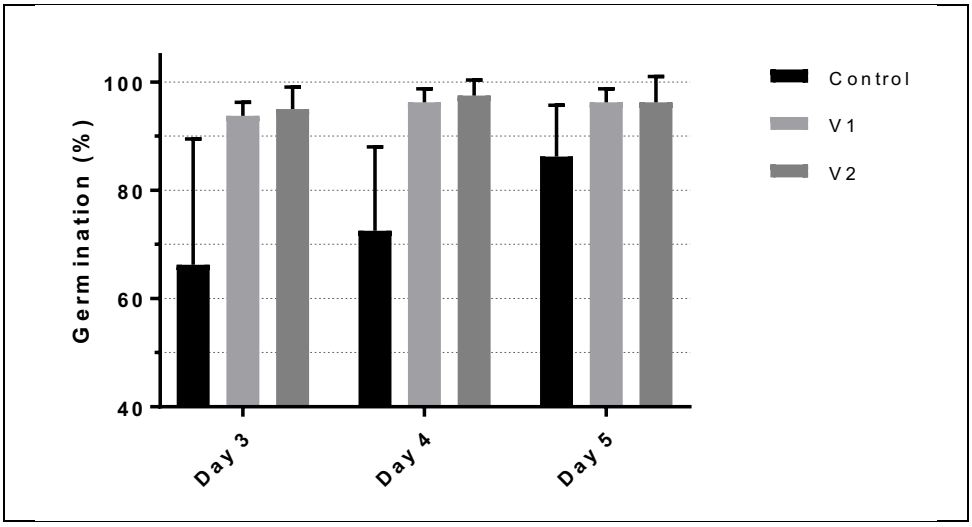

Figure 8. Germination percentage of sunflower seeds treated with a plant-based decoction

Effect of Repel Aves treatment on sunflower seed germination

Germination of sunflower seeds treated with Repel Aves at concentrations of 125, 250, 500 and 1000 ppm showed generally high germination percentages, ranging from 65 to 100%, depending on treatment concentration and evaluation time (Figure 9). One-way analysis of variance (ANOVA) revealed statistically significant differences among treatments on day 3 ($F(4.15)$, $p < 0.05$). At this early evaluation stage, treatments applied at 125 and 1000 ppm resulted in significantly lower germination percentages compared with the control. Tukey's HSD post hoc test confirmed significant differences between the control and these two concentrations. By day 4, the differences among treatments diminished, and ANOVA no longer indicated statistically significant effects ($p > 0.05$). By day 5, all treated variants reached high germination levels, ranging from 90 to 100%, which were comparable to those of the control, with no statistically significant differences detected. These results indicate that Repel Aves treatment may induce a slight and concentration-dependent delay in sunflower seed germination at early stages, particularly at the lowest and highest concentrations tested.
However, this effect was transient and did not affect the final germination capacity under the experimental conditions applied.

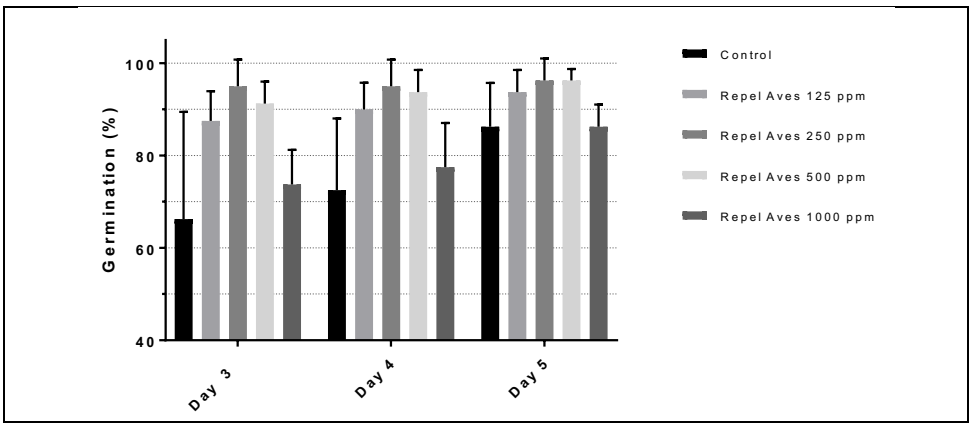

Figure 9. Germination percentage of sunflower seeds treated with Repel Aves

Effect of Requiem Prime treatment on sunflower seed germination

Germination of sunflower seeds treated with Requiem Prime at concentrations ranging from 125 to 1000 ppm was evaluated throughout the incubation period. Analysis of variance (ANOVA) indicated a significant overall treatment effect ($F = 3.08$, $p = 0.023$), whereas the effect of evaluation day was not statistically significant ($F = 2.87$, $p = 0.065$). However, post hoc analysis did not reveal statistically significant differences among individual treatment variants or between treated seeds and the control (Figure 10). All treatments exhibited high germination percentages, approaching 100% and comparable to those of the untreated control. Germination progressed rapidly over time (days 3-5), reaching complete germination (100%) by day 5 across all variants, indicating uniform and vigorous seed germination. These results demonstrate that Requiem Prime did not negatively affect the germination of sunflower seeds, irrespective of the concentration applied. All tested concentrations supported complete germination, confirming the absence of phytotoxic effects on seed viability under the experimental conditions.

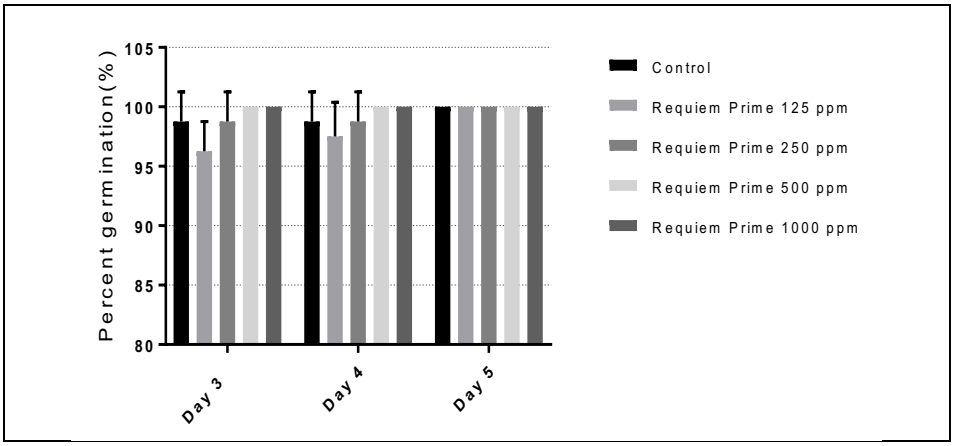

Figure 10. Germination of sunflower seeds treated with Requiem Prime

Radicle elongation

Effect of plant-based decoction treatment on radicle length in sunflower

Measurements of radicle length performed on day 5 after incubation showed that treatment with the plant-based decoction, applied either in undiluted form or diluted 1:1 (50% decoction + 50% distilled water), did not result in statistically significant differences compared with the control. One-way analysis of variance (ANOVA) indicated the absence of a significant treatment effect on radicle length ($F(2.9) = 1.86$, $p = 0.21$), and Tukey's HSD post hoc test confirmed that none of the decoction-treated variants differed significantly from the control. Mean radicle lengths ranged from 1.9 to 2.4 cm in the decoction-treated seeds, whereas the control exhibited a mean radicle length of approximately 2.7 cm (Figure 11). Although radicles from decoction-treated seeds tended to be shorter than those of the control, this tendency was not statistically supported. These results indicate that the plant-based decoction, irrespective of the application form (undiluted or diluted 1:1), did not significantly affect radicle elongation in sunflower during the first five days of germination.

Effect of Repel Aves treatment on radicle length in sunflower

Measurements of radicle length performed on day 5 after incubation showed that treatment with Repel Aves at concentrations of 125, 250, 500, and 1000 ppm did not result in statistically significant differences compared with the control. One-way analysis of variance (ANOVA) indicated the absence of a significant treatment effect on radicle length ($F(4.15) = 1.12$, $p = 0.386$), and Tukey's HSD post hoc test did not reveal significant differences between the control and any of the treated variants. Mean radicle lengths ranged from approximately 2.1 to 3.8 cm (Figure 12). Although some variability among replicates was observed, radicle length values overlapped across all treatments. These results indicate that Repel Aves applied to sunflower seeds, irrespective of the concentration used, did not significantly influence radicle elongation during the first five days of germination.

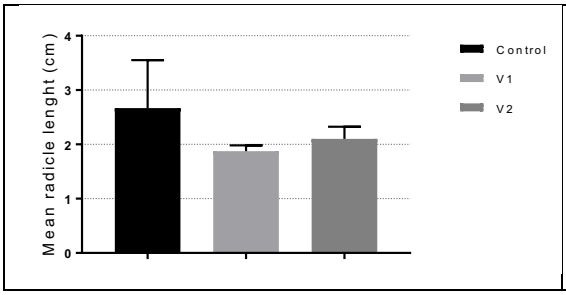

Figure 11. Effect of plant-based decoction treatment on radicle length in sunflower

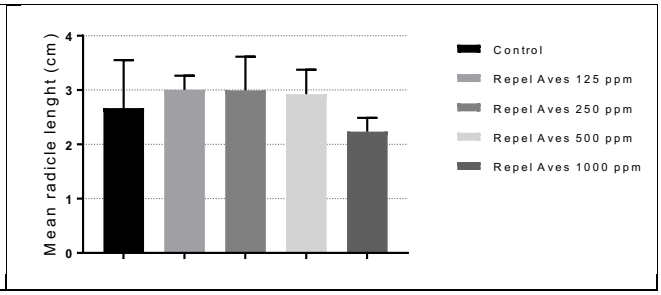

Figure 12. Effect of Repel Aves treatment on radicle length in sunflower

Effect of Requiem Prime treatment on radicle length in sunflower

Mean radicle length values recorded on day 5 ranged from 1.57 to 1.84 cm across all treatments, indicating relatively uniform early root development in sunflower seedlings (Figure 13). Seeds treated with Requiem Prime exhibited slightly higher mean radicle lengths compared with the control, particularly at concentrations of 500 and 1000 ppm. One-way analysis of variance (ANOVA) suggested the presence of a significant overall treatment effect; however, Tukey's HSD post hoc test did not reveal statistically significant differences between individual treatment pairs at the $p < 0.05$ level. These results indicate that Requiem Prime treatment did not negatively affect radicle development in sunflower. Although higher concentrations were associated with numerically greater mean radicle lengths relative to the control, these differences were not statistically significant.

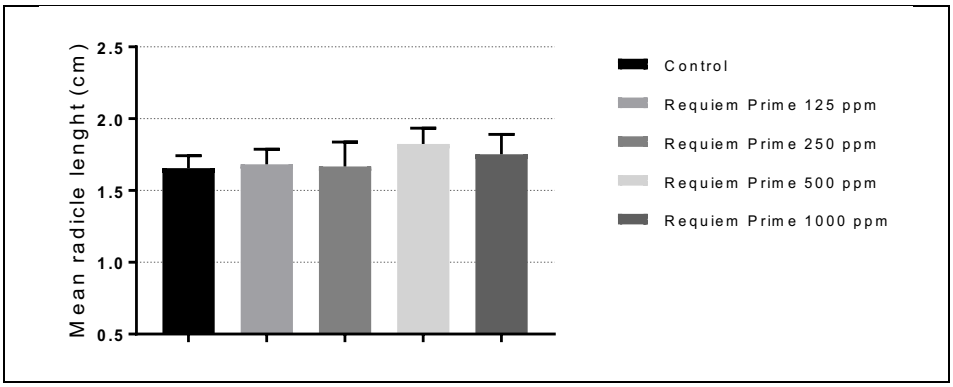

Figure 13. Effect of Requiem Prime treatment on radicle length in sunflower

DISCUSIONS

The primary objective of the present study was to evaluate the compatibility of selected biological and commercial seed treatment products with seed germination and early seedling development under controlled laboratory conditions. The results clearly indicate that the tested treatments, applied at the evaluated concentrations and assessed shortly after application, did not negatively affect the germination capacity of maize, oilseed rape, or sunflower seeds. This outcome is consistent with previous findings showing that seed treatments can remain safe for germination and viability when appropriately formulated and evaluated under short-term laboratory conditions, in the absence of prolonged storage (Teixeira & Pereira, 2019; Carvalho et al., 2020; Axinti et al., 2025).

Reductions in germination or seed vigor reported in the literature are most frequently associated with high application rates, complex mixtures of active ingredients, or extended storage periods, particularly in industrial seed treatments combining multiple fungicides and insecticides (Camargo et al., 2022; Oliveira et al., 2021). In contrast, simpler or well-balanced formulations have been shown to maintain germination within acceptable quality standards, even in crops known for their sensitivity to chemical inputs (Axinti et al., 2025). The consistently high germination percentages observed across all treatments in the present study therefore support the conclusion that the tested products, including plant-based decoctions and commercial formulations, were compatible with seed viability during the early stages of germination.

While germination percentage proved to be a reliable indicator of seed viability, additional biologically relevant information emerged from the evaluation of early seedling development. The results demonstrate that early radicle elongation was more sensitive to seed treatments than final germination percentage. Germination remained high and largely unaffected across treatments and crops; however, significant and concentration-dependent effects on radicle growth were detected, particularly following vacuum-assisted application of the plant-based decoction in maize. This response pattern aligns with numerous studies reporting that radicle or primary root elongation is a more sensitive endpoint than germination for detecting sublethal effects of chemical, biological, or environmental stressors applied to seeds (Hillis et al., 2011; Li et al., 2023; Ugolini et al., 2021). In many cases, germination, defined as radicle protrusion, remains unchanged even at concentrations that substantially affect subsequent root development, indicating that early post-germination growth represents a critical window of sensitivity (Luo et al., 2020). The heightened sensitivity of radicle elongation has been attributed to intense cell division and elongation processes occurring immediately after germination, which are particularly vulnerable to disturbances in water uptake, hormonal balance, and metabolic regulation. Consequently, seed treatments may exert inhibitory or stimulatory effects on early root growth without compromising seed viability, a phenomenon widely reported across crops and treatment types (Khelil et al., 2022; Kummerová et al., 2024). These observations emphasize that evaluations based solely on germination percentage may underestimate biologically relevant treatment effects expressed during early seedling development and support the inclusion of radicle growth parameters in seed treatment assessments.

In addition to treatment-specific responses, several variants induced temporary delays in germination during early evaluation stages without affecting final germination percentages. Such transient effects were observed at specific concentrations and early time points, while by day 5 germination levels converged across treatments and remained comparable to the control. The distinction between temporary germination delay and persistent inhibition is well

documented in the literature. Seed treatments may intentionally or unintentionally slow the onset of germination while preserving seed viability, a phenomenon commonly described as germination retardation rather than phytotoxicity (Richardson et al., 2019; Baughman et al., 2022). In certain agronomic contexts, delayed germination can even be advantageous, preventing premature seedling emergence under unfavorable environmental conditions and improving subsequent establishment (Laumann et al., 2023). Conversely, transient delays may also arise from mild stress induced by allelochemicals or osmotic and physiological constraints, where germination speed is reduced before any measurable loss of final germination occurs (Araniti et al., 2022; Chenyin et al., 2023). The transition from reversible delay to true inhibition is strongly dose dependent and typically accompanied by subsequent reductions in total germination and seedling vigor. In the present study, early delays did not progress toward persistent inhibition, as final germination percentages remained high across all treatments. This suggests that the tested products acted, at most, as mild germination retarders under the applied conditions rather than causing irreversible damage to seed viability. Therefore, short-term delays observed during early germination assessments should be interpreted cautiously and in conjunction with final germination and early seedling performance.

The observed compatibility of the tested commercial and plant-based products with seed germination further supports this interpretation. Treatments based on methyl anthranilate did not negatively affect germination or early seedling development in oilseed rape and sunflower, corroborating previous reports indicating that this compound can be used as a seed-applied bird repellent without compromising seed viability (Avery et al., 1995; Esther et al., 2011; Ahmad et al., 2018). Botanical extracts applied as seed treatments are known to exhibit variable effects on germination and early growth, depending on crop species, extract composition, and application rate. While many plant-derived coatings enhance germination and seedling vigor, inhibitory effects have also been reported under specific crop–dose combinations, highlighting the importance of preliminary laboratory screening (Barthwal et al., 2025; Pumnuan et al., 2022). The absence of negative effects on germination observed in the present study indicates that the applied plant-based decoction was within a safe concentration range for the tested crops, although concentration-dependent responses at the level of radicle growth emphasize the need for species-specific evaluation. Similarly, biological seed treatments based on beneficial microorganisms are generally reported to be compatible with germination and capable of enhancing early seedling performance, provided that formulation factors such as binders, carriers, and pH are appropriately managed (Rocha et al., 2019; Cardarelli et al., 2022; Amoroso et al., 2025). Collectively, these findings support the feasibility of integrated seed treatment strategies combining botanical compounds, microbial products, and repellents, while underscoring the importance of careful formulation design and dose optimization to avoid unintended effects on germination and early growth.

Vacuum-based seed treatments are increasingly investigated as physical or physicochemical pre-treatments aimed at improving seed permeability, invigoration, and uniformity of liquid penetration, rather than as routine methods for applying insecticidal or fungicidal seed dressings. In major crops such as maize, sunflower, and oilseed rape, most studies describe the use of vacuum for seed degassing, plasma-based activation, or biological infiltration, generally reporting high germination rates and, in some cases, enhanced early seedling performance (Mildažienė et al., 2019; Li et al., 2018; Ipate et al., 2024). Experimental systems employing vacuum infiltration have demonstrated the capacity of reduced pressure to facilitate deep penetration of aqueous solutions into seed tissues, including embryos (Zhang et al., 2017; O'Keefe et al., 2025). Despite this technical potential, vacuum-assisted delivery has not yet been adopted as a standard approach for pesticide or biopesticide seed treatments in

these crops, and its agronomic application remains largely conceptual.

The present study contributes to the growing body of evidence showing that commercially available seed treatment products, such as methyl anthranilate - and terpenoid-based formulations, can be applied without compromising seed germination or early seedling development when used at appropriate concentrations. Beyond this confirmatory aspect, the study further advances the field by demonstrating that vacuum-assisted application of a plant-based decoction to maize seeds did not impair germination, even at high concentrations, although concentration-dependent effects on early radicle growth were observed. These findings indicate that vacuum treatment itself is compatible with seed viability, while highlighting the importance of dose optimization when internal exposure to biologically active compounds is increased. To date, no studies have reported the use of vacuum systems to apply plant-derived extracts as seed treatments in maize, sunflower, or oilseed rape, identifying a clear research gap. Existing vacuum technologies could potentially be adapted for such applications; however, further research is required to optimize pressure–time parameters, assess internal dose effects, and evaluate agronomic benefits relative to conventional seed treatment methods.

CONCLUSIONS

The present study demonstrates that the evaluated biological and commercial seed treatment products were compatible with seed germination in maize, oilseed rape, and sunflower under controlled laboratory conditions. Across all treatments, final germination percentages remained high and comparable to the untreated control, indicating that seed viability was not adversely affected at the tested concentrations. Assessment of early seedling development revealed that radicle elongation was more sensitive to treatment effects than final germination, particularly in maize seeds subjected to vacuum-assisted application of the plant-based decoction. These responses were concentration dependent and did not result in reduced final germination, suggesting transient biological effects rather than persistent phytotoxicity.

Commercial formulations based on methyl anthranilate and terpenoid compounds, as well as the tested plant-based decoction, proved compatible with germination and early root development in oilseed rape and sunflower when applied within appropriate concentration ranges. In addition, vacuum-assisted seed treatment was technically feasible and did not compromise seed viability, highlighting its potential as an alternative delivery approach for biologically active compounds.

These findings support the feasibility of integrating botanical and commercial seed treatment products, as well as alternative application technologies, into seed treatment strategies. Further research under extended storage conditions and field environments is required to assess long-term performance, crop establishment and agronomic relevance.

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