

Interrelationships of morphological, productive and biotic attributes in flaxseed crops

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Abstract: The cultivation of flaxseed (*Linum usitatissimum* L.) has gained prominence because of its agronomic potential and nutritional benefits. In Brazil, its cultivation is expanding, particularly in the southern region, where it serves as an alternative crop during colder seasons. However, even with its increased production, information on crop management remains scarce, especially regarding the use of biological products in its cultivation. The objective of this study was to evaluate the morphological, productive and biotic characteristics of the flaxseed crop in response to the application of biological inputs. The experiment was conducted in the municipality of Augusto Pestana, RS, Brazil, using a randomized block design, with two experiments: one in an area with application of combined chemical + biological inputs and another with only chemical inputs. Each experiment had 10 sampling grids, and variables such as plant stand, plant height, number of branches, soil cover, compaction, productivity and presence of insect pests, diseases and weeds were evaluated. The data were subjected to the Kruskal-Wallis test at 5% probability and Pearson's linear correlation analysis. The results indicated that the combined application of chemical + biological inputs improved variables related to crop growth and development compared to chemical inputs alone. The correlations highlighted the interaction between morphological and productive factors, demonstrating that the use of biologicals can be a sustainable alternative in crop management.

Keywords: *Linum usitatissimum* L.; bioinputs; agronomic variables; productivity; sustainable management.

Abbreviations: BB_Basal Branching; BF_Beginning of Flowering; CL_Compaction Level; EM_Emergence; FB_Flower Bud; FVC_First Visible Capsule; GWP_Grain Weight per Plant; HIFC_Height of Insertion of the First Capsule; LD_Leaf Development; MAP_Monoammonium Phosphate; NC_Number of Capsules; NBB_Number of Basal Branches; NFC_Number of Fertile Capsules; NPB_Number of Productive Branches; PE_Plant Emergence; PH_Plant Height; PHENS_Phenological Stage; PLT_Plantability; PS_Plant Stand; QIPE_Quantification and Identification of Insect Pests; QIPL_Quantification and Identification of Invasive Plants; RS_Row Spacing; SC_Soil Cover; SN_Senescence; SO_Sowing; ST_Straw Volume; TGW_Thousand Grain Weight.

Introduction

Flaxseed (*Linum usitatissimum* L.), belonging to the Lineaceae family, is a fibrous and dicotyledonous plant, with an erect and branched stem, that gives rise to leaves, flowers, and seed capsules (Kausar et al., 2023). It is self-pollinating, diploid ($2n = 30$), hermaphrodite and hypogynous species, with the characteristic of the species being the opening of the flowers only in the morning, with the loss of the petals close to noon (Barroso et al., 2014; Azevedo et al., 2022). Its high self-pollination capacity is due to the fact that its sticky pollen is rarely transported by insects. Furthermore, it is of great importance both in the agricultural context, for grain production and crop rotation, and in the food context, as it presents nutraceutical compounds (Almeida, 2019; Pradebon et al., 2023). Its benefits come from the presence of essential fatty acids, as well as minerals, vitamins, proteins, fibers and phenolic components (Brestenský et al., 2016).

Currently, there are two genotypes, differentiated by the color of their grains: one golden and the other brown. Both are similar in their composition, and the color of these genotypes is determined by the number of pigments in their external coating, influenced by genetic and environmental factors (Souza, 2021). Brown flaxseed performs better in warmer, more humid climates like Brazil, whereas golden flaxseed thrives in colder climates, such as in Canada (Fioreze et al., 2022; Scarton et al., 2023). In general, the crop prefers low temperatures for its development (Stanck, 2017), which makes the southern region of Brazil an area with great productive potential for flaxseed cultivation (Tomassoni, et al., 2013; Muraro et al., 2018).

The crop requires lower levels of fertilization when compared to other crops, such as wheat, soybeans, and corn, which facilitates its insertion in agricultural cultivation (Carvalho et al., 2023). Sowing can occur from April to June, and harvesting depends on the crop cycle and its thermal requirements. Brown flaxseed has a cycle of approximately 120 days, and golden flaxseed has a cycle of 130 days and can reach 150 days (Fioreze et al., 2022; Pradebon et al., 2023). However, sowing also occurs in the months of November and December in southern Brazil, where temperatures are warmer (Stanck, 2017). Flaxseed cultivation has some gaps, mainly due to the lack of adequate agricultural zoning and the small variability of genotypes available on the market. This makes sowing more difficult and limits the expansion of the crop, which continues to have little presence in cultivated areas, even where soil and climate conditions are appropriate (Peripolli et al., 2024).

Biotic and abiotic factors directly affect flaxseed productivity. Among the biotic factors, invasive plants are considered highly harmful as they compete for resources and reduce crop yield (Acharya et al., 2024). In addition, the productivity of oilseed flaxseed is impacted by fungal diseases such as rust (*Melampsora lini*), anthracnose (*Colletotrichum lini*), fusarium leaf spot (*Fusarium oxysporum* sp. *lini*), and spasm caused by the pathogen *Septoria linicola*, and negatively impact seed production and oil yield (Bruinsma et al., 2022; Yadav et al., 2022;).

The crop is also vulnerable to pests such as aphids and beetles, which affect plant development and reduce grain quality, making the use of efficient management strategies essential (CABI, 2019). Biological control appears to be a promising alternative, as beneficial microorganisms compete with pathogens and promote plant health. In addition, adequate soil management and crop rotation can help reduce the incidence of diseases and maintain flaxseed productivity in sustainable agricultural systems.

Flaxseed cultivation is a viable alternative due to the low demand for inputs, as it requires fewer fertilizers and pesticides, and the use of biological inputs can further contribute to reducing production costs (Osmari, 2024). Biological agents have been widely used in agriculture to increase productivity and improve grain quality, but there are still no concrete results on their effectiveness in flaxseed management. Studies have shown that, however, that biocontrol agents act through several mechanisms, including inducing tolerance in plants, antibiosis, competition for resources, parasitism, predation, and can also promote plant growth (Teixeira, 2015).

Despite facing challenges, flaxseed cultivation continues to expand annually, being an important alternative for crop rotation and presenting high added value for the food industry (Bruinsma et al., 2022; Kauser et al., 2023). This growth highlights the need for studies and morphological analyses of flaxseed characteristics, especially in a scenario of exponential population growth, which demands food in greater quantity and quality (Souza, 2021). Thus, the importance of research on this crop is highlighted, including its nutraceutical and pharmacological potentials, aiming not only at optimizing production, but also for the sustainability of the ecosystem. Thus, the objective of this work was to evaluate the morphological, productive and biotic characteristics of flaxseed crops based on the use of biological products based on microorganisms.

Results and discussion

Climatic influence on grain productivity

According to Furlan et al. (2022), variations in weather conditions throughout the flaxseed cycle can significantly affect yield components. Their study highlighted that sharp fluctuations between maximum and minimum temperatures have a negative impact on grain productivity, underscoring the importance of thermal stability for crop performance.

Soil structure, sowing configuration, and early plant development

As shown in Table 1, the chemical + biological treatment resulted in a higher level of soil compaction, with an average of 6.4. The spacing between rows was also higher in this treatment, reaching 18.7 cm, oscillation between rows at sowing. On the other hand, in the variables plant height and number of branches, the chemical treatment presented higher averages, with 78.8 cm and 2.6 branches, respectively. The variable volume of straw in the soil in the chemical + biological treatment presented a higher average of 2.6, however the variable soil cover showed an average of 3 for both treatments, while in the variable plantability the chemical + biological treatment was superior with an average of 10.

Table 1. Averages for the quantitative variables evaluated.

Variable	Chemical	Chemical + Biological
Compression level (0-10)	6.1	6.4
Line spacing (cm)	18	18.7
Plant height (cm)	75.6	78.8
Number of branches	2.5	2.6
Volume of straw in the soil (0-10)	2.3	2.6
Ground cover (0-10)	3	3
Plantability (0-10)	9.8	10
Quantification of invasive plants (m ²)	7.5	4.5
Quantification of insect pests (m ²)	0	0

Source: Created by the Authors.

The quantification of invasive plants variable showed a higher incidence in the chemical treatment, with an average of 7.5 plants per square meter, while the chemical + biological treatment obtained a lower average (4.5). In the quantification of insect pests, both treatments presented values equal to zero, indicating the absence of these organisms at the times of evaluation.

Reproductive development and grain yield components

In the variables analyzed in (Table 2), it was observed that the height of the insertion of the first capsule (HIFC) shows that the chemical + biological treatment had the first insertion at a height of 65 cm on average, while the chemical treatment obtained the insertion at a height of 73 cm, on average, which indicating improved performance when using combined management, since the earlier capsule production begins, the greater the filling of capsules and consequently grains throughout the plant. This can be explained by the biological

treatment having stimulated plant growth, increasing the production of plant hormones that promote the higher insertion of the first capsule, allowing for healthier growth. In studies carried out with flaxseed by Pradebon et al., (2023), the average height of insertion of the first capsule are similar and/or close to those found in this study, which guarantees the reliability of the data.

Table 2. Results of the analyses for the variables analyzed in contrast with two chemical and chemical+biological treatments, in Flaxseed CISJU21, UNIJUÍ, 2024.

Treatment	Variable	CV (%)	Maximum	Mean	Minimum
Chemical	HIFC	9.84	90.00	72.98	58.00
Chemical+Biological		11.36	85.00	65.74	49.00
Chemical	GWP	41.99	0.10	0.05	0.01
Chemical+Biological		55.20	0.11	0.04	0.01
Chemical	TGW	30.65	7.17	3.74	0.93
Chemical+Biological		35.87	5.71	3.17	1.35
Chemical	NC	48.75	3.00	14.06	3.00
Chemical+Biological		45.74	27.00	13.90	4.00
Chemical	NFC	43.80	31.00	13.89	4.00
Chemical+Biological		45.41	26.00	13.13	4.00
Chemical	NBB	33.99	4.00	2.50	1.00
Chemical+Biological		36.89	4.00	2.60	2.00
Chemical	NPB	37.10	11.00	5.70	3.00
Chemical+Biological		39.26	11.00	5.77	3.00

CV: Coefficient of variation; height of insertion of the first capsule (HIFC, cm), grain weight per plant (GWP, unit), thousand grain weight (TGW, unit), number of capsules (NC, unit), number of fertile capsules (NFC, unit), number of basal branches (NBB, units), number of productive branches (NPB, unit) Performed by the Kruskal-Wallis test at 0.05% probability. Source: Performed by the Authors.

In the variable grain weight per plant (GWP), the chemical treatment presented a higher average grain weight per plant with 0.0492 g and the chemical + biological treatment with a weight of 0.0407 grams. In the thousand grain weight (TGW), the chemical treatment demonstrated superiority, with an average of 3.7424 g. The variables number of capsules (NC) and number of fertile capsules (NFC) also presented higher values in this treatment, with averages of 14.0625 and 1.5826, respectively. Similar results were reported by Stanck (2017), showing that the absence of fungicides can increase plant stress, negatively impacting productivity. As pointed out by Bosco et al. (2021), several factors at specific stages of development can reduce growth, prolong the cycle and, consequently, compromise production.

Branch architecture, statistical significance, and trait correlations

In the behavior of the variable number of basal branches (NBB), the average was higher in the chemical + biological treatment, presenting an average of 2.6, while in the chemical treatment the average was 2.5. The variable number of productive branches (NPB), the average was higher in the chemical + biological treatment, presenting an average of 5.7692, and in the chemical treatment it presented an average of 5.7021.

Table 3 presents the results of the statistical analysis performed using the Kruskal-Wallis test, a nonparametric approach used to assess significant differences, selected to evaluate between the managements applied to the flaxseed crop. This test is recommended when the assumptions of data normality are not met, providing a robust comparison between treatments (Nasir et al., 2022).

Table 3. Kruskal-Wallis analysis to assess contrast between management for variables in the Flaxseed crop CISJU21, UNIJUÍ, 2024.

Variable	chi-squared	p-value
Number of Basal Branches (NBB)	533.49	< 2.2e-16
Height of the insertion of the first capsule (HIFC)	533.49	< 2.2e-16
Number of Productive Branches (NPB)	533.49	< 2.2e-16
Number of Capsules (NC)	533.49	< 2.2e-16
Number of Fertile Capsules (NFC)	533.49	< 2.2e-16
Grain Weight Per Plant (GWP)	533.49	< 2.2e-16
Thousand Grain Weight (TGW)	533.49	< 2.2e-16

chi-squared – Kruskal-Wallis analysis: higher values indicate greater differences between the managements. p-value – Probability of occurrence of the observed (or most extreme) result under the null hypothesis. Values less than 0.05 indicate statistically significant differences between the managements. Source: Carried out by the Authors.

The results show a chi-square (χ^2) value of 533.49 for all variables analyzed, accompanied by a p-value lower than 2.2×10^{-16} , which indicates that there are statistically significant differences between the managements for the variables number of basal branches (NBB), height of the insertion of the first capsule (HIFC), number of productive branches (NPB), number of capsules (NC), number of fertile capsules (NFC), grain weight per plant (GWP) and thousand grain weight (TGW). These differences may be associated with factors such as nutrient availability, growth conditions and possible interactions between chemical + biological treatments, impacting morphological and productive variables. Thus, the statistical analysis confirms the relevance of the managements employed in the crop, reinforcing the need for adequate strategies to maximize crop performance and grain productivity.

According to Sriram (2006), strong correlations occur when Pearson's correlation coefficient (r) varies between 0.60 and 0.90, while very strong correlations are observed for r values between 0.90 and 1.0. Pearson's linear correlation analysis applied to the variables of agronomic interest showed significant associations between different morphological and productive traits in the evaluated treatments.

In the chemical + biological treatment (Table 4), correlations were observed between the following variables: number of productive branches (NPB) and number of basal branches (NBB), number of capsules (NC) and height of insertion of the first capsule (HIFC), number of fertile capsules (NFC) and number of capsules (NC), number of fertile capsules (NFC) and height of insertion of the first capsule (HIFC), number of fertile capsules (NFC) and Number of capsules (NC), grain weight per plant (GWP) and number of fertile capsules (NFC), grain weight per plant (GWP) and number of capsules (NC).

Table 4. Pearson's linear correlation graph for chemical+biological treatment.

Variable	NBB	HIFC	NPB	NC	NFC	GWP	TGW
Number of basal branches (NBB)	-	0.09	0.70*	0.26	0.27	-0.15	-0.53
Height of insertion of the first capsule (HIFC)		-	0.24	0.64*	0.66*	0.22	-0.60
Number of poductive branches (NPB)			-	0.33	0.37	-0.08	-0.46
Number of capsules (NC)				-	0.99*	0.74*	-0.24
Number of fertile capsules (NFC)					-	0.75*	-0.26
Grain weight per plant (GWP)						-	0.43
Thousand grain weight (TGW)							-

*degree of linear association between them where values close to +1 or -1 represent strong, positive or negative correlations, respectively. Source: Created by the authors.

These correlations indicate the interaction between the variables, suggesting that one factor may influence one another's expression or impact. According to Filho and Júnior (2009), two variables are associated or correlated when their frequency distributions present similar behavior, which can vary between 1 and -1. The correlation sign indicates whether the relationship is positive or negative, while values close to zero suggest the absence of a linear association.

For the chemical treatment, correlations were identified between the following variables: height of insertion of the first capsule (HIFC) and number of basal branches (NBB), number of capsules (NC) and height of insertion of the first capsule (HIFC), number of fertile capsules (NFC) and number of capsules (NC), number of fertile capsules (NFC) and height of insertion of the first capsule (HIFC), grain weight per plant (GWP) and number of fertile capsules (NFC), grain weight per plant (GWP) and number of capsules (NC), grain weight per plant (GWP) and number of fertile capsules. The identification of these correlations reinforces the importance of the management adopted in determining the development and productivity of the crop, highlighting the interaction between the morphological and productive components (HIFC), grain weight per plant (GWP) x number of fertile capsules (NFC), grain weight per plant (GWP) x number of capsules (NC).

Table 5. Pearson's linear correlation graph for chemical treatment.

Variable	NBB	HIFC	NPB	NC	NFC	GWP	TGW
Number of basal branches (NBB)	-	0.87*	0.12	0.61	0.59	0.54	-0.39
Height of insertion of the first capsule (HIFC)		-	0.47	0.91*	0.89*	0.81	-0.69
Number of poductive branches (NPB)			-	0.69	0.72	0.61	-0.75
Number of capsules (NC)				-	1.00*	0.95*	-0.69
Number of fertile capsules (NFC)					-	0.95*	-0.7
Grain weight per plant (GWP)						-	-0.45
Thousand grain weight (TGW)							-

* degree of linear association between them where values close to +1 or -1 represent strong, positive or negative correlations, respectively. Created by the authors

Materials and methods

Plant materials

The experiment was conducted at the Regional University of Northwestern Rio Grande do Sul (UNIJUÍ), located in the municipality of Augusto Pestana, RS, Brazil (28° 26' 30" S latitude and 54° 00' 58" W longitude, altitude of 390 m). The soil of the area is classified as Typical Dystroferic Red Latosol (Santos et al, 2018), and the climate of the region, according to the Köppen classification, is humid subtropical (Alvares et al., 2014).

The brown flaxseed cultivar CISJU21 was used for the study, with a cycle of approximately 130 days, with the predecessor crop being soybean. Sowing was carried out on May 21, 2024, using a seeder-fertilizer. Based on the soil analysis for the experimental area, the results are as follows: clay content at 53%, pH at 4.8, SMP at 4.9 cmolc/dm³, phosphorus at 45.5 mg/dm³, potassium at 231 mg/dm³, organic matter at 2.6%, and aluminum at 1.0 cmolc/dm³. The pH, ranging from 1 to 14, indicates soil acidity (pH < 7) or alkalinity (pH > 7), while SMP represents Base Saturation.

Based on the soil analysis, the appropriate fertilization was defined: In the base fertilization, 120 kg ha⁻¹ of N-P-K (5-20-20) were applied, providing 6 kg ha⁻¹ of nitrogen (N), 24 kg ha⁻¹ of phosphorus (P₂O₅) and 24 kg ha⁻¹ of potassium (K₂O). Topdressing was carried out on 07/08/2024, with the application of 50 kg ha⁻¹ of Monoammonium Phosphate (MAP), 50 kg ha⁻¹ of Potassium Chloride and 100 kg ha⁻¹ of Urea.

Weather conditions

Based on the meteorological data for 2024, during the growing months starting in June, the mean air temperatures ranged from a minimum of 3°C to a maximum of 32.83°C, with significant fluctuations throughout the phenological stages of the crop. According to Stanck (2017), flaxseed thrives at an ideal growing temperature between 10°C and 18°C, requiring lower temperatures for germination, with a minimum of up to 4.8°C recommended during this phase. During its development, it needs temperatures between 18°C and 25°C, while temperatures above 30°C can hinder full development.

Regarding precipitation, the total accumulated between May and October was 781 mm, with the highest amounts recorded in June (195.12 mm) and October (192.72 mm), and the lowest in June at 64.14 mm. These precipitation levels are considered suitable for the flaxseed crop to express its genetic potential when well distributed (Pradebon et al., 2023).

Treatments

In all areas of the experiment, applications were carried out for the control of invasive plants and phytosanitary management. In the first application, on 06/27/2024, Nativo (Trifloxystrobin + Tebuconazole) were applied at a dose of 500 mL/ha, mineral oil 300 mL/ha and pH reducer 30 mL/ha. In the second application, on 07/22/2024, ALLY (Metsulfuron Methyl) was used at a dose of 6 g/ha and pH reducer 50 mL/ha. In the third application, the doses of the first application were repeated. In the fourth application, carried out on 09/02/2024, Nativo was used at a dose of 600 mL/ha.

In the experiment with application of chemical + biological inputs, in addition to the chemical products mentioned, biological inputs were applied on 08/14/2024, being Tripel (Trichoderma) at a dose of 1 L/ha, Rootland (phosphorus solubilizer) 1 L/ha, Assertivo (Metarhizium, Beauveria) 1.5 L/ha, Ct Green 200 mL/ha, Azospirillum 2 L/ha, Bacillus pumilus 2 L/ha and Bacillus megaterium 2 L/ha. All these bioinputs are commonly used in the routine of agronomic recommendations.

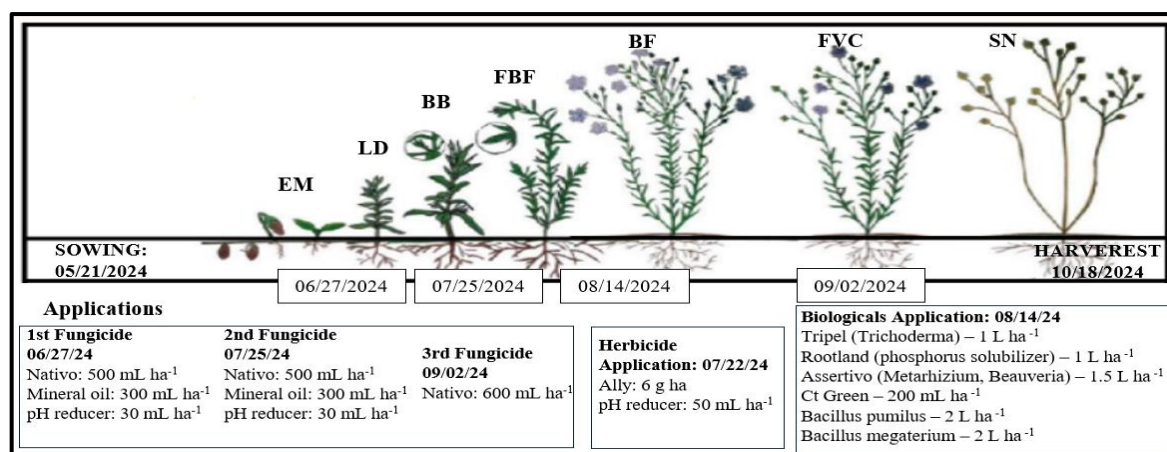


Fig. 1. Phenological stages of flaxseed and agrochemical management practices performed on the crop. Source: Produced by the authors. Scale adapted from Smith & Froment, 1998.

Conduction of study and experimental design

The study was conducted in two experiments, both using a randomized block design, with ten replications per plot. The plots were sown using a 17-row seeder, with a spacing of 0.20 m between rows, totaling a width of 3.40 m and a length of 50 meters, resulting in a total area of 170 m² per plot. For the evaluations, the central 2 meters were considered, disregarding the edges. Thus, the useful area for evaluation was 6.80 m² per evaluation grid, totaling 10 samples in the total area. However, two experiments were conducted, one with 10 grids in an area with application of combined chemical + biological inputs and another with 10 grids in an area with application of only chemical inputs.

According to the phenological scale proposed by Smith and Froment (1998), the main development stages of oilseed flax include: emergence (EM), leaf development (LD), basal branching (BB), flower bud formation (FBF), beginning of flowering (BF), emergence of the first visible capsules (FVC) and senescence (SN) (Fig. 1).

Measured variables

In the experiment, the following evaluations were performed: plant stand, soil compaction level, phenological stage, straw volume, soil cover, spacing between rows, plantability, emergence, number of tillers, plant height, invasive plants, quantification of invasive plants, diseases and insect pests. The method of performing the evaluations is described below.

Plant Stand (PS, units): Quantification was performed by counting plants in 5 linear meters, and the average was subsequently calculated, according to the methodology of Lisson and Mendham (2000).

Compaction Level (CL, levels): According to Schoenholtz, Van Miegroet and Burger (2000), the compaction level can be conventionally assessed by uprooting the plants, using a scale from 1 to 10, where 10 represents the most severe compaction and 1 the least soil compaction.

Phenological Stage (PHENS, units): Ten plants were selected per experiment, totaling 20 plants in the experiments. The phenological stages were identified according to Bosco et al. (2021), using the phenological scale of flaxseed, with the following stages: SO (sowing), EM (emergence), LD (leaf development), BB (appearance of basal branches), FB (appearance of flower bud), BF (beginning of flowering), FVC (first visible capsules) and SN (senescence).

Volume of straw in the soil (ST, levels): The quantification of the volume of straw in the soil was performed visually, following the methodology of Furlani et al. (2007). For this, an area of 1 m² was delimited at random and a scale of 1 to 10 was used, where 10 represents the largest increase in straw and 1 the absence of straw.

Soil cover (SC, levels): The soil cover assessment followed the methodology of Alvarenga et al. (2001), through visual quantification in a randomly delimited area of 1 m². The scale used varied from 1 to 10, with 10 for soil completely covered by straw and 1 for completely uncovered soil or bare soil.

Row spacing (RS, meters): Row spacing was done by measuring the plants every 2 meters in a total of 10 linear meters. In order to obtain better homogeneity, the procedure was carried out 3 times in each grid.

Plantability (PLT, unit): Plantability was weighted by evaluating the presence or absence of repeated plants, plant failure in the determined space and homogeneity of spacing between plants.

Plant Emergence (PE, unit): The assessment of plant emergence was carried out through counting, with the number of plants that emerged being counted in 5 linear meters.

Basal Branching (BB, unit): To evaluate the quantification of tillers in plants, the count was performed manually on different plants.

Plant Height (PH, centimeters): For plant height, measure the plants with a tape measure from their base to the end of their main stem.

Quantification and Identification of Invasive Plants (QIPL, square meters): To assess the quantification of invasive plants, 1 m² plots were selected and the number of invasive plants was counted.

Quantification and Identification of Insect Pests (QIPE, square meters): The methodology explained by Sturmer et al. (2014) was followed, using the beating cloth, which consists of a 1 m × 1 m white fabric or plastic fixed to wooden handles. The crop leaves were shaken over the cloth to collect and identify the insects.

Statistical analysis

The data obtained did not meet the assumption of normality of errors and homogeneity of variances, tested by Shapiro-Wilk and Bartlett, respectively. Therefore, a nonparametric analysis was performed using the Kruskal-Wallis test, at 5% probability. For variables with a significant treatment effect, position and dispersion measures were used to represent the differences between treatments. In a complementary manner, Pearson's linear correlation coefficients were obtained for the quantitative traits measured, stratified for chemical and chemical + biological treatment. All analyses were performed using R software (R Core Team, 2023).

Conclusion

The comparative analysis between treatments revealed statistically significant differences in key agronomic traits, such as basal branch number, capsule insertion height, and grain yield components. The inclusion of biological inputs led to enhanced performance in several traits, supporting the potential of integrated management for optimizing flaxseed production.

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Statement of Contributions

MES, VHBW, GAS, JVRL, and LBP were responsible for field data preparation, experimental design, and conducting evaluations. JAGS, IRC, and DAM contributed to methodological support, organization of the experimental design, and technical interpretation of the data.

CMB and JPS contributed to statistical analysis, manuscript formatting, and technical and grammatical revision.

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