

Article

Effects of Conventional and Alternative Seed Treatments on Winter Wheat Establishment, Yield and Economic Performance

Laura Șopterean ¹, Ana-Maria Vălean ¹, Adina Tărașu ¹, Ioana Crișan ¹, Florin Russu ¹, Ionuț Racț ^{1,2}, Nicolae Tritean ¹, Alina Șimon ¹, Camelia Urdă ¹, Loredana Suciș ^{2,*} and Ana-Cristina Fătu ^{3,*}

¹ Agricultural Research and Development Station Turda, 27 Agriculturii Street, 401100 Turda, Romania; laura.sopterean@scdaturda.ro (L.Ș.); anamaria.valean@scdaturda.ro (A.-M.V.); adina.tarau@scdaturda.ro (A.T.); ioana.crisan@scdaturda.ro (I.C.); florin.russu@scdaturda.ro (F.R.); ionut.racz@usamvcluj.ro (I.R.); nicu.tritean@scdaturda.ro (N.T.); alina.simon@scdaturda.ro (A.Ș.); camelia.urda@scdaturda.ro (C.U.)

² Department of Crop Science, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăstur Street, No. 3-5, 400372 Cluj-Napoca, Romania

³ Research—Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd., District 1, 013813 Bucharest, Romania

* Correspondence: loredana.suciu@usamvcluj.ro (L.S.); cristina.fatu@icdpp.ro (A.-C.F.)

Abstract

Sustainable alternatives to conventional seed treatments require validation under field conditions. This study compared two conventional chemical seed treatments (a fungicide and an insecticide) and three alternative treatments (a microbiological formulation, a plant-extract-based formulation and a biostimulant) with an untreated control in winter wheat over two growing seasons at ARDS Turda, Romania. The field experiment was arranged in a randomized complete block design with three replicates. Germination, crop establishment, cereal fly damage, yield components, grain quality and economic performance were evaluated. Data were analysed using linear mixed-effects models and Tukey-adjusted pairwise comparisons. All treatments significantly increased germination energy and germination capacity relative to the untreated control. Seed treatment significantly influenced field emergence and cereal fly damage but not tiller density or productive spike density. Biosem produced the highest field emergence (89.58%), whereas Bioseed was associated with the lowest incidence of cereal fly damage (4.02%). The overall treatment effect on grain yield was not significant ($p = 0.063$), although Signal 300 ES produced the highest yield (7909 kg ha⁻¹) and differed significantly from the untreated control in the Tukey-adjusted comparison. Seed treatment significantly influenced thousand-kernel weight but not test weight, protein content, wet gluten content, or Zeleny sedimentation value. No significant growing season $\times$ treatment interactions were detected. Signal 300 ES generated the highest estimated net benefit, whereas Redigo Pro 170 FS achieved the highest return on investment. The alternative treatments did not differ significantly in grain yield from either conventional reference treatment, although this should not be interpreted as evidence of equivalence. Further multi-environment validation is required.

Keywords: *Triticum aestivum* L. subsp. *vulgare*; seed treatment; biological products; biostimulant; crop establishment; cereal fly damage; grain yield and quality; economic efficiency



Received: 20 August 2026

Revised: 19 September 2026

Accepted: 23 September 2026

Published: 26 September 2026

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1. Introduction

Winter wheat (*Triticum aestivum* L.) is one of the most widely cultivated cereal crops worldwide and represents a major source of calories and protein for the global population. Ensuring stable wheat production has become increasingly difficult due to climate change, greater weather variability, the intensification of agricultural systems, the spread of seed- and soil-borne pathogens and pests, and the increasing demand for environmentally sustainable crop protection practices [1,2]. Consequently, developing effective and sustainable technologies capable of maintaining crop productivity while reducing the environmental impact of agricultural inputs has become a major challenge for modern cereal production. The successful establishment of the crop is one of the most critical stages determining the final productivity of winter wheat [3]. Rapid and uniform seedling emergence, vigorous early plant growth and adequate tillering provide the basis for the development of an optimal number of productive spikes and increase crop competitiveness against weeds while improving tolerance to environmental stresses. Conversely, poor crop establishment may reduce plant density, limit spike formation and ultimately decrease grain yield. The quality and health status of seed therefore play a fundamental role in ensuring successful crop establishment and yield stability [4,5].

Seeds may carry numerous fungal and bacterial pathogens capable of reducing germination, impairing seedling development and serving as primary inoculum for diseases occurring later in the growing season [6,7]. Among the most frequently reported seed-borne fungi in wheat are species of *Alternaria*, *Fusarium*, *Bipolaris*, *Curvularia*, *Aspergillus* and *Penicillium*, many of which negatively affect seed vigour, plant establishment and grain quality [8–10]. Consequently, seed treatment has become a standard agronomic practice aimed at protecting seedlings during the most vulnerable stages of crop development [11,12].

Conventional fungicidal seed treatments remain one of the most reliable methods for controlling seed- and soil-borne pathogens and improving crop establishment. Nevertheless, increasing concerns regarding pesticide residues, environmental contamination, biodiversity conservation, the development of pathogen resistance and progressively stricter regulations on pesticide use have accelerated the search for safer and more sustainable alternatives. As a result, biological products based on beneficial microorganisms, ecological formulations containing natural compounds and plant extracts, and plant biostimulants have attracted increasing scientific and practical interest [13,14].

Unlike conventional fungicides, biological products reduce pathogen activity through mechanisms such as competition, antibiosis and induced systemic resistance, whereas biostimulants primarily enhance plant physiological processes by stimulating root development, improving nutrient use efficiency and increasing tolerance to abiotic stress [15–17]. When applied as seed treatments, these products may promote faster and more uniform emergence, stimulate tillering and improve crop performance during the early stages of development. Although numerous studies have demonstrated the potential of these products, their efficacy under field conditions remains variable because it depends on environmental conditions, product composition and interactions with the soil microbiome [18–21].

Despite the growing interest in alternative seed treatments, most published studies have evaluated only one category of products or have focused on a limited number of response variables, such as seed germination, disease suppression or grain yield [22–25]. Comparative field studies simultaneously assessing conventional chemical treatments, biological products, formulations based on naturally derived compounds and plant biostimulants under identical environmental and management conditions remain scarce. Moreover, relatively few studies have monitored treatment performance throughout successive stages of crop development, from seedling emergence and tillering to productive spike formation and final grain yield [26]. The possible influence of these treatments on early-season pest

damage, including cereal fly damage, has also received limited attention [13]. Multi-season field evaluations are therefore needed to determine the consistency of their effects under contrasting environmental conditions [26,27].

The present study aimed to compare conventional chemical seed treatments with alternative formulations containing microorganisms, naturally derived compounds or plant biostimulants in winter wheat over two consecutive growing seasons in the Transylvanian Plateau of Romania. Treatment performance was assessed through laboratory germination tests and an integrated evaluation of field emergence, crop development, cereal fly damage, yield components, grain yield, grain quality and economic returns. By evaluating these treatment categories under identical field conditions across two growing seasons, the study assessed the consistency of their agronomic effects and their potential contribution to more sustainable winter wheat production systems.

2. Materials and Methods

2.1. Study Site, Weather and Soil Conditions

To evaluate the effects of conventional chemical and alternative seed treatments on crop establishment, early-season pest damage and grain yield of winter wheat, a field experiment was conducted during the 2023–2024 and 2024–2025 growing seasons at the Agricultural Research and Development Station (ARDS) Turda, Romania (46°34′57.4″ N, 23°49′01.2″ E), located in the Transylvanian Plateau. The experimental site has a temperate continental climate characterized by marked interannual variability in temperature and precipitation. From October to June, mean air temperatures were 9.69 °C in 2023–2024 and 7.80 °C in 2024–2025, while cumulative precipitation amounted to 268.6 and 258.1 mm, respectively (Figure 1A,B). Compared with the long-term climatic averages, the two experimental seasons were characterized by marked deviations in both precipitation and air temperature. During the periods corresponding to crop emergence and grain setting, precipitation was below the long-term average, while mean air temperatures were generally higher. The simultaneous occurrence of reduced rainfall and elevated temperatures likely decreased soil water availability and increased crop water demand during these critical developmental stages. These conditions may have contributed to the seasonal variability observed in crop establishment, stand development, and grain yield.

These meteorological data were provided by the Turda Weather Station, operated by the Romanian National Meteorological Administration (ANM), which is located adjacent to the experimental field [28].

The experiment was conducted on a clay-textured Chernozem [29]. The soil properties presented below were obtained from a previously published characterization of the same agricultural field at ARDS Turda, although the two experiments were not established at precisely the same location within the field. At a depth of 0–20 cm, the soil contained 56.07% clay, 19.15% fine silt, 9.15% silt, 14.90% fine sand and 0.73% coarse sand, with a bulk density of 1.13 g cm^{−3}, total porosity of 58%, humus content of 3.73%, pH (H₂O) of 6.81, total nitrogen content of 0.205%, available phosphorus of 35 mg kg^{−1} and available potassium of 320 mg kg^{−1} [30].

2.2. Experimental Design and Seed Treatments

The experiment was arranged in a randomized complete block design with three replications. Each experimental plot had a total area of 7 m², including a net harvest area of 6 m² used for grain yield determination. Six seed treatment variants were included: an untreated control and five commercial seed treatment products representing different categories, namely a chemical fungicide, a chemical insecticide, a biological insecto-fungicide, an organic insecto-fungicide, and a biostimulant. All products were applied to the seed

immediately before sowing at the manufacturers' recommended application rates. Detailed information on the evaluated products, their active ingredients and application rates is provided in Table 1.

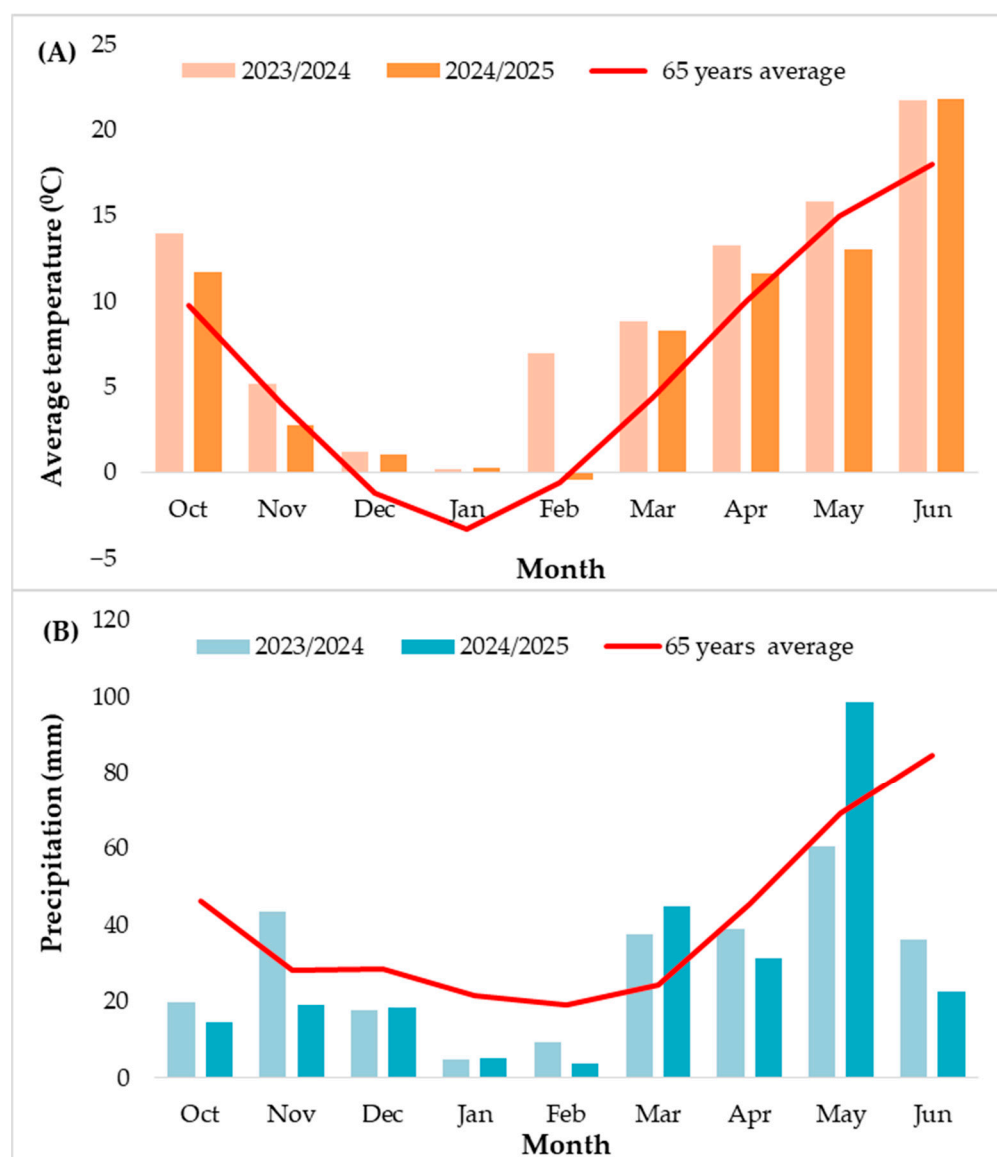


Figure 1. Monthly mean air temperature (A) and monthly precipitation (B) recorded during the 2023/2024 and 2024/2025 growing seasons compared with the long-term average at ARDS Turda, Romania.

Table 1. Seed treatment products evaluated in the winter wheat experiment.

Treatment No.	Product	Active Ingredient/Composition	Product Type	Dose (L t ⁻¹)	Seed Treatment Cost (€ t ⁻¹ Seed)
1	Untreated control	–	–	–	–
2	Redigo Pro 170 FS [31]	Prothioconazole 150 g L ⁻¹ + tebuconazole 20 g L ⁻¹	Chemical fungicide	0.5	20.08
3	Signal 300 ES [32]	Cypermethrin 300 g L ⁻¹	Chemical insecticide	2.5	83.75

Table 1. Cont.

Treatment No.	Product	Active Ingredient/Composition	Product Type	Dose (L t ⁻¹)	Seed Treatment Cost (€ t ⁻¹ Seed)
4	Biosem [33]	Beneficial microorganisms, neem seed extract, salicylic acid, and plant-derived components	Microbiological seed treatment with insecticidal and fungicidal effects	1.5	84.89
5	Bioseed [34]	Natural plant and fruit extracts and oils	Organic seed treatment with insecticidal and fungicidal effects	1.5	70.75
6	Raiza Mix [35]	Free amino acids (12.0%), N (1.92%), B (0.2%), Cu (0.1%), Fe (1.1%), Mn (0.5%), Mo (0.02%), Zn (0.2%)	Biostimulant	2.0	51.63

Product composition was reported according to the manufacturers' technical information. Treatment costs refer exclusively to the commercial products and exclude labour and seed-treatment application costs.

2.3. Crop Management

The experiment was conducted under a conventional tillage system, with field pea (*Pisum sativum* L.) as the preceding crop in both growing seasons. Primary tillage consisted of autumn mouldboard ploughing to a depth of approximately 28 cm, followed by seedbed preparation before sowing. The winter wheat cultivar Luminița, developed by ARDS Turda and officially registered in Romania in 2023, was used in both growing seasons. The cultivar is characterized by good lodging resistance, moderate resistance to major foliar diseases and high yield potential under the environmental conditions of the Transylvanian Plateau [36,37]. The crop was sown on 18 October 2023 and 23 October 2024 at a density of 550 viable seeds m⁻², with a row spacing of 12.5 cm and a sowing depth of approximately 4 cm. Before sowing, basal fertilization was applied uniformly to all plots at a rate of 300 kg ha⁻¹ of an NPK 16–16–16 (Azomureș, Mureș, Romania) compound fertilizer, corresponding to 48 kg ha⁻¹ each of N, P₂O₅, and K₂O. No additional nitrogen fertilizer was applied in spring, and no foliar fungicides were used during either growing season. All subsequent crop-management practices were performed uniformly across the experimental plots according to the standard practices of ARDS Turda.

Crop growth stages were identified according to the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale. At the two-node stage (BBCH 32), weed control was achieved using a tank mixture of Sekator PROGRES OD (Agro Bayer, Bucharest, Romania), (amidosulfuron, 100 g L⁻¹; iodosulfuron-methyl-sodium, 25 g L⁻¹; and mefenpyr-diethyl, 250 g L⁻¹, as a safener) at 100 mL ha⁻¹ and DMA-6 (2,4-D, 660 g L⁻¹ acid equivalent, formulated as 825 g L⁻¹ dimethylamine salt) at 400 mL ha⁻¹. At the same growth stage, all plots received a uniform foliar application of Leptostar 200 SL (Qemetica, Bucharest, Romania), (acetamiprid, 200 g L⁻¹) at 200 mL ha⁻¹ to minimize the influence of subsequent insect infestations on crop development and grain yield. The crop was harvested at full grain maturity (BBCH 89) on 5 July 2024 and 10 July 2025 in the 2023/2024 and 2024/2025 growing seasons, respectively.

2.4. Data Collection and Laboratory Analyses

Seeds used in both growing seasons originated from the same untreated seed lot of the winter wheat cultivar 'Luminița'. Between the two experimental seasons, the remaining untreated seeds were stored in a dry and ventilated seed-storage room under ambient non-controlled conditions; temperature and relative humidity were not continuously monitored. Seed treatments were applied separately immediately before sowing in each growing season. Germination energy and germination capacity were determined

before sowing under controlled laboratory conditions according to the International Rules for Seed Testing [38] using three replicates of 100 seeds per treatment and growing season. Seeds were placed on moistened paper and incubated at 20 °C. Normal seedlings were counted four days after test establishment to determine germination energy and after eight days to determine germination capacity. Results are expressed as percentages of the total number of tested seeds.

All experimental plots were visually monitored throughout the seedling emergence period for treatment-related phytotoxicity symptoms, including delayed emergence, chlorosis, necrosis, seedling deformation, and growth suppression. Field emergence was evaluated at BBCH 11, after emergence had stabilized, by counting all emerged plants within a 1 m² quadrat placed in the central area of each plot, avoiding the border rows and plot ends. Emergence is expressed as a percentage of the target sowing density of 550 viable seeds m⁻². Tiller density and cereal fly damage were assessed at BBCH 31, before the uniform foliar insecticide application at BBCH 32. Tiller density was determined by counting all living shoots, including the main stems and primary and secondary tillers, irrespective of their subsequent productive status. Because the insects responsible for the observed symptoms were not collected and taxonomically identified, the damage could not be attributed to a particular species. Therefore, cereal fly damage was assessed as the percentage of plants showing characteristic symptoms. All tillers and cereal showing cereal fly damage were counted within the same 1 m² quadrat in each experimental plot. Tiller density is expressed as tillers m⁻², while cereal fly damage is expressed as the percentage of attacked plants relative to the total number of plants counted within the quadrat.

Productive spike density was determined at full grain maturity (BBCH 89), immediately before harvest by counting all productive spikes within a 1 m² quadrat in each experimental plot, and is expressed as spikes m⁻². Grain yield was determined by harvesting the 6 m² net area of each plot and was converted to kg ha⁻¹ at a standardized grain moisture content of 14%.

Thousand-kernel weight (TKW) was determined using a 500 g grain sample collected from each experimental plot. Two subsamples of 1000 kernels were counted and weighed separately, and their mean was calculated for each plot and expressed in grams. Grain quality analyses were performed in the Laboratory of Biotechnologies and Physico-Chemical Analysis at the Agricultural Research and Development Station Turda. Test weight, grain protein content, wet gluten content and Zeleny sedimentation value were estimated non-destructively using Fourier-transform near-infrared (FT-NIR) spectroscopy with a calibrated NIR TANGO spectrophotometer (Bruker Optik GmbH, Ettlingen, Germany). Test weight is expressed as kg hL⁻¹, protein and wet gluten contents as percentages, and the Zeleny sedimentation value in mL.

2.5. Economic Analysis

Economic efficiency was evaluated using a partial budget analysis based on commercial prices and costs applicable in Romania in 2025. Treatment cost per hectare was calculated based on the commercial product cost per tonne of treated seed and an actual seeding rate of 270 kg ha⁻¹. Additional revenue was calculated by multiplying the grain-yield increase relative to the untreated control by a wheat grain price of €244.25 t⁻¹. Net benefit was calculated as the difference between additional revenue and treatment cost. Return on investment (ROI) was calculated as the ratio of net benefit to treatment cost and is expressed as a percentage. The break-even yield increase was calculated by dividing the treatment cost per hectare by the wheat grain price and represents the minimum additional grain yield required to recover the seed-treatment cost. Because the same seed-treatment procedure was used for all products, labour and application costs were excluded from the

analysis. All other production costs were identical across treatments and were therefore not included in the partial budget analysis.

2.6. Statistical Analysis

Prior to ANOVA, germination energy and germination capacity percentages were arcsine square-root transformed. Homogeneity of variances for the remaining variables was assessed using the median-based Levene test (Brown–Forsythe test) in PAST software 5.3 [39]. No significant heterogeneity was detected ($p = 0.644–0.979$); therefore, these variables were analysed without transformation. Untransformed means are reported for all variables to facilitate biological interpretation. Field data were analysed using linear mixed-effects models in R version 4.6.1 [40] within RStudio version 2026.07.1+147 [41] with growing season, seed treatment, and their interaction as fixed effects and blocks nested within growing season as a random effect. Germination energy and germination capacity were analysed by two-way ANOVA. Estimated marginal means were compared using Tukey-adjusted pairwise tests (emmeans), with compact letter displays at $p \leq 0.05$. Tukey-adjusted pairwise comparisons contrasted the untreated control and each alternative treatment with the two conventional reference treatments, Redigo Pro 170 FS and Signal 300 ES. The FactoMineR package version 2.16 [42] was used to perform the PCA, whereas factoextra version 2.2.0 [43] was used for visualization.

3. Results

3.1. Effect of Seed Treatments on Germination Energy and Germination Capacity

Seed treatment significantly affected both germination energy and germination capacity of winter wheat ($p \leq 0.001$), whereas neither the main effect of growing season nor the growing season $\times$ treatment interaction was significant (Table 2). Germination energy ranged from 89.67% in the untreated control to 96.83% following treatment with Redigo Pro 170 FS. All seed treatments significantly increased germination energy compared with the untreated control. Redigo Pro 170 FS produced the highest numerical value and did not differ significantly only from Biosem among the treated variants. Bioseed recorded the lowest value among the treated variants but did not differ significantly from Signal 300 ES, Biosem, or Raiza Mix.

Table 2. Effect of seed treatments on germination energy and germination capacity of winter wheat during the 2023/2024 and 2024/2025 growing seasons.

Seed Treatment	Germination Energy (%)	Germination Capacity (%)
Untreated control	89.67	93.00 C
Redigo Pro 170 FS	96.83 A	99.33 A
Signal 300 ES	95.67 AB	98.00 B
Biosem	96.33 AB	98.50 AB
Bioseed	94.50 B	98.67 AB
Raiza Mix	95.83 AB	97.67 B
Source of variation		
Year (Y)	ns	ns
Seed treatment (T)	***	***
Year $\times$ Treatment (Y $\times$ T)	ns	ns

Values are untransformed means across two growing seasons, with three replicates per treatment in each season. Percentage data were transformed to angular degrees using the arcsine square-root transformation [$\arcsin\sqrt{(x/100)}$] before ANOVA. Within each column, means followed by at least one common letter are not significantly different according to Tukey-adjusted pairwise comparisons of estimated marginal means at $p \leq 0.05$. ns, not significant; ***, $p \leq 0.001$.

A similar response was observed for germination capacity, which ranged from 93.00% in the untreated control to 99.33% following treatment with Redigo Pro 170 FS. All seed treatments significantly increased germination capacity compared with the untreated control. Redigo Pro 170 FS produced the highest value and significantly exceeded Signal 300 ES and Raiza Mix but did not differ significantly from Biosem or Bioseed. No significant differences were detected among Signal 300 ES, Biosem, Bioseed and Raiza Mix.

3.2. Effect of Seed Treatments on Crop Establishment and Early Crop Development

No symptoms of treatment-related phytotoxicity, such as chlorosis, necrosis, seedling deformation, or growth suppression, were observed during the seedling emergence period in either growing season. Seed treatment significantly influenced seedling emergence and cereal fly damage but did not significantly affect tiller density or productive spike density (Table 3). Growing season significantly affected cereal fly damage but not seedling emergence, tiller density, or productive spike density. No significant growing season $\times$ treatment interactions were detected for any of the evaluated traits. Across treatments, mean field emergence decreased from 85.07% in 2023/2024 to 82.45% in 2024/2025, whereas the incidence of cereal fly damage increased from 5.10% to 6.03%.

Table 3. Effect of seed treatments on crop establishment and early crop development of winter wheat (mean of the 2023/2024 and 2024/2025 growing seasons).

Seed Treatment	Emergence (%)	Tillers (No. m ⁻²)	Cereal Fly Damage (%)	Productive Spikes (No. m ⁻²)
Untreated control	76.12 C	1231 A	6.55 A	506 B
Redigo Pro 170 FS	82.79 ABC	1272 A	5.84 AB	580 A
Signal 300 ES	87.76 AB	1290 A	5.40 B	556 A
Biosem	89.58 A	1234 A	5.53 AB	550 A
Bioseed	80.73 BC	1229 A	4.02 C	564 A
Raiza Mix	85.58 AB	1283 A	6.08 AB	572 A
Source of variation				
Year (Y)	ns	ns	*	ns
Seed treatment (T)	***	ns	***	ns
Year $\times$ Treatment (Y $\times$ T)	ns	ns	ns	ns

Values are estimated marginal means across two growing seasons, with three replicates per treatment in each season. Within each column, means sharing at least one uppercase letter are not significantly different according to Tukey-adjusted pairwise comparisons at $p \leq 0.05$. ns, not significant; *, $p \leq 0.05$; ***, $p \leq 0.001$.

Seedling emergence ranged from 76.12% in the untreated control to 89.58% following Biosem treatment. Biosem, Signal 300 ES, and Raiza Mix significantly increased emergence compared with the untreated control, whereas the increases obtained with Redigo Pro 170 FS and Bioseed were not statistically significant.

Seed treatment also significantly affected cereal fly damage. The untreated control recorded the highest attack (6.55%), while Bioseed produced the lowest value (4.02%) and differed significantly from all other treatments. Signal 300 ES also significantly reduced cereal fly damage compared with the untreated control. Redigo Pro 170 FS, Biosem and Raiza Mix produced intermediate values that did not differ significantly from the untreated control.

Tiller density ranged from 1229 to 1290 tillers m⁻², but neither seed treatment nor growing season had a significant effect on this trait.

Productive spike density ranged from 506 spikes m⁻² in the untreated control to 580 spikes m⁻² following Redigo Pro 170 FS treatment. Neither seed treatment nor growing season significantly affected this trait.

Tiller density included all living shoots at BBCH 31, whereas productive spike density included only ear-bearing culms at BBCH 89. The ratio of productive spikes to tillers ranged from 41.1% to 45.9% and was numerically lowest in the untreated control. However, because the treatment effect on productive spike density was not significant, this pattern should be interpreted descriptively.

3.3. Effect of Seed Treatments on Grain Yield and Grain Quality

Seed treatment significantly affected thousand-kernel weight but did not significantly influence grain yield, test weight protein content, wet gluten content and Zeleny sedimentation value (Table 4). Growing season significantly affected grain yield, test weight, and thousand-kernel weight, whereas no significant growing season $\times$ treatment interactions were detected for any of the evaluated traits.

Table 4. Effect of seed treatments on grain yield and grain quality characteristics of winter wheat (mean of the 2023/2024 and 2024/2025 growing seasons).

Seed Treatment	Grain Yield (kg ha ⁻¹)	Test Weight (kg hL ⁻¹)	Thousand Kernel Weight (g)	Protein (%)	Wet Gluten (%)	Zeleny Sedimentation Value (mL)
Untreated control	7400 B	75.52 A	47.87 B	10.86 A	19.43 A	30.33 A
Redigo Pro 170 FS	7745 AB	76.18 A	49.40 AB	10.78 A	19.80 A	29.67 A
Signal 300 ES	7909 A	76.18 A	49.70 A	10.93 A	20.28 A	29.83 A
Biosem	7685 AB	75.73 A	49.40 AB	10.85 A	19.45 A	29.67 A
Bioseed	7656 AB	75.68 A	48.87 AB	10.46 A	17.88 A	27.50 A
Raiza Mix	7741 AB	76.42 A	49.54 AB	10.84 A	19.72 A	28.33 A
Source of variation						
Year (Y)	*	***	**	ns	ns	ns
Seed treatment (T)	ns	ns	*	ns	ns	ns
Year $\times$ Treatment (Y $\times$ T)	ns	ns	ns	ns	ns	ns

Values are estimated marginal means across two growing seasons, with three replicates per treatment in each season. Within each column, means sharing at least one uppercase letter are not significantly different according to Tukey-adjusted pairwise comparisons at $p \leq 0.05$. ns, not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Grain yield ranged from 7400 kg ha⁻¹ in the untreated control to 7909 kg ha⁻¹ following treatment with Signal 300 ES. Although the overall effect of seed treatment was not significant ($p = 0.063$), the Tukey-adjusted comparison indicated that Signal 300 ES produced a significantly higher yield than the untreated control. The remaining treatments produced intermediate values that did not differ significantly from either Signal 300 ES or the untreated control.

Test weight ranged from 75.52 kg hL⁻¹ in the untreated control to 76.42 kg hL⁻¹ following treatment with Raiza Mix. These differences were numerical, as neither the overall treatment effect nor any Tukey-adjusted pairwise comparison was significant.

Thousand-kernel weight ranged from 47.87 g in the untreated control to 49.70 g following treatment with Signal 300 ES. Signal 300 ES produced a significantly higher thousand-kernel weight than the untreated control. Redigo Pro 170 FS, Biosem, Bioseed and Raiza Mix produced significantly higher thousand-kernel weights than the untreated control.

Protein content, wet gluten content and Zeleny sedimentation value were not significantly affected by seed treatment, growing season or their interaction. Thus, numerical differences in grain yield observed for some treatments were not accompanied by significant changes in the evaluated grain protein and technological quality parameters.

Tukey-adjusted pairwise contrasts with the two conventional reference treatments are presented in Figure 2. None of the evaluated treatments differed significantly from Redigo Pro 170 FS for seedling emergence, productive spike density, grain yield, or thousand-kernel weight (Figure 2a,c,e,g). Compared with Signal 300 ES, the untreated control showed significantly lower seedling emergence (-11.64 percentage points; $p \leq 0.001$), grain yield (-509 kg ha $^{-1}$; $p \leq 0.05$), and thousand-kernel weight (-1.8 g; $p \leq 0.05$) (Figure 2b,f,h). No significant differences relative to Signal 300 ES were detected for productive spike density (Figure 2d). Biosem, Bioseed, and Raiza Mix did not differ significantly from either conventional reference treatment for any of the traits included in the figure.

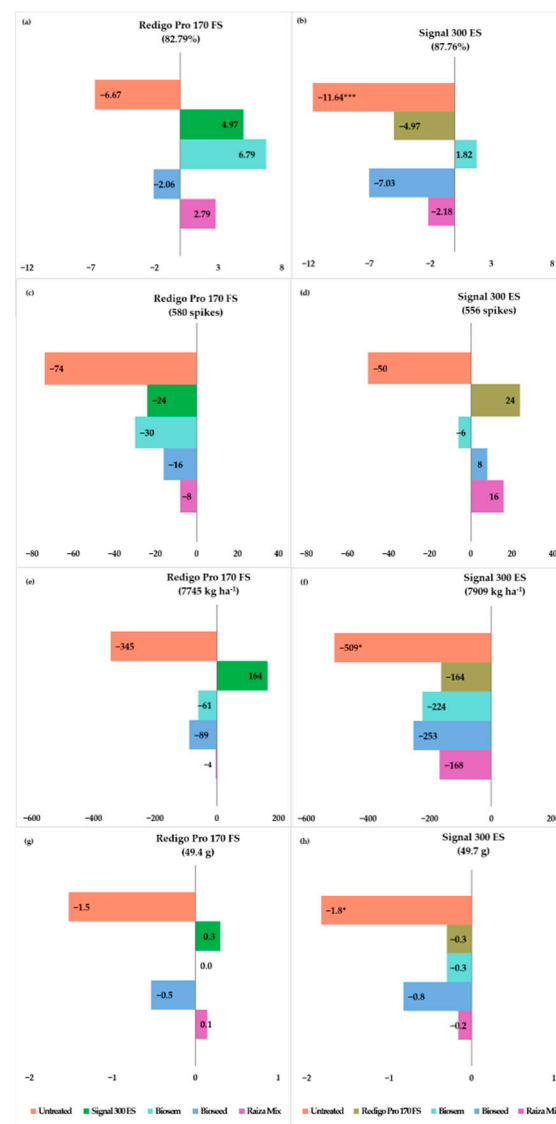


Figure 2. Differences in seedling emergence (a,b), productive spike density (c,d), grain yield (e,f), and thousand-kernel weight (g,h) relative to the conventional reference treatments Redigo Pro 170 FS (left column) and Signal 300 ES (right column). Positive and negative values indicate increases and decreases, respectively, relative to the corresponding reference treatment. Asterisks indicate significant Tukey-adjusted pairwise comparisons: * $p \leq 0.05$ and *** $p \leq 0.001$.

3.4. Multivariate Relationships Among Treatments and Agronomic Traits

The first three principal components explained 68.3% of the total variance (Figure 3). Grain yield, tiller density, and productive spike density showed similar orientations, indicating positive associations, whereas cereal fly damage was oriented in the opposite direction. Field emergence, test weight, and thousand-kernel weight were mainly associ-

ated with the positive side of PC1. The three-dimensional PCA provided an integrated representation of trait relationships and treatment distribution.

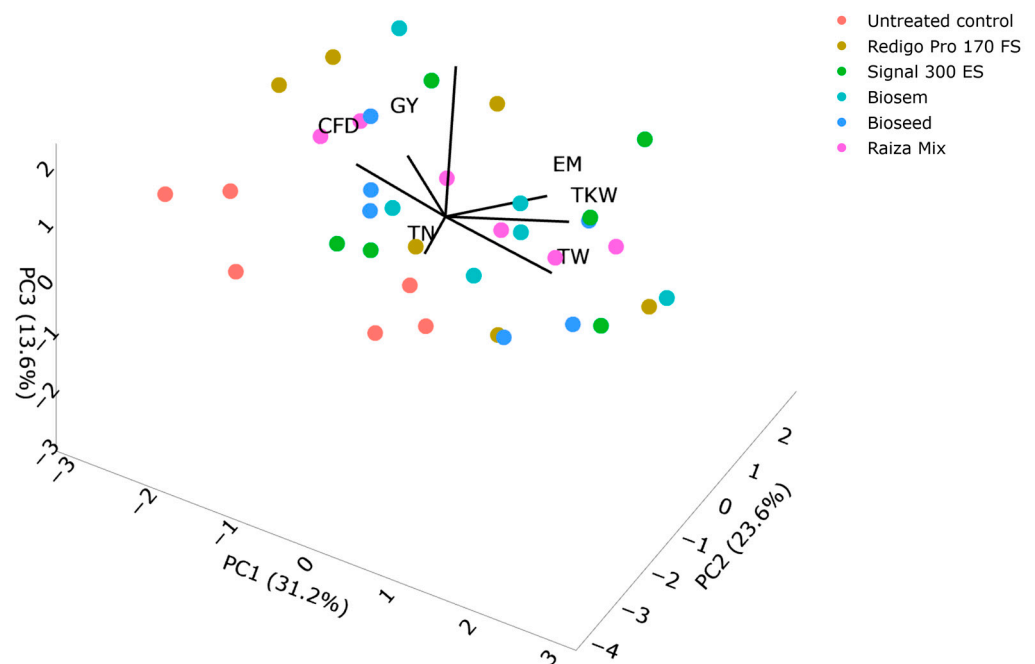


Figure 3. Three-dimensional principal component analysis (PCA) biplot based on standardized plot-level agronomic variables. PC1, PC2, and PC3 accounted for 31.2%, 23.6%, and 13.6% of the total variance, respectively, explaining 68.3% cumulatively. EM, field emergence; TN, tiller density; CFD, cereal fly damage; PSD, productive spike density; GY, grain yield; TW, test weight; TKW, thousand-kernel weight. Colors indicate the six seed treatments.

3.5. Economic Efficiency of Seed Treatments

Based on the observed marginal yields, all seed treatments generated positive estimated net benefits relative to the untreated control (Table 5). Numerical yield increases ranged from 256.18 kg ha⁻¹ with Bioseed to 508.43 kg ha⁻¹ with Signal 300 ES. Only Signal 300 ES produced a statistically significant yield increase compared with the untreated control; the differences obtained with Redigo Pro 170 FS, Biosem, Bioseed, and Raiza Mix were not statistically significant.

Additional revenue ranged from €62.57 ha⁻¹ for Bioseed to €124.28 ha⁻¹ for Signal 300 ES, while estimated net benefits ranged from €43.47 to €101.57 ha⁻¹, respectively. Signal 300 ES generated the highest net benefit, followed by Redigo Pro 170 FS (€78.87 ha⁻¹) and Raiza Mix (€69.30 ha⁻¹).

Redigo Pro 170 FS achieved the highest return on investment (1455.17%) because of its comparatively low product cost, despite producing a lower net benefit than Signal 300 ES. The break-even yield increase ranged from 22.19 kg ha⁻¹ for Redigo Pro 170 FS to 93.84 kg ha⁻¹ for Biosem. The numerical yield increases exceeded the corresponding break-even values for all evaluated treatments. Except for Signal 300 ES, these economic estimates should be interpreted descriptively because the corresponding yield differences were not statistically significant. These estimates should be interpreted within the scope of the partial budget analysis, which included product costs but excluded labour and seed-treatment application costs.

Table 5. Partial budget analysis of seed treatments in winter wheat based on mean grain yields across the 2023–2024 and 2024–2025 growing seasons.

Seed Treatment	Mean Yield (kg ha ⁻¹)	Yield Increase (kg ha ⁻¹)	Treatment Cost (€ ha ⁻¹)	Additional Revenue (€ ha ⁻¹)	Net Benefit (€ ha ⁻¹)	Return on Investment (%)	Break-Even Yield Increase (kg ha ⁻¹)
Untreated control	7400.28	–	0.00	–	–	–	–
Redigo Pro 170 FS	7745.38	345.10	5.42	84.29	78.88	1455.17	22.19
Signal 300 ES	7908.71	508.43	22.61	124.18	101.67	449.23	92.57
Biosem	7684.71	284.43	22.92	69.47	46.57	203.10	93.84
Bioseed	7656.45	256.18	19.10	62.57	43.47	227.59	78.20
Raiza Mix	7741.08	341.81	13.94	83.24	69.35	497.13	57.07

Treatment costs include only commercial product costs and exclude labour and seed-treatment application costs. All prices and costs refer to 2025. Additional revenue and net benefit were calculated using the unrounded estimated marginal yield differences relative to the untreated control. Only the yield increase obtained with Signal 300 ES was statistically significant according to the Tukey-adjusted comparison at $p \leq 0.05$; the remaining economic estimates should be interpreted descriptively.

Compared with the conventional reference treatments, Raiza Mix showed the closest economic performance to Redigo Pro 170 FS, producing a similar grain yield but a €9.57 ha⁻¹ lower net benefit because of its higher product cost. Signal 300 ES generated the highest net benefit, whereas Redigo Pro 170 FS achieved the highest return on investment owing to its comparatively low cost. Biosem and Bioseed also generated positive estimated net benefits, although their economic returns were lower than those of both conventional reference treatments. Despite these economic differences, none of the alternative treatments differed significantly from Redigo Pro 170 FS or Signal 300 ES in grain yield; therefore, these economic comparisons should be interpreted descriptively.

4. Discussion

4.1. Interpretation of Seed Treatment Effects on Germination Energy and Germination Capacity

Seed treatments improved both germination energy and final germination capacity, with consistent responses across growing seasons. Redigo Pro 170 FS recorded the highest numerical values, which may reflect the protection provided by prothioconazole and tebuconazole against seed-borne pathogens [44], although pathogen loads were not quantified. The responses to the alternative treatments are consistent with studies showing that *Trichoderma*, plant extracts, humic substances and amino acids can support cereal germination and early seedling development [45–47]. However, these mechanisms were not directly investigated.

4.2. Interpretation of Seed Treatment Effects on Crop Establishment and Early Crop Development

Growing season significantly influenced cereal fly damage but not field emergence with lower emergence and greater damage in 2024/2025, probably reflecting differences in environmental conditions during crop establishment. The absence of a growing season $\times$ treatment interaction indicates that treatment responses were generally consistent across seasons.

All seed treatments numerically improved field emergence, but significant increases relative to the untreated control were detected only for Signal 300 ES, Biosem, and Raiza Mix. These product-dependent responses are consistent with previous studies [24,48] and with observations in spring barley under the conditions of ARDS Turda [49]. The strong response to Biosem may be associated with its microbial and bioactive components, although their individual contributions were not evaluated.

Cereal fly damage was lowest following Bioseed treatment, which differed significantly from all other treatments. Signal 300 ES also significantly reduced damage relative to the untreated control, consistent with the protective activity of insecticidal seed treatments against cereal flies and other early-season pests [50–52]. Redigo Pro 170 FS, Biosem, and Raiza Mix produced intermediate values that did not differ significantly from the untreated control. The mechanism responsible for the response to Bioseed remains unclear because insect mortality and feeding or host-selection behaviour were not evaluated.

Neither tiller density nor productive spike density was significantly affected by seed treatment. Although all treated variants showed numerically higher productive spike densities than the untreated control, these differences were not statistically significant. Tiller density included all living shoots at BBCH 31, whereas productive spike density included only ear-bearing culms at BBCH 89. The reduction between these stages is consistent with tiller mortality [53], and compensatory development in winter wheat [54], although tiller survival was not directly monitored.

4.3. Interpretation of Seed Treatment Effects on Grain Yield and Grain Quality

Grain-yield responses differed among products, although the overall effect of seed treatment was not significant ($p = 0.063$). Signal 300 ES produced the highest yield and differed significantly from the untreated control according to the Tukey-adjusted comparison. Redigo Pro 170 FS, Biosem, Bioseed, and Raiza Mix produced intermediate yields that did not differ significantly from either the untreated control or Signal 300 ES. No significant yield differences were detected among the treated variants. Variation in crop establishment and the numerically higher productive spike density recorded in the treated plots may have contributed to the observed yield responses, although these relationships were not directly examined.

Positive effects of conventional and alternative seed treatments on cereal yield have also been reported previously [5,13,24]. Because the target soil pests and seed-borne pathogen populations were not quantified, the yield responses to Signal 300 ES and Redigo Pro 170 FS cannot be attributed exclusively to insect or pathogen control. Their numerical performance suggests that protection during crop establishment may contribute to maintaining subsequent productivity, but this interpretation requires further validation. Previous field studies have similarly shown that fungicidal and combined fungicide–insecticide seed treatments can improve winter wheat stand establishment and grain yield, particularly under less favourable establishment conditions [3,55].

Seed treatment significantly influenced thousand-kernel weight but not test weight. Signal 300 ES produced the highest thousand-kernel weight and differed significantly from the untreated control, whereas the remaining treatments produced intermediate values. Raiza Mix recorded the highest numerical test weight, but no significant differences were detected among treatments. Similar effects of fungicidal and insecticidal seed treatments on wheat establishment, thousand-kernel weight and grain yield were reported by Mendes et al. [56]. Protein content, wet gluten and Zeleny sedimentation value were unaffected, indicating that treatment effects were primarily expressed in yield formation and physical grain characteristics rather than protein-related technological quality. Grain protein concentration and related technological quality traits are strongly influenced by genotype and environmental conditions during grain filling. In a multi-environment field study, Soba et al. [57] showed that drought and heat stress altered grain and flour protein, gluten content and dough rheological properties, with responses differing between cultivars.

4.4. Multivariate Relationships Among Seed Treatments and Agronomic Traits

Principal component analysis provided an integrated representation of the relationships among treatments and agronomic traits. Grain yield, tiller density and productive spike density were oriented in similar directions, suggesting positive associations among these variables, whereas cereal fly damage showed an opposing orientation. These patterns are consistent with previous studies reporting relationships among crop establishment, early-season pest damage and cereal productivity [13].

The untreated control tended to occupy a different region of the multivariate space from most treated variants, whereas Signal 300 ES was positioned in the direction of higher field emergence and thousand-kernel weight. Biosem, Bioseed and Raiza Mix showed partially overlapping positions, indicating treatment-specific but broadly comparable multivariate responses. The first three principal components explained 68.3% of the total variance (PC1: 31.2%, PC2: 23.6%, and PC3: 13.6%). PCA is exploratory and does not test statistical significance or establish causal relationships.

4.5. Interpretation of the Economic Efficiency of Seed Treatments

Based on the estimated marginal grain yields, all seed treatments generated positive estimated net benefits. Only the yield increase obtained with Signal 300 ES was statistically significant relative to the untreated control. The projected economic advantages of Redigo Pro 170 FS, Biosem, Bioseed, and Raiza Mix were based on numerical yield differences that were not statistically significant and therefore require confirmation across additional seasons, locations, and cultivars.

Profitability reflected the balance among treatment cost, grain price and the magnitude and consistency of the yield response. Previous field studies have similarly shown that seed treatments can increase winter wheat yield and net returns, although their economic benefits vary with genotype, crop establishment and environmental conditions [58,59]. The comparatively modest numerical yield responses of Biosem and Bioseed, together with their treatment costs, resulted in lower net benefits than those of the best-performing conventional treatments. Positive cost–benefit ratios have also been reported for biological and combined disease-control treatments in winter wheat, although the products and application strategies differed from those evaluated here [60].

Among the alternative treatments, Raiza Mix showed the most favourable economic performance. Its grain yield did not differ significantly from that obtained with either conventional reference treatment, while its lower cost relative to Signal 300 ES contributed to a higher return on investment. Nevertheless, the yield difference between Raiza Mix and the untreated control was not statistically significant; therefore, its projected economic advantage should be interpreted descriptively. Positive economic returns from foliar biostimulant application have also been reported in commercial wheat production, although profitability varied among price scenarios [61]. The economic ranking observed in the present study may change with grain and product prices. Because labour and seed-treatment application costs and potential environmental benefits were excluded, these results represent a short-term, site-specific economic assessment.

4.6. Study Limitations

This study was conducted at a single location using one winter wheat cultivar over two growing seasons, with three field blocks per treatment in each season, which limits the broader applicability of the findings. Although the seeds used in both seasons originated from the same initial lot and were stored under the same conditions, storage temperature and relative humidity were not continuously monitored. Furthermore, the seeds evaluated in the second season had undergone a longer storage period. Consequently, potential

effects of storage duration and conditions cannot be completely separated from growing-season effects, and interannual differences should be interpreted with caution. Moreover, several alternative products contained complex mixtures of microorganisms, plant extracts, oils, amino acids or nutrients, preventing the contribution of individual components from being distinguished. Seed-borne pathogen loads and the physiological mechanisms underlying the observed responses were not directly assessed. In addition, the cereal fly species responsible for the observed symptoms were not taxonomically identified, and pest pressure was evaluated only as the incidence of damaged plants. Wheat diseases were not systematically assessed because, under natural field infection, the effects of seed-borne pathogens could not be separated from foliar infections originating from crop residues or airborne inoculum. Consequently, the findings should be validated across additional cultivars, locations, seed lots and growing seasons.

5. Conclusions

Across the two growing seasons 2023/2024 and 2024/2025, all evaluated seed treatments significantly increased germination energy and germination capacity relative to the untreated control. Seed treatment significantly influenced field emergence and cereal fly damage but did not significantly affect tiller density or productive spike density. Biosem produced the highest field emergence (89.58%), while Bioseed was associated with the lowest incidence of cereal fly damage (4.02%). The overall effect of seed treatment on grain yield was not significant, although Signal 300 ES produced the highest yield and differed significantly from the untreated control in the Tukey-adjusted comparison. Seed treatment significantly influenced thousand-kernel weight but not test weight, protein content, wet gluten content, or Zeleny sedimentation value.

Under the economic assumptions used in this study, Signal 300 ES generated the highest estimated net benefit, while Redigo Pro 170 FS achieved the highest overall return on investment. Among the alternative treatments, Raiza Mix showed the most favourable projected economic performance, although its yield increase relative to the untreated control was not statistically significant. No significant growing season $\times$ treatment interactions were detected, providing no evidence that relative treatment responses were markedly modified by growing season under the conditions of this experiment. Although no significant grain-yield differences were detected between the alternative treatments and the two conventional reference treatments, this result should not be interpreted as evidence of equivalence. The single-location, single-cultivar and two-season design, together with the absence of seed-borne pathogen quantification and taxonomic identification of the cereal fly, limits the broader generalization of the findings. Further multi-location and multi-season studies involving additional cultivars and seed lots are therefore required.

Author Contributions: Conceptualization, L.S., L.S. and A.-C.F.; methodology, L.S., A.-M.V., A.T. and F.R.; validation, L.S., A.S., C.U. and L.S.; formal analysis, I.C. and N.T.; investigation, L.S., A.-M.V., A.S. and C.U.; resources, L.S., L.S., I.R. and A.-C.F.; data curation, L.S. and I.C.; writing—original draft preparation, L.S., A.-M.V. and A.T.; writing—review and editing, L.S., A.-M.V., A.T., L.S., I.C., N.T., A.S., C.U., F.R., I.R. and A.-C.F.; visualization, I.C., I.R. and N.T.; supervision, L.S. and A.-C.F.; project administration, N.T., F.R. and A.-C.F.; funding acquisition, L.S., I.R. and A.-C.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Romanian Ministry of Agriculture and Rural Development through the ADER Project No. 2.1.3, under the contract signed on 18 July 2023, project: “Testing and analyzing the technical and economic efficiency of using eco-friendly/biological insecto fungicide products for the treatment of maize, sunflower, wheat/fall cereals, and rapeseed seeds, as an alternative to conventional products”.

Data Availability Statement: The original contributions presented in this study are included in the article, and further inquiries can be directed to the corresponding authors.

Acknowledgments: We would like to thank the Agricultural Research and Development Station Turda for organizing research in the experimental field.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ANM	National Meteorological Administration
ANOVA	Analysis of Variance
ARDS	Agricultural Research and Development Station
BBCH	Biologische Bundesanstalt, Bundessortenamt und Chemical Industry Growth Scale
CFD	Cereal Fly Damage
EM	Field Emergence
FT-NIR	Fourier-Transform Near-Infrared Spectroscopy
GC	Germination Capacity
GE	Germination Energy
GY	Grain Yield
NPK	Nitrogen–Phosphorus–Potassium
PCA	Principal Component Analysis
PSD	Productive Spike Density
ROI	Return On Investment
TKW	Thousand-Kernel Weight
TN	Tiller Density
TW	Test Weight

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