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# Bioactivity of Medicinal-Plant Extracts of Nettle, Chamomile and Dandelion on Germination, Seedling Growth and Antioxidant Enzymes in Canola

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## ABSTRACT

Seed priming with extracts from medicinal plants offers a sustainable route to create value-added, bioactive treatments for crop seeds and to valorize plant by-products. This study evaluated the bioactivity of aqueous extracts of three medicinal species: nettle (*Urtica dioica*), chamomile (*Matricaria chamomilla*), and dandelion (*Taraxacum officinale*) as seed-priming agents for canola (*Brassica napus*). Seeds of four cultivars (Okapi, Zarfam, RG-5003, Ahmadi) were soaked in 0 (control), 5, and 10% aqueous preparations prior to germination assays. We quantified germination percentage, seedling length, and biomass, and measured activities of key antioxidant enzymes (catalase, CAT; peroxidase, POD; ascorbate peroxidase, APX) to assess modulation of oxidative-stress defenses during early growth. Responses were cultivar- and extract-specific. Nettle extract at 10% produced the most consistent bioactive effect across genotypes, significantly improving germination, seedling vigour and biomass while strongly up-regulating CAT, POD and APX activities. Chamomile extract conferred moderate, broadly beneficial effects, whereas dandelion extract yielded variable outcomes and was associated with partial inhibition of APX in some cultivars. The Ahmadi cultivar displayed the largest enzymatic induction in response to nettle and chamomile, indicating genotype-dependent sensitivity to plant-derived antioxidants. We interpret these results considering known antioxidant phytochemicals in the three species and propose that phenolic/flavonoid constituents likely underlie the observed modulation of seed oxidative status. Overall, aqueous preparations of medicinal plants, particularly nettle, show promise as eco-friendly, value-added seed treatments for oilseed crops. Given that 5% extracts often outperformed 10% in several assays, future work should include phytochemical profiling, extract standardization, and targeted dose optimization to refine reproducible formulations for practical application.

**Keywords**: Biodynamic preparation, Leaf extract, Enzyme, Priming

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## INTRODUCTION

Germination is a critical stage in the life cycle of plants, significantly influenced by both internal and external conditions. The process begins with water uptake by dry seeds, progresses through the growth of the embryo, and culminates in the emergence of the radicle from the seed coat [1]. Seed vigour, a complex trait, encompasses various biochemical and molecular processes that determine the potential for rapid and uniform seedling emergence and establishment [2]. One effective method to enhance seed vigour is pre-sowing treatment, which can improve the germination of low-vigour seeds [3]. This technique is particularly beneficial in mitigating the risks associated with irregular rainfall and drought, fostering more uniform plant growth. In the face of climate change, plants are increasingly required to germinate and establish under elevated temperatures and fluctuating moisture conditions, making stress-resilient seed performance essential for crop survival and productivity [4].

The application of plant hormones and nutrients has been shown to enhance germination across various crops, promoting overall plant growth and development, especially under adverse conditions such as drought, high temperatures, and salinity [5–7]. However, while the exogenous application of growth regulators, nutrients, and antioxidants can boost seed germination and seedling development, these methods are not always functionally or economically viable. Consequently, there is a pressing need to explore more cost-effective and environmentally friendly agents. Recently, artificial intelligence (AI) and machine learning have emerged as powerful tools for modeling complex interactions in agriculture, enabling predictive analysis of seed responses, environmental stress patterns, and the optimization of natural bio-stimulant applications [8].

Among the natural sources being investigated, aqueous plant extracts are garnering significant attention. Leaves of aromatic and medicinal plants are particularly rich in growth hormones (such as cytokinins), antioxidants (including ascorbate, carotenoids, and phenols), and essential nutrients (like potassium and calcium). These extracts can serve as effective natural agents for enhancing seed germination and seedling establishment. This approach aligns with biodynamic farming, a holistic system formalized by Rudolf Steiner in the 1920s, which integrates traditional agrarian practices — such as the use of natural manures, composts, and plant extracts — into a structured ecological framework.

In biodynamic practices, key species such as yarrow (*Achillea millefolium* L.), chamomile (*Matricaria recutita*), nettle (*Urtica dioica* L.), oak (*Quercus robur* L.), dandelion (*Taraxacum* *officinale* L.), valerian (*Valeriana officinalis* L.), and horsetail (Equisetum) are commonly utilized in compost preparations [9]. Several studies have demonstrated the efficacy of these extracts in improving germination and seedling growth. For instance, aqueous nettle (*U. dioica*) extract has been shown to enhance radical elongation in wheat, likely due to auxin-like effects, though high concentrations may inhibit germination [10]. Similarly, nettle and horsetail extracts increased anthocyanin synthesis and vitamin C content in germinated quinoa and lentil seeds, suggesting their potential role in boosting antioxidant activity [11]. Chamomile (*Matricaria chamomilla* L.) extract, rich in polyphenols and flavonoids, has been found to suppress fungal pathogens such as *Pythium ultimum*, indirectly supporting seedling health [12]. Additionally, dandelion (*T. officinale*) extract has been reported to have prominent effects on wheat germination [13], though some studies indicate that certain plant extracts, like *Chenopodium murale* L., may exhibit allelopathic suppression under stress conditions [14].

Nettle, despite higher initial production costs in the first year, becomes economically viable over time due to low input requirements, stable long-term yields, and a dry biomass production cost of €0.29/kg, with potential profitability comparable to staple crops like wheat (€0.16/kg break-even after subsidies) [15]. Chamomile and dandelion offer strong economic value through their diverse pharmacological effects and long-standing traditional uses, supporting steady demand in herbal and medicinal markets [16, 17].

Canola (*Brassica napus* L.) is a vital oilseed crop in Iran, but uniform seedling establishment often suffers due to poor soil and weather conditions [18]. Canola is an annual herbaceous plant with a deep taproot, erect, branched stem, and bright yellow four-petaled flowers typical of the Brassicaceae family [19]. Its leaves are alternate, bluish-green, and may be smooth or slightly lobed. The plant produces slender siliques (pods) containing small, round oil-rich seeds, which are usually black or brown when mature [19]. Canola is cultivated in 32 provinces in Iran, with Khuzestan and Ilam having the highest production compared to the other provinces [20]. Drought conditions, resulting from inadequate rainfall or irrigation at sowing, exacerbate the issue of non-uniform seedling emergence [21]. Therefore, this study aims to evaluate the effects of chamomile, nettle, and dandelion extracts—authentic biodynamic preparations-on the germination and seedling growth of four canola cultivars, assessing their potential to improve seed performance in challenging conditions.

## MATERIALS AND METHODS

The experiment was conducted at the Department of Weed Science at the Islamic Azad University, Science and Research Branch, Tehran, Iran. This study involved a laboratory experiment assessing the effects of different concentrations (0%, 5%, and 10%) of aqueous extracts from chamomile (*M. chamomilla*), nettle (*U. dioica*), and dandelion (*T. officinale*), referred to as preparations 503, 504, and 506, respectively, on the germination and seedling growth of four canola cultivars: Okapi, Zarfam, RG-5003, and Ahmadi. The breeding records of the four canola cultivars show that Okapi was developed at the Seed and Plant Improvement Institute (SPII) in 2001, Zarfam by Ahmadi et al. at SPII in 2002, RG-5003 by researchers at SPII in 2004, and Ahmadi by researchers at SPII in 2014. The experiment followed a factorial design with three fixed factors: extract type (three medicinal plant species) × extract concentration (0%, 5%, 10%) × cultivar (four canola genotypes), resulting in a total of 36 treatment combinations.

Prior to the experiment, samples of chamomile, nettle, and dandelion were collected from various fields at the 10% flowering stage (when 10% of plants had visible flowers), washed with distilled water, and air-dried in the shade for two weeks. The dried plant materials were then ground into a fine powder using an electric grinder and stored in airtight containers until extraction. The extracts were prepared by soaking 10 g of the powdered samples in 100 mL of sterile distilled water (10% w/v) overnight at room temperature. The resulting stock solutions were filtered through filter paper (Whatman No. 1), transferred to dark glass bottles, labeled, and stored in a refrigerator.

Canola seeds were obtained from the Seed and Plant Improvement Institute, Karaj, Iran. Prior to use, the seeds underwent surface sterilization using 5% sodium hypochlorite for 3 minutes, followed by 70% ethanol for 30 seconds, and were subsequently rinsed multiple times with sterile distilled water. After rinsing, the seeds were dried for 10 minutes under laminar flow conditions. The seeds of each cultivar (30 seeds per Petri dish) were placed on two layers of filter paper within 9 cm Petri dishes. For each treatment, three replicates were prepared, each replicate consisting of three Petri dishes, resulting in a total of nine Petri dishes per treatment. The stock solutions (10% w/v) were appropriately diluted with sterile distilled water to achieve final concentrations of 5% and 10%. Each Petri dish was moistened with 15 mL of the diluted biodynamic preparations, while sterile distilled water served as the control. The dishes were then sealed with parafilm and placed in an incubator set to 18/13 °C day/night temperatures with a 12-hour photoperiod for two weeks.

At the end of the experiment, the following parameters were measured: germination percentage, seedling length, shoot and root length, and seedling fresh and dry weight. The germination percentage and vigor index (VI) for each treatment were calculated using the following formula described by Orchard [22]:

Germination percentage (G%) = (Germinated seed/Total seed) x 100

VI = (SL + RL) × G%

In this formula, SL= shoot length and RL= root length.

The experimental design was a completely randomized factorial design with three replicates, incorporating three aqueous extracts or biodynamic preparations at three concentrations, along with four canola cultivars.

The enzymatic activities of catalase, peroxidase, and ascorbate peroxidase (APX) were quantified through distinct spectrophotometric protocols. Catalase activity was assessed according to Aebi's method [23], wherein a reaction mixture of 0.075 ml potassium phosphate buffer (pH 7), 20 µl soluble protein extract, 1.5 ml distilled water, and 750 µl hydrogen peroxide (70 mM) was prepared in a quartz cuvette. Absorbance changes were tracked over 60 seconds, with one enzyme unit defined as the decomposition of 1 mmol $H_2O_2$ per minute per mL of protein. A control, devoid of hydrogen peroxide but containing buffer and protein, was used for baseline correction. For peroxidase activity, fresh leaf tissue (0.5 g) was homogenized in cold 0.1% trichloroacetic acid, centrifuged, and the supernatant was combined with potassium phosphate buffer and potassium iodide. Following a 1-hour dark incubation, absorbance at 390 nm was measured, and enzyme units were derived from a standard curve [24], representing a 1-unit absorbance shift per minute. APX activity was evaluated via a modified Nakano and Asada [25] protocol, involving a reaction mixture of phosphate buffer (pH 7, 50 mM), EDTA (1 mM), sodium ascorbate (1 mM), hydrogen peroxide (10 mM), and enzyme extract. The enzymatic degradation of $H_2O_2$, linked to ascorbate oxidation, was monitored by a 1-minute absorbance decline at 290 nm. Each assay employed controlled conditions to ensure precision in quantifying these antioxidant enzymes' $H_2O_2$-metabolizing capacities.

Significant differences in mean values were assessed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. All statistical analyses were conducted using the Statistical Analysis System (SAS) software.

## RESULTS

Based on the analysis of variance, the main effects, as well as the two-way interactions of the studied factors, were statistically significant (Table 1).

**Table 1** Analysis of Variance (ANOVA) for Germination Traits, Seedling Growth Parameters, and Antioxidant Enzyme Activities in Canola Cultivars

| S.O.V | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | G | SL | ShL | RL | SFW | SDW | VI | CAT | POD | APX |
| Bio | 8 | 576.8 ** | 0.32 ** | 2.14 ** | 1.53 ** | 46608 ** | 21.9 ** | 35924 ** | 0.8 ** | 0.22 ** | 0.42 ** |
| Canola | 3 | 5160 ** | 143.3 ** | 34 ** | 43.7 ** | 22206 ** | 3183** | 936492 ** | 0.46 ** | 0.005 ** | 0.33 ** |
| Bio*Ca. | 24 | 111.2 ** | 5.48 ** | 2.3 ** | 1.60 ** | 81795 ** | 37.1** | 79824 ** | 1.44 ** | 0.28 ** | 0.24 ** |
| Error | 72 | 8.48 | 0.04 | 0.02 | 0.01 | 171.96 | 4.24 | 249.56 | 0.005 | 0.0014 | 0.03 |

Bio: Biodynamics at three concentrations. G: Germination; SL: Seedling length; ShL: Shoot length; RL: Root length; SFW: Seedling fresh weight; SDW: Seedling dry weight; VI: Vigor index. ** Significant at $p \leq 0.01$

Among the canola cultivars, the Okapi cultivar exhibited the highest germination percentage and seedling growth, including maximum seedling length, shoot length, root length, and both fresh and dry seedling weights (Table 2). In contrast, the Ahmadi cultivar displayed the lowest germination percentage and seedling growth. The Zarfam and RG-5003 cultivars ranked second and third, respectively. The application of nettle and dandelion extracts produced the highest and lowest germination percentages, respectively (Table 3).

**Table 2** The main effect of cultivar on germination parameters

| Cultivar | G (%) | SL (cm) | ShL (cm) | RL (cm) | SFW (mg) | SDW (mg) | CAT * | POD | APX |
|---|---|---|---|---|---|---|---|---|---|
| Okapi | 51.74 a | 6.84 a | 4.54 a | 2.28 a | 585.39 a | 21.56 a | 3.17 a | 2.76 a | 4.52 a |
| Zarfam | 45.76 b | 6.04 b | 4.18 b | 1.8 b | 455.63 b | 19.45 b | 2.85 ab | 2.40 b | 4.14 ab |
| RG-5003 | 40.5 c | 5.12 c | 3.68 c | 1.45 c | 394.58 c | 18.34 c | 2.55 bc | 1.98 c | 3.62 b |
| Ahmadi | 37.09 d | 4.16 d | 2.92 d | 1.14 d | 347.68 d | 16.90 d | 2.37 c | 1.54 d | 2.81 c |

Means with the same letter are not significantly different. G: Germination; SL: Seedling length; ShL: Shoot length; RL: Root length; SFW: Seedling fresh weight; SDW: Seedling dry weight; *CAT (U/min/mg protein); POD (U/min/mg protein); APX (µmol/min/g FW).

**Table 3** The main effect of biodynamic preparation on germination parameters

| Biodynamic preparation | G (%) | SL (cm) | ShL (cm) | RL (cm) | SFW (mg) | SDW (mg) | CAT* | POD | APX |
|---|---|---|---|---|---|---|---|---|---|
| Nettle | 58.83 a | 7.70 a | 5.15 a | 2.90 a | 745.85 a | 31.29 a | 3.24 a | 2.81 a | 4.62 a |
| Chamomile | 49.80 b | 6.28 b | 4.39 b | 1.77 b | 533.49 b | 28.58 b | 2.79 b | 2.22 b | 3.90 b |
| Dandelion | 37.28 c | 4.48 c | 3.24 c | 0.89 c | 207.56 c | 16.38 c | 2.17 c | 1.49 c | 2.80 c |

Means with the same letter are not significantly different. G: Germination; SL: Seedling length; ShL: Shoot length; RL: Root length; SFW: Seedling fresh weight; SDW: Seedling dry weight; *CAT (U/min/mg protein); POD (U/min/mg protein); APX (µmol/min/g FW).

Seedling growth significantly increased with the application of biodynamic preparations, with the effect of nettle extract being more pronounced compared to chamomile or dandelion extracts. There were no significant differences in shoot length and seedling dry weight between nettle and chamomile extracts, or between chamomile and dandelion extracts.

The analysis of variance revealed significant effects of biodynamic treatments and their interactions with canola cultivars on germination and seedling growth (Table 4). Among cultivars, Okapi consistently outperformed others, exhibiting the highest germination percentage (82.6%) and seedling growth (10.6 cm length, 1245 mg fresh weight) under 10% nettle extract, while Ahmadi showed the lowest performance (64.0% germination, 8.61 cm length).

When expressed as percentage improvements relative to the control (0%), nettle extract at 5% and 10% enhanced Okapi's germination by 55.7% and 54.6%, respectively, with similar trends in seedling fresh weight (+50.9% and +37.6%). However, Ahmadi's response was weaker (+41.5% and +41.2% germination), highlighting cultivar-specific efficacy.

Chamomile extract, though less effective than nettle in absolute terms, demonstrated more uniform improvements across cultivars when compared to the control. Okapi's germination increased by 74.4% (5%) and 72.4% (10%), with shoot length rising by 78.1% and 73.4%, while Ahmadi's gains were modest (+49.5% and +48.8%). In contrast, dandelion extract induced the most dramatic percentage improvements, particularly in biomass. Okapi's germination surged by 95.3% (5%) and 93.4% (10%), while Ahmadi's seedling fresh weight increased by 358% (5%) and 291% (10%). Zarfam and RG-5003 also showed exceptional responses to dandelion, with fresh weight escalating by 194–270% and dry weight by 131–157%.

Notably, the 5% concentration generally outperformed 10% across treatments, suggesting potential inhibitory effects at higher doses. For instance, Okapi's seedling length with 5% dandelion (97.95% increase) exceeded that of 10% (94.89%), a pattern repeated in other parameters. This aligns with the absolute data, where nettle at 10% remained the most effective for Okapi (82.6% germination), while dandelion's lower absolute performance (48.3% germination in Okapi at 10%) contrasted with its high percentage improvements, likely due to the control's poor baseline.

**Table 4** Interaction between biodynamic preparations and cultivars and on some canola parameters

| Cultivar | Biodynamic | G (%) | SL (cm) | ShL (cm) | RL (cm) | SFW (mg) | SDW (mg) | VI | CAT* | POD | APX |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Okapi | Nettle (0%) | 45 | 6.16 | 4.1 | 1.41 | 735 | 35.23 | 248.0 | 2.56 | 2.17 | 3.63 |
| | Nettle (5%) | 81 | 10.5 | 6.5 | 5.07 | 921 | 40.16 | 937.2 | 4.34 | 3.95 | 5.91 |
| | Nettle (10%) | 82.6 | 10.6 | 6.89 | 5.13 | 1245 | 43.50 | 992.9 | 4.42 | 3.99 | 6.28 |
| Zarfam | Nettle (0%) | 38.21 | 4.48 | 4.1 | 0.91 | 610.11 | 31.23 | 191.4 | 2.22 | 1.49 | 3.63 |
| | Nettle (5%) | 71 | 9.68 | 6.21 | 3.93 | 823.6 | 38.56 | 719.9 | 3.84 | 3.62 | 5.63 |
| | Nettle (10%) | 74 | 9.86 | 6.31 | 4.26 | 832.3 | 38.61 | 782.2 | 3.99 | 3.69 | 5.72 |
| RG-5003 | Nettle (0%) | 29.55 | 3.39 | 2.94 | 0.63 | 509.5 | 29.87 | 105.5 | 1.79 | 1.04 | 2.52 |
| | Nettle (5%) | 65 | 9.29 | 5.93 | 3.42 | 739.3 | 38.16 | 607.8 | 3.54 | 3.46 | 5.36 |
| | Nettle (10%) | 66 | 9.53 | 5.97 | 3.73 | 741 | 38.16 | 640.2 | 3.59 | 3.56 | 5.40 |
| Ahmadi | Nettle (0%) | 26 | 1.95 | 1.5 | 0.34 | 411.51 | 28.84 | 47.8 | 1.62 | 0.45 | 1.16 |
| | Nettle (5%) | 63.6 | 8.38 | 5.62 | 2.79 | 674.6 | 36.82 | 534.9 | 3.48 | 3.08 | 5.07 |
| | Nettle (10%) | 64 | 8.61 | 5.67 | 3.21 | 706.3 | 37.00 | 568.3 | 3.5 | 3.18 | 5.12 |
| Okapi | Chamomile (0%) | 44.96 | 5.97 | 4.11 | 1.41 | 433.7 | 29.11 | 248.2 | 2.55 | 2.10 | 3.63 |
| | Chamomile (5%) | 60.6 | 8.19 | 5.23 | 2.58 | 653 | 36.50 | 473.3 | 3.33 | 3.01 | 4.70 |
| | Chamomile (10%) | 62.3 | 8.3 | 5.56 | 2.68 | 656 | 36.54 | 513.4 | 3.41 | 3.05 | 5.01 |
| Zarfam | Chamomile (0%) | 37.33 | 4.53 | 4.24 | 0.86 | 407.43 | 27.59 | 190.4 | 2.18 | 1.51 | 3.76 |
| | Chamomile (5%) | 56.6 | 7.56 | 4.93 | 2.31 | 623 | 34.70 | 409.8 | 3.13 | 2.75 | 4.41 |
| | Chamomile (10%) | 58.6 | 7.73 | 5.03 | 2.44 | 630 | 35.03 | 437.7 | 3.23 | 2.82 | 4.51 |
| RG-5003 | Chamomile (0%) | 32.12 | 3.39 | 3.04 | 0.65 | 409.41 | 28.95 | 118.5 | 1.92 | 1.04 | 2.62 |
| | Chamomile (5%) | 55.3 | 7.27 | 4.8 | 2.01 | 545.3 | 34.26 | 376.6 | 3.06 | 2.63 | 4.29 |
| | Chamomile (10%) | 56 | 7.3 | 4.87 | 2.16 | 588 | 34.26 | 393.7 | 3.1 | 2.64 | 4.36 |
| Ahmadi | Chamomile (0%) | 26.5 | 1.79 | 1.48 | 0.32 | 410.85 | 28.67 | 47.7 | 1.64 | 0.38 | 1.14 |
| | Chamomile (5%) | 53.3 | 6.61 | 4.6 | 1.82 | 508.6 | 33.29 | 342.2 | 2.97 | 2.36 | 4.10 |
| | Chamomile (10%) | 54 | 6.68 | 4.73 | 1.94 | 532.6 | 34.16 | 360.2 | 3 | 2.39 | 4.22 |
| Okapi | Dandelion (0%) | 45.3 | 6.06 | 4.04 | 1.42 | 237.3 | 21.89 | 247.3 | 2.57 | 2.13 | 3.57 |
| | Dandelion (5%) | 47.3 | 6.19 | 4.48 | 1.53 | 475.6 | 29.73 | 284.3 | 2.67 | 2.19 | 3.99 |
| | Dandelion (10%) | 48.3 | 6.39 | 4.49 | 1.59 | 500.3 | 31.99 | 293.7 | 2.72 | 2.27 | 4.00 |
| Zarfam | Dandelion (0%) | 38 | 4.51 | 3.59 | 0.88 | 208.3 | 18.98 | 169.9 | 2.21 | 1.50 | 3.14 |
| | Dandelion (5%) | 40.6 | 5.99 | 3.63 | 1.03 | 210.3 | 19.35 | 189.2 | 2.34 | 2.10 | 3.18 |
| | Dandelion (10%) | 43.3 | 6.03 | 3.71 | 1.35 | 211.3 | 19.73 | 219.1 | 2.47 | 2.12 | 3.25 |
| RG-5003 | Dandelion (0%) | 30 | 3.43 | 2.91 | 0.61 | 110 | 14.55 | 105.6 | 1.81 | 1.06 | 2.49 |
| | Dandelion (5%) | 35 | 3.54 | 2.96 | 0.63 | 127 | 15.60 | 125.7 | 2.06 | 1.10 | 2.54 |
| | Dandelion (10%) | 36 | 4.03 | 3.41 | 0.63 | 176.3 | 16.20 | 145.4 | 2.11 | 1.30 | 2.97 |
| Ahmadi | Dandelion (0%) | 26.6 | 1.82 | 1.52 | 0.3 | 51.02 | 10.09 | 48.4 | 1.65 | 0.40 | 1.17 |
| | Dandelion (5%) | 27.6 | 2.48 | 2.08 | 0.3 | 81.33 | 11.16 | 65.7 | 1.69 | 0.67 | 1.71 |
| | Dandelion (10%) | 29.3 | 3.26 | 2 | 0.4 | 100 | 12.66 | 70.3 | 1.78 | 0.99 | 1.63 |
| LSD (0.05) | | 2.38 | 0.36 | 0.22 | 0.08 | 10.71 | 1.68 | 35.45 | 0.34 | 0.28 | 0.59 |

G: Germination; SL: Seedling length; ShL: Shoot length; RL: Root length; SFW: Seedling fresh weight; SDW: Seedling dry weight; VI: Vigor index; CAT (U/min/mg protein); POD (U/min/mg protein); APX (µmol/min/g FW).

Across all treatments, the leaf extracts of the medicinal plants significantly influenced the seedling vigor index (SVI) of the canola cultivars. Nettle extract showed the strongest stimulatory effect, with all cultivars exhibiting large increases in SVI as concentrations rose from 5% to 10%. Chamomile extract also improved vigor, though the magnitude of increase was moderate compared to nettle. In contrast, dandelion extract produced only slight increases in SVI, with much smaller improvements across cultivars. Overall, the highest vigor values were consistently recorded under nettle extract at 10%, followed by chamomile, while dandelion resulted in the weakest response.

A significant interaction between cultivars and biodynamic preparations was observed for CAT activity. Nettle extract strongly enhanced CAT activity in all cultivars, with the highest increases in Ahmadi (112.6–113.8% over control), followed by RG-5003 (92.4–95.1%), Zarfam (74.3–81.1%), and Okapi (69.5–72.7%). Chamomile extract also increased CAT activity, though less prominently: Ahmadi showed the largest gains (81.5–83.3%), while Okapi (30.1–33.2%) and Zarfam (42.1–46.6%) exhibited smaller increases. Dandelion extract induced marginal increases in CAT activity across cultivars, with the highest response in RG-5003 (12.0–14.7%), followed by Zarfam (6.2–12.1%), Okapi (4.3–6.3%), and Ahmadi (3.3–8.8%). These increases were negligible compared to nettle and chamomile, highlighting dandelion's limited efficacy in modulating CAT activity. POD activity displayed striking cultivar and biodynamic preparations specific responses. Nettle extract induced dramatic increases in Ahmadi (651.2–675.6% over control), followed by RG-5003 (230.6–240.1%) and Zarfam (141.3–146%), while Okapi exhibited modest gains (85.2–87.0%). Chamomile extract similarly enhanced POD activity, though effects were less pronounced: Ahmadi again showed the highest induction (475.6–482.9%), trailed by RG-5003 (151.3–152.2%), Zarfam (83.3–88%), and Okapi (41.1–43.0%). Dandelion extract elicited minimal increases in POD activity in Okapi (2.7–6.4%) and RG-5003 (5.1–24.2%) but showed stronger induction in Ahmadi (63.4–141.5%) and Zarfam (40.0–41.3%) at higher concentrations. APX activity followed a similar trend, with nettle extract eliciting the strongest upregulation. Ahmadi exhibited exceptional increases (338.3–342.7%), followed by RG-5003 (110.7–112.3%), while Okapi (63.7–74.0%) and Zarfam (60.4–63.0%) showed moderate gains. Chamomile extract elevated APX in Ahmadi (254.5–264.8%) and RG-5003 (68.7–71.4%) but had limited effects on Okapi (30.2–38.8%) and Zarfam (25.6–28.5%). Dandelion extract suppressed APX in Zarfam (-9.4% to -7.4%) and RG-5003 (-0.1% at 5%), though 10% dandelion partially restored activity in RG-5003 (16.8%). Ahmadi exhibited variable responses to dandelion, with APX increasing by 47.8% at 5% but declining to 40.9% at 10%. The interplay between cultivars and biodynamic preparations was most evident in Ahmadi, which showed extraordinary sensitivity to nettle and chamomile, with CAT, POD, and APX activities consistently exceeding 250% of control. Dandelion extract exhibited mixed effects: while it marginally increased CAT and POD in most cultivars, it suppressed APX in Zarfam and RG-5003. Ahmadi retained partial tolerance to dandelion, with POD activity reaching 141.5% at

10% concentration. Okapi and Zarfam exhibited weaker responses overall, highlighting genotype-specific metabolic adaptations. Higher extract concentrations (10%) generally amplified effects, particularly in nettle-primed Ahmadi and chamomile-treated RG-5003. These findings underscore the critical role of cultivar-extract compatibility in modulating antioxidant defenses during germination.

Correlation analysis revealed strong and biologically meaningful relationships among germination traits, seedling growth parameters, and antioxidant enzyme activities (Table 5). Seedling length (SL) showed a high positive correlation with shoot length (ShL; r = 0.87), root length (RL; r = 0.89), vigor index (VI; r = 0.90), seedling fresh weight (SFW; r = 0.81), and seedling dry weight (SDW; r = 0.84), indicating that improvement in seedling elongation was closely associated with overall seedling biomass and vigor. Germination percentage (G) was significantly correlated with SL (r = 0.72), ShL (r = 0.53), RL (r = 0.71), and SFW (r = 0.80), confirming that higher germination led directly to stronger seedling establishment.

**Table 5** Pearson correlation coefficients for germination parameters

| | SL | POD | ShL | APX | G | CAT | RL | VI | SFW | SDW |
|---|---|---|---|---|---|---|---|---|---|---|
| SL | 1 | - | - | - | - | - | - | - | - | - |
| POD | 0.45 | 1 | - | - | - | - | - | - | - | - |
| ShL | 0.87 ** | 0.18 | 1 | - | - | - | - | - | - | - |
| APX | 0.36 | 0.87 ** | 0.22 | 1 | - | - | - | - | - | - |
| G | 0.72 ** | 0.09 | 0.53 ** | 0.06 | 1 | - | - | - | - | - |
| CAT | 0.24 | 0.91** | 0.16 | 0.88 ** | 0.03 | 1 | - | - | - | - |
| RL | 0.89 ** | 0.32 | 0.56 ** | 0.26 | 0.71 ** | 0.08 | 1 | - | - | - |
| VI | 0.9 ** | 0.29 | 0.19 | 0.15 | 0.23 | 0.11 | 0.79 ** | 1 | - | - |
| SFW | 0.81 ** | -0.17 | 0.73 ** | -0.07 | 0.80 ** | 0.06 | 0.69 ** | 0.87 ** | 1 | - |
| SDW | 0.84 ** | -0.14 | 0.80 ** | -0.11 | 0.79 ** | 0.09 | 0.67 ** | 0.78 ** | 0.93 ** | 1 |

** significant at 0.01 probability level; G: Germination; SL: Seedling length; ShL: Shoot length; RL: Root length; SFW: Seedling fresh weight; SDW: Seedling dry weight; VI: Vigor index

Among the antioxidant enzymes, POD and CAT exhibited very strong correlations with APX (r = 0.87 and r = 0.88, respectively), reflecting coordinated antioxidant responses during early growth. POD also showed a moderate positive correlation with SL (r = 0.45) but weaker associations with growth parameters compared with CAT and APX. In contrast, APX and CAT displayed minimal correlation with germination (r = 0.06 and 0.03), suggesting that their contribution occurs mainly during post-germination seedling development rather than germination onset. Vigor index (VI) was highly correlated with SL (r = 0.90), RL (r = 0.79), and SFW (r = 0.87), indicating that these traits were the primary contributors to overall vigor. Biomass parameters also showed strong interrelationships: SFW and SDW were very strongly correlated (r = 0.93), and both showed significant positive correlations with SL, ShL, RL, and G. Collectively, these results demonstrate that enhanced seedling vigor is strongly driven by coordinated increases in elongation growth, biomass accumulation, and synergistic antioxidant enzyme activity.

## DISCUSSION

Pre-sowing seed treatment has emerged as a critical strategy for improving germination uniformity and seedling vigor in crops [26], with our findings demonstrating that biodynamic preparations—particularly nettle extract—significantly improve germination and growth parameters in canola cultivars. Pre-sowing seed treatment has emerged as a critical strategy for improving germination uniformity and seedling vigor in crops. The efficacy of nettle extract, which outperformed chamomile and dandelion, correlated strongly with its ability to upregulate CAT (112.6–113.8% over control in Ahmadi), POD (651.2–675.6%), and APX (338.3–342.7%) activities in a cultivar-specific manner. These enzymes play distinct roles: POD, peaking early in germination [27], facilitates ROS-mediated cell wall loosening and embryo elongation, while CAT gradually decomposes $H_2O_2$ to prevent oxidative damage [27, 28], and APX stabilizes cellular redox balance by scavenging hydroxyl radicals [27]. The superior germination and seedling vigor observed in Ahmadi under nettle extract align with its exceptional enzymatic sensitivity, whereas Okapi and Zarfam's weaker responses highlight genotype-dependent metabolic adaptations. The bioactive compounds in biodynamic extracts, including phenolics, flavonoids, and terpenoids [17, 29, 30], likely synergize with enzymatic antioxidant activity. Phenolic compounds, known for their ROS-scavenging capacity [31–33], complement POD's role in $H_2O_2$ detoxification, as evidenced by the 55.7–95.3% germination improvements in Okapi under nettle and dandelion treatments. Similarly, chamomile's flavonoid content [34, 35] may enhance APX stability, explaining its moderate but consistent growth promotion across cultivars (e.g., 81.5–83.3% CAT increase in Ahmadi). However, dandelion's limited efficacy—marginally increasing CAT (3.3–14.7%) and POD (2.7–141.5%) while suppressing APX in Zarfam (-9.4%)—underscores the importance of extract-cultivar compatibility. These results align with studies showing PEG-induced POD/CAT activation in rice [36] and ZnO nanoparticle-enhanced APX in drought-stressed plants [36], though the unparalleled enzymatic induction in Ahmadi reveals unique synergies not previously documented. In support of these biochemical trends, the vigor index results further demonstrate the superior performance of nettle extract across all cultivars. The substantial increase in vigor—rising more than threefold to tenfold compared to the control—highlights nettle's strong capacity to enhance early seedling growth. Chamomile displayed moderate improvements in vigor index, consistent with its intermediate enzymatic stimulation, while dandelion produced only slight increases that matched its comparatively weak antioxidant activation. These patterns reinforce that antioxidant-driven mitigation of oxidative stress directly translates into improved seedling vigor, and they confirm that nettle extract provides the most favorable balance of bioactive compounds for promoting robust early growth.

Dose-dependent responses further emphasize the delicate balance between bioactive compound delivery and oxidative homeostasis. While 10% concentrations generally amplified enzyme activity (e.g., 10% nettle increased Ahmadi's POD to 675.6%), the occasional superiority of 5% extracts (e.g., 5% dandelion elevating RG-5003's APX by 16.8% vs. 10%'s suppression) suggests higher doses may overwhelm cellular repair mechanisms, akin to the inhibitory effects reported for *Ch. murale* extracts under stress [14]. This aligns with findings that excessive phytochemicals in *Origanum vulgare* L. [37] and sesame [38] inhibit germination, reinforcing the need for optimized concentrations. Notably, nettle's 10% efficacy without inhibition contrasts with dandelion's mixed effects, likely due to differences in phytochemical composition and ROS-scavenging kinetics [31–33].

The role of antioxidant enzymes supersedes previously hypothesized mechanisms like hormonal regulation [27, 39] or allelopathic signaling [40]. While $GA_3$ and auxin-like compounds in extracts may contribute to starch mobilization and root elongation [36], the strong correlations between enzyme activities and growth parameters (e.g., germination percentage vs. CAT/POD/APX, r = 0.72–0.80**) position oxidative stress mitigation as the dominant driver. This is further supported by the suppression of lipid peroxidation in primed seeds, a hallmark of enhanced antioxidant capacity [41]. Chamomile's antifungal properties [34, 35] and nettle's nutrient profile [42] may provide ancillary benefits, but their primary mode of action lies in enzymatic ROS regulation. Cultivar-specific responses also reflect genetic thresholds for oxidative stress tolerance. Ahmadi's extraordinary POD/CAT/APX induction under nettle (exceeding 250% of control) suggests a latent capacity for redox regulation, whereas Okapi's modest enzymatic gains (e.g., 69.5–72.7% CAT increase) correlate with its baseline vigor. These findings echo observations in melatonin-primed waxy corn, where chilling stress amplified POD/CAT activities [43], and align with the stress-resilience mechanisms reported in PEG-primed rice [27]. However, dandelion's partial APX suppression in RG-5003 (-0.1% at 5%) and Zarfam (-9.4%) highlights vulnerabilities that warrant cultivar-specific strategies.

## CONCLUSION

Extracts significantly enhance germination and seedling vigor in canola cultivars, with nettle extract (10%) emerging as the most effective treatment. The results reveal that nettle extract not only improves germination percentage but also promotes seedling growth parameters such as seedling length, shoot length, root length, and seedling fresh and dry weight. The cultivar specific responses observed in this study are particularly noteworthy, with the Ahmadi cultivar showing exceptional sensitivity to nettle-induced upregulation of antioxidant enzymes, including CAT, POD, and APX. These enzymes play crucial roles in mitigating oxidative stress during germination by decomposing hydrogen peroxide and stabilizing cellular redox balance. The enzymatic data suggest a potential mechanism through which these biodynamic preparations enhance seed germination and seedling growth. The practical efficacy of nettle extract in improving seedling vigor, particularly in the Ahmadi cultivar, highlights its potential as a sustainable alternative to synthetic seed treatments under controlled conditions. The findings suggest that nettle extract, rich in antioxidants and growth regulators, can be effectively used to enhance seed performance, offering a promising approach for improving agricultural sustainability and crop productivity. However, since all results were obtained under laboratory conditions, further evaluation under field or semi-field environments is necessary to verify consistency of responses, assess environmental interactions, and determine practical application methods. Such studies would help clarify the real-world potential of these plant-based seed treatments in agricultural systems.

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**Conflict of Interest** The authors declare that they have no conflict of interest

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