The effects of different topdressing conditions on the growth, yield, and medicinal quality of *Ophiopogon japonicus* and corn in an intercropping system

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ABSTRACT

Ophiopogon japonicus is an important medicinal plant, while corn is a significant economic crop. Due to land resource issues in suitable production areas, an ecological planting model based on "Corn-*Ophiopogon japonicus*" intercropping has emerged, accounting for over 90% of the *Ophiopogon japonicus* planting area in these regions. Choosing appropriate chemical fertilizers is key to optimizing the management of the "Corn-*Ophiopogon japonicus*" intercropping system and improving the yield and quality of silage corn and medicinal *O. japonicus*. This study, based on an investigation of the fertilization practices in the "Corn-*Ophiopogon japonicus*" intercropping system, examines the effects of three factors—types of nitrogen fertilizer (urea and ammonium nitrate), micronutrients (magnesium and manganese), and topdressing (P+K)—using a completely randomized block design with a total of eight treatments. The study assesses these factors' impact on the growth, yield, and medicinal quality of corn and *Ophiopogon japonicus*. The results indicate that for corn, nitrogen fertilizer type significantly affects plant height, with corn treated with ammonium nitrate exhibiting superior height. In terms of fresh biomass, both nitrogen fertilizer type and topdressing significantly influence the fresh biomass of corn leaves, stems, husked corn, and silage corn yield, while micronutrients primarily affect the fresh biomass of corn stems. Cluster analysis and PCA results align, showing that treatments T5 (Urea+P+K), T7 (Urea+Mn+Mg+P+K), and T8 (Ammonium nitrate+Mn+Mg+P+K) perform best. For *Ophiopogon japonicus*, only micronutrient fertilizers significantly affect the root tubers, while topdressing impacts plant height and nutritive roots. All three factors—types of nitrogen fertilizers, micronutrient fertilizers, and topdressing—significantly influence the fresh biomass of root tubers, thereby affecting the yield of medicinal *Ophiopogon japonicus*. Again, cluster analysis and PCA confirm that treatment T8 is the most

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The entire manuscript is long. Rephrase the manuscript. Do not use T1, T2, ... because it makes it difficult to understand. Since the study involved three factors, it should be written in a way that makes it easier to understand.¶
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effective, followed by T5. Regarding the quality of medicinal *Ophiopogon japonicus*, the combination of cluster analysis, correlation analysis, and PCA indicates that treatment T5 yields the highest quality. These results suggest that the optimal fertilization combination to significantly enhance the yield of silage corn and medicinal *Ophiopogon japonicus* is “150 kg N/ha of ammonium nitrate, 45 kg/ha of magnesium sulfate ($MgSO_4·7H_2O$), 15 kg/ha of manganese sulfate ($MnSO_4·H_2O$), 75 kg P_2O_5/ha of superphosphate ($P_2O_5 \geq 12\%$), and 450 kg K_2O /ha of potassium sulfate ($K_2O \geq 52\%$). For improving the quality of medicinal *Ophiopogon japonicus*, it is recommended to prioritize the nutrient supplementation scheme of “150 kg N/ha of urea, 75 kg P_2O_5/ha of superphosphate ($P_2O_5 \geq 12\%$), and 450 kg K_2O /ha of potassium sulfate ($K_2O \geq 52\%$). This scheme not only ensures the yields of silage corn and medicinal *Ophiopogon japonicus* but also significantly enhances the quality of the medicinal product.

Keywords: *Ophiopogon japonicus*; Corn; Intercropping system; Topdressing; Medicinal quality

INTRODUCTION

With the rising global health awareness and shifts in medical paradigms, the efficacy and safety of natural medicines are increasingly recognized in the global market. Traditional Chinese medicine, as a significant component of natural medicine, has also gained widespread acknowledgment for its effectiveness and safety. Modern research has identified the main chemical constituents of *Ophiopogon japonicus*, including steroid saponins(Sun et al. 2013; Wang et al. 2011), high isoflavonoids (Lin et al. 2014; Zhou et al. 2013), and polysaccharides (Fang et al. 2018; Xiong et al. 2011), which exhibit various pharmacological effects such as lowering blood sugar (Ding et al. 2012; Wang 2013), protecting the cardiovascular system (Lan et al. 2013; Zheng et al. 2009), enhancing immunity (li Xiong et al. 2011), anti-aging (li Xiong et al. 2011), anti-inflammatory (Song et al. 2010), and anti-tumor activities (Chen et al. 2013; Zhang et al. 2012). Due to its efficacy and safety (Jin et al. 2024), *Ophiopogon japonicus* and its products are widely used in at least 25 countries, including China, Japan, Germany, Vietnam, India, the United States, Malaysia, and South Korea. According to customs data, China exported over 6,000 tons of *Ophiopogon japonicus* from 2018 to 2022. Santai County in Sichuan Province is a major production area for *Ophiopogon japonicus* with a long cultivation history, yielding approximately 15,000 tons annually, which accounts for over 90% of the domestic market. The development of regional planting and specialized production has made *Ophiopogon japonicus* a leading industry in local rural areas and a significant source of income for farmers. However, the suitable land for *Ophiopogon japonicus* cultivation is limited, compounded by policies aimed at preventing the "non-food" use of arable land (Dong et al. 2024; Li et al. 2024a; Su et al. 2024), leading to competition for space between medicinal and food crops. Additionally, *Ophiopogon japonicus* cultivation faces challenges such as soil pH imbalance, pollution, and biodiversity loss (Bertness & Callaway 1994; Moonen & Bàrberi 2008). To address these issues, a site-specific approach combining traditional agriculture with modern technology has led to the development of a "Corn-*Ophiopogon japonicus* " ecological planting model. This ecological approach plays a crucial role in enhancing biodiversity within agricultural systems and significantly improves land utilization.

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It increases the economic benefits for farmers in the production area (Li et al. 2024b), with this method now accounting for over 90% of *Ophiopogon japonicus* cultivation.

This ecological planting model influences the growth and yield of intercropped species through spatial and temporal arrangements (Yang et al. 2015). However, due to differences in crop structure, nutrient promotion and competition may arise (Davis & Liebman 2001; Ghosh et al. 2009). Soil nutrients are essential for crop growth, yet improper management and fertilization practices can lead to low nutrient use efficiency and significant nutrient loss, threatening production safety and wasting agricultural resources while causing environmental pollution. Current research on fertilization for *Ophiopogon japonicus* primarily focuses on monoculture (Deng et al. 2021; Lei et al. 2019), with little guidance on nutrient supplementation in the " Corn-*Ophiopogon japonicus* " intercropping system. As a result, subsequent fertilization lacks direction, and the types and ratios of fertilizers applied are often arbitrary. Given that corn and *Ophiopogon japonicus* are complementary crops, their spatial distribution can enhance light interception, resulting in higher light utilization and yield advantages (Awal et al. 2006; Gao et al. 2010).

Therefore, developing a rational nutrient supplementation strategy for the " Corn-*Ophiopogon japonicus* " intercropping system is crucial. Preliminary studies indicate that both crops have significant nitrogen demands, with corn exhibiting a competitive advantage in nitrogen uptake, which further impacts *Ophiopogon japonicus*'s nitrogen utilization efficiency (Li et al. 2001). Current fertilization practices primarily target *Ophiopogon japonicus* and do not adequately meet the nutrient needs of both crops, especially nitrogen. Urea and ammonium nitrate are the main nitrogen sources in the region, and their different forms affect plant physiological functions and root development. Identifying the most suitable nitrogen source for this intercropping system warrants further investigation. Additionally, the inclusion of micronutrients like magnesium and manganese is essential, as their supplementation can alleviate nutrient competition and ensure the quality of the medicinal material (Aulakh & Malhi 2005).

Currently, research on nutrient supplementation in the " Corn-*Ophiopogon japonicus* " intercropping system is scarce. This study aims to investigate the effects of different nitrogen fertilizers, the application of phosphorus and potassium, and the supplementation of micronutrients on the growth of corn and *Ophiopogon japonicus* as well as the quality of *Ophiopogon japonicus*. The findings will provide a foundation for nutrient management and achieving high, stable, and quality yields in *Ophiopogon japonicus* production.

MATERIAL & METHODS

Experimental design

This study was conducted at the *Ophiopogon japonicus* Research Demonstration Park in Santai County, Sichuan Province (Longitude 104°57′44″, Latitude 31°24′35″). The area has a subtropical monsoon climate with humid conditions and sandy loam soil. Key soil characteristics include a pH of 7.05, Organic matter at 10.3 g/kg, Ammo-nium nitrogen at 28.2 mg/kg, Available phosphorus at 81.2 mg/kg, and Available po-tassium at 124.8 mg/kg.

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Uniform basal fertilizer management was implemented across all treatments, uti-lizing 1.5 t/hm² of commercial organic fertilizer ($N+P_2O_5+K_2O \geq 5\%$, organic matter $\geq 45\%$, produced by Mianyang Keya Agricultural Development Co., Ltd.) and 600 kg/hm² of compound fertilizer (N: P_2O_5: K_2O = 17: 17: 17, total nutrients $\geq 51\%$, pro-duced by Guizhou Xiyang Industry Co., Ltd.).

The topdressing experiment employed a complete randomized block design with three factors and two levels, resulting in eight treatments, each replicated three times across 24 plots, each measuring 24 m². The first factor examined types of nitrogen fer-tilizers, comparing Urea ($N \geq 46\%$) and Ammonium nitrate ($N \geq 34.8\%$), applied at 150 kg N/hm². The second factor focused on micronutrient fertilizers, specifically magne-sium and manganese, with treatments for magnesium sulfate ($MgSO_4 \cdot 7H_2O$) applied at 45 kg/hm² and manganese sulfate ($MnSO_4 \cdot H_2O$) at 15 kg/hm², both as foliar applica-tions. The third factor evaluated topdressing with phosphorus and potassium fertiliz-ers, applying Calcium superphosphate ($P_2O_5 \geq 12\%$) at 75 kg P_2O_5/hm² and Potassium sulfate ($K_2O \geq 52\%$) at 450 kg K_2O/hm² (Table 1).

Corn was planted at a density of 45,000 plants/hm², with ten rows of *Ophiopogon japonicus* interspersed between two rows of corn, resulting in a planting density of 1.5 million plants/hm². The *Ophiopogon japonicus* variety used was "Chuanmaidong No. 1", the local cultivar, while the corn variety was "Chengdan 99", intended for silage production. Both *Ophiopogon japonicus* and corn were sown on April 22, 2022, with management practices following the standard (2020). The corn was harvested on July 26, 2022, and the *Ophiopogon japonicus* on March 25, 2023.

Methods for determination of agronomic indicators

The height of *Ophiopogon japonicus* and corn was measured using a measuring tape, while the stem circumference of corn was measured with a flexible tape. The number of corn leaves was recorded, excluding those with over 50% wilting. The quantities of storage roots, nutritive roots, and root tubers of Ophiopogon japonicus were noted. Fresh weights of various parts—corn (including corn with husks, leaves, and stems) and *Ophiopogon japonicus* (leaves, rhizomes, storage roots, nutritive roots, and root tubers)—were measured using a 0.01 g electronic balance. The fresh weight of the root tuber represents the yield of *Ophiopogon japonicus* (Figure 2). The medicinal material of Ophiopogon japonicus was graded according to standards (2023).

Methods for Determining Quality Indicators of *Ophiopogon japonicus* Medicinal Material

The determination of moisture, extractives, and total saponins for *Ophiopogon japonicus* was conducted according to the methods outlined in the 2020 edition of the Pharmacopoeia of China (Commission 2020). The total flavonoid content in the medicinal material was measured using ultraviolet spectrophotometry, with hesperidin as the reference standard (Lin 2014). The total polysaccharide content was determined using the sulfuric acid-anthrone colorimetric method (Wang et al. 2016).

Standards for Ophiopogon saponin D (reference substance number Wkg22061609), Methylophiopogonanone A (reference substance number Wkq24021807), and Methylophiopogonanone B (reference substance number Wkq23072006) were purchased from Sichuan Weikegi Biological Technology Co., Ltd. (Chengdu, China). High-performance liquid

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chromatography (HPLC)-grade acetonitrile was obtained from Sichuan Cologne Chemical Co., Ltd. (Chengdu, China). HPLC was employed to determine the content of Ophiopogon saponin D, Methylophiopogonanone A, and Methylophiopogonanone B in the samples.

For the extraction of Ophiopogon saponin D, 3 g of *Ophiopogon japonicus* powdered material was placed in a round-bottom flask with 50 mL of methanol. The mixture was refluxed for 2 hours, filtered, and the filtrate was concentrated to dryness. Afterward, 10 mL of water was added to dissolve the residue. The solution was then extracted with water-saturated n-butanol, shaking five times with 12 mL each time. The n-butanol extracts were combined and washed twice with 5 mL of ammonium hydroxide solution. The ammonium solution was discarded, and the n-butanol solution was evaporated to dryness. Finally, the residue was dissolved in 80% methanol, transferred to a 5 mL volumetric flask, and filtered through a 0.45 μm membrane filter (Wu et al. 2015).

The chromatographic conditions for Ophiopogon saponin D included using a C18-bonded silica gel column (250 mm length, 46 mm inner diameter, 5 μm particle size). The mobile phase consisted of water (A) and acetonitrile (B) with a gradient elution as follows: 0–5 min, 38%–55% acetonitrile; 5–15 min, 55%–70% acetonitrile; 15–17 min, 38%–55% acetonitrile; 17–20 min, 70%–38% acetonitrile. The flow rate was 1.0 mL/min, the column temperature was set at 25°C, the drift tube temperature at 105°C, and the gas flow rate at 3.0 L/min. The retention time for Ophiopogon saponin D was 12.3 minutes.

For the extraction of Methylophiopogonanone A and Methylophiopogonanone B, 3.0 g of *Ophiopogon japonicus* root tuber powder was placed in a conical flask with 25 mL of water and allowed to stand for 24 hours. The mixture was then ultrasonicated (500 W, 40 kHz) for 60 minutes, shaken well, and filtered to obtain the filtrate (Wu et al. 2016).

The chromatographic conditions for Methylophiopogonanone A and Methylophiopogonanone B also utilized a C18-bonded silica gel column (250 mm length, 46 mm inner diameter, 5 μm particle size). The mobile phase consisted of water (A) and acetonitrile (B) at 60% acetonitrile, employing isoelution. Detection was carried out at 296 nm, with a flow rate of 1.0 mL/min and a column temperature of 25°C. The retention times for Methylophiopogonanone A and Methylophiopogonanone B were 13.5 minutes and 15.3 minutes, respectively.

Statistical Analysis

All data were processed using Excel. Stacked bar charts and radar charts were created using Origin 2024b. Additionally, principal component analysis (PCA) of the quality of *Ophiopogon japonicus* was conducted using Origin 2024b, producing a 3D PCA plot. Prior to calculating scores for different treatments, the quality data were standardized. Bar charts and stacked bar charts were created using GraphPad Prism 8.

Data standardization for growth indicators of corn and *Ophiopogon japonicus* was performed using the '*Stats*' package in R. Clustering was conducted with the '*Pheatmap*' package, which generated a circular clustering heatmap using complete linkage and Euclidean distance. A 2D PCA analysis was created using stats, while inter-group correlation analysis employed the Pearson method, utilizing the '*psych*' package. Correlation plots were visualized using the '*ggplot2*' package, along with additional visualizations created through the online platform www.chiplot.online (accessed September 27, 2024).

One-way and multi-factor analysis of variance (ANOVA) were performed using SPSS 26.0, with multiple comparisons conducted using Duncan's new multiple range test.

RESULTS

Effects of different topdressing conditions on corn growth and yield

Under different topdressing treatments, the height of corn reached between 288.76 and 306.58 cm per plant. Treatments T2 and T4 were significantly taller than T5, with increases of 6.17% and 4.91%, respectively. The type of nitrogen fertilizer had a significant effect on corn height, with corn treated with ammonium nitrate showing superior growth.

The stem circumference varied between 7.96 and 9.20 cm per plant across the treatments, with treatments T1 and T4 significantly outperforming T2 by 15.64% and 14.25%, respectively. All three factors demonstrated a significant interactive effect on corn stem circumference. The number of leaves per plant ranged from 12.67 to 13.56, with T4 significantly exceeding T8 by 7.02%. Micronutrient fertilizers and topdressing showed a significant interactive effect on the number of corn leaves.

Under different topdressing treatments, the fresh biomass of corn leaves reached between 28.13 and 39.38 t/hm². Treatment T5 was significantly higher than the other treatments, surpassing them by 34.62% (T1), 40.00% (T2), 16.67% (T3), 25.00% (T4), 29.63% (T6), 12.90% (T7), and 12.90% (T8). Both the type of nitrogen fertilizer and the topdressing had significant effects on the fresh biomass of corn leaves, with a notable interaction among the three factors (N×M×T).

The fresh biomass of corn stems ranged from 31.50 to 37.13 t/hm², with treatments T4, T5, and T8 significantly higher than T3 by 17.86%, 17.86%, and 14.29%, respectively. There was a significant interaction between nitrogen and micronutrient treatments (N×M) regarding the fresh biomass of corn stems.

For corn with husks, the fresh biomass ranged from 22.50 to 27.00 t/hm², with treatments T3, T5, and T8 significantly exceeding T1 and T2. Both nitrogen and topdressing had significant effects on the fresh biomass of corn with husks, and these two factors exhibited a notable interaction.

Overall, the fresh yield of silage corn varied from 84.38 to 103.50 t/hm², with T5 significantly outperforming the other treatments (except for T7 and T8). T5 exceeded the other treatments by 16.46% (T1), 22.67% (T2), 12.20% (T3), 12.20% (T4), and 15.00% (T6). Both nitrogen and topdressing had significant effects on the fresh yield of silage corn, with a significant interaction between nitrogen and micronutrient treatments (N×M).

According to the circular clustering diagram of corn, the variables corn plant height, corn stem girth, and number of corn leaves formed one cluster, while the fresh biomass of corn and corn yield from different parts grouped together in another cluster. The results of the hierarchical clustering analysis indicate that the eight treatments can be divided into three categories. The first

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category consists solely of T5, where the fresh biomass of corn parts and corn silage yield performed the best, while corn plant height, stem girth, and number of leaves showed the worst results. The second category includes T7 and T8, where the fresh biomass and corn silage yield were inferior compared to the first category, but corn plant height and stem girth exhibited improvements relative to the first cluster. The remaining treatments, T1, T2, T3, T4, and T6, fall into the third category, which overall showed relatively high values for corn plant height, stem girth, and number of leaves, but the lowest performance for the fresh biomass of corn parts and corn silage yield.

PCA analysis was conducted using corn agronomic traits, biomass from different parts, and corn silage yield. The first principal component (PC1) accounted for 45.62% of the variance, while the second principal component (PC2) accounted for 18.62%, resulting in a cumulative variance contribution of 64.24% for the two principal components. The P-value of 0.2230 indicates that the differences between groups are not significant.

In the analysis, T5 was categorized as a distinct group, while T7 and T8 were grouped together. The remaining treatments, T1, T2, T3, T4, and T6, formed another cluster. These findings are consistent with the results of the circular clustering analysis.

Effects of different topdressing conditions on the growth and quality of *Ophiopogon japonicus*

Effects of different topdressing conditions on the growth of Ophiopogon japonicus

Under different topdressing treatments, the number of root tubers for *Ophiopogon japonicus* ranged from 12.7 to 16.4, with T8 significantly higher than T1 by 29.65%. The micronutrient fertilizers (M) had a significant impact on the number of root tubers. The plant height of *Ophiopogon japonicus* reached 22.9 to 26.7 cm/plant, with T1 and T4 significantly taller than T5, T7, and T8. The number of nutritive roots varied from 6.6 to 9.8, where T2 and T4 were significantly higher than T7 by 38.24% and 48.45%, respectively, with no significant differences among the other treatments. The topdressing (T) significantly influenced both plant height and the number of nutritive roots. The storage root count ranged from 16.8 to 19.8, with no significant differences observed between treatments.

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Under different topdressing treatments, the fresh biomass of root tubers ranged from 11.00 to 17.91 t/hm², with T4 to T8 significantly higher than T1 to T3, achieving increases of 48.86% to 62.75% compared to T1, and 18.45% to 29.50% compared to T2 and T3. The types of nitrogen fertilizers, micronutrient fertilizers, and topdressing all had significant effects on the fresh biomass of root tubers, with notable interactions between N and T.

The fresh biomass of *Ophiopogon japonicus* leaves ranged from 19.50 to 23.45 t/hm², while the fresh biomass of rhizomes varied from 2.22 to 3.21 t/hm². For nutritive roots, it ranged from 2.88 to 4.69 t/hm², and storage roots ranged from 6.63 to 9.27 t/hm². Among these, the N, M, and T showed significant interaction effects only on the fresh biomass of storage roots, with no significant impact on the other indicators.

According to the circular clustering analysis of *Ophiopogon japonicus*, the fresh biomass of root tubers, OJ storage roots, and OJ root tubers were grouped together, while OJ plant height and fresh biomass of OJ leaves formed another group. A third group included OJ nutritive roots, fresh biomass of OJ nutritive roots, fresh biomass of OJ rhizomes, and fresh biomass of OJ storage roots.

The results indicate that the eight treatments can be categorized into three main groups. The first group consists of T8, which performed optimally in terms of root quantity and biomass but showed poorer results in OJ plant height and fresh biomass of OJ leaves. The second group includes T5, T6, and T7, which excelled primarily in fresh biomass of root tubers but were less effective in other measures. The remaining treatments, T1, T2, T3, and T4, form the third group, which exhibited overall poorer performance in fresh biomass of root tubers, OJ storage roots, and OJ root tubers, while showing better results in OJ plant height, fresh biomass of OJ leaves, OJ nutritive roots, fresh biomass of OJ nutritive roots, fresh biomass of OJ rhizomes, and fresh biomass of OJ storage roots.

In the PCA analysis based on *Ophiopogon japonicus* plant height, root quantity, and biomass of various parts, PC1 accounted for 34.91% and PC2 accounted for 24.10%, resulting in a cumulative variance contribution rate of 59.01%. The p-value was 0.2670, indicating no significant differences between the groups. Treatment T8 was categorized as a separate group, while treatments T5, T6, and T7 formed another group, and treatments T1, T2, T3, and T4 were grouped together. This classification aligns with the results of the circular clustering analysis.

Effects of different topdressing conditions on the quality of *Ophiopogon japonicus*

Under different topdressing treatments, the extract reached 82.78% to 85.03%, with T2 significantly higher than other treatments (except T3) by 1.31% to 2.72%. Nitrogen type (N) and topdressing (T) had significant effects on the extract, and there was a significant interaction between N and micronutrient fertilizers (M). Total polysaccharide content ranged from 61.52% to 67.54%, with T1 and T3 significantly higher than T2, T7, and T8 by 4.37% to 9.79%. Micronutrient fertilizers (M) and topdressing (T) had significant effects on total polysaccharides, and there were significant interactions among N, M, and T. Total saponin content varied from 0.15% to 0.23%, with T4, T7, and T8 significantly higher than other treatments by 30.13% to 56.72%. All factors (N, M, T) significantly affected total saponin, with significant interactions among all factors except for the interaction between M and T.

Total flavonoid content ranged from 0.16% to 0.19%, with T7 significantly higher than other treatments by 5.89% (T1), 3.93% (T2), 22.12% (T3), 14.60% (T4), 3.47% (T5), 8.24% (T6), and 9.70% (T8). N, M, and T significantly influenced total flavonoid content, with significant interactions among all factors except for the interaction between N and M. Ophiopogon saponin D levels reached 0.19 to 0.48 mg/g, with T4 and T7 significantly higher than other treatments, where T4 was 36.59% to 150.42% higher and T7 was 27.52% to 133.79% higher than others. All factors (N, M, T) significantly affected Ophiopogon saponin D, and significant interactions were observed among all factors. Methylophiopogonanone A ranged from 52.69 to 103.88 μg/g, with T5 significantly higher than other treatments (except T7) by 63.29% (T1), 21.48% (T2), 97.17% (T3), 38.86% (T4), 17.69% (T6), and 50.25% (T8). Micronutrient fertilizers (M) and topdressing (T) significantly affected Methylophiopogonanone A, with a significant interaction between N and T. Methylophiopogonanone B varied from 32.37 to 65.51 μg/g, with T6 significantly higher than other treatments (except T5) by 56.55% (T1), 29.29% (T2), 102.39% (T3), 44.79% (T4), 14.23% (T7), and 59.27% (T8). Both M and T significantly influenced Methylophiopogonanone B, with significant interactions among all factors except for the interaction between N and M.

Based on the circular clustering heatmap of Ophiopogon, Extract and Total polysaccharide were grouped together, while Total saponin and Ophiopogon saponin D formed another group. Total flavonoid, Methylophiopogonanone A, and Methylophiopogonanone B were clustered as a third group. The hierarchical clustering analysis indicated that the eight treatments could be broadly categorized into three groups. The first group included T4 and T8, which exhibited the best overall performance in Total saponin and Ophiopogon saponin D but performed poorly in Total flavonoid, Methylophiopogonanone A, and Methylophiopogonanone B. The second group comprised T5, T6, and T7, which showed superior results in Total flavonoid, Methylophiopogonanone A, and Methylophiopogonanone B, but had lower performance in other aspects. The remaining treatments formed the third group, including T1, T2, and T3, which performed well in Extract and Total polysaccharide but were less effective in the other five quality indicators.

In the correlation analysis, Total saponin and Ophiopogon saponin D showed a highly significant positive correlation with *Ophiopogon japonicus* (OJ) yield, while Methylophiopogonanone A demonstrated a significant positive correlation with OJ yield. Extract was significantly positively correlated with both OJ nutritive root and fresh biomass of OJ rhizome. Conversely, Extract and Total polysaccharide exhibited a significant negative correlation with OJ yield. Additionally, Total flavonoid and Methylophiopogonanone A showed a significant negative correlation with OJ plant height, and Total flavonoid was significantly negatively correlated with OJ nutritive root.

Under different topdressing treatments, the first-grade *Ophiopogon japonicus* yield reached 33.85% to 40.77%, with T3 significantly outperforming T1, T2, T4, and T5 by 7.64% (T1), 8.39% (T2), 20.43% (T4), and 13.56% (T5), while showing no significant correlation with T6, T7, and T8. The second-grade yield varied between 47.39% and 54.08%, with T4 and T5 significantly higher than the other treatments, where T4 exceeded other groups by 4.27% to 12.42%, and T5 by 5.85% to 14.13%. The third-grade yield ranged from 10.02% to 14.55%, with T1 significantly higher than all other groups by 28.83% (T2), 35.75% (T3), 13.04% (T4), 45.28% (T5), 31.57% (T6), 32.30% (T7), and 15.26% (T8).

Principal component analysis (PCA) was performed on the quality indicators and medicinal properties of *Ophiopogon japonicus* root tuber.

The first principal component (PC1) accounted for 47.00%, the second (PC2) for 29.53%, and the third (PC3) for 11.73% of the variance. Together, these three principal components explained 88.26% of the variance, representing most of the information content in the quality and medicinal indicators of *Ophiopogon japonicus* root tuber. Therefore, these three principal components can be used to evaluate the quality and medicinal properties of *Ophiopogon japonicus* root tuber from the 8 treatment groups.

PC1 primarily integrates the information from extract, total polysaccharide, total saponin, total flavonoid, Ophiopogon saponin D, Methylophiopogonanone A and Methylophiopogonanone B. These indicators show a positive distribution on the first principal component, meaning that the higher the PC1 value, the higher the values of these indicators. PC2 mainly reflects the total saponin.

Based on the principal component analysis model, the eigenvectors for each indicator were calculated and used as weights to derive the scoring formulas for the three principal components (H_1, H_2, H_3). SPSS software was used to standardize the quality data of the *Ophiopogon japonicus* root tuber and, in putting it into these scoring formulas, we obtained the scores of *Ophiopogon japonicus* root tuber from different treatment groups on seven principal components. The weights for these principal components were based on their respective variance contribution rates, and the comprehensive quality score (H_0) of the *Ophiopogon japonicus* root tuber was calculated accordingly.

$$H_1 = -0.417Z_1 - 0.307Z_2 + 0.266Z_3 + 0.406Z_4 + 0.293Z_5 + 0.480Z_6 + 0.425Z_7$$

$$H_2 = 0.150Z_1 + 0.145Z_2 - 0.598Z_3 + 0.355Z_4 - 0.529Z_5 + 0.274Z_6 + 0.343Z_7$$

$$H_3 = -0.129Z_1 + 0.865Z_2 - 0.001Z_3 - 0.173Z_4 + 0.335Z_5 + 0.147Z_6 + 0.267Z_7$$

$$H_0 = 0.533H_1 + 0.335H_2 + 0.133H_3$$

The comprehensive scores for each treatment group are ranked as follows: T5 > T7 > T6 > T2 > T1 > T8 > T4 > T3. Among the 8 treatment groups, the *Ophiopogon japonicus* root tuber from T5 treatment group has the highest quality. The *Ophiopogon japonicus* root tuber from the treatment groups of T1, T8, T4, and T3 has relatively poor quality.

DISCUSSION

In the future, sustainable development of traditional Chinese medicine (TCM) agriculture will primarily rely on ecological planting, with intercropping emerging as a key production method within this framework. Generally, intercropping optimizes the existing natural resource environment (Kheroar & Patra 2013; Latati et al. 2013; Nasar et al. 2020), including land, water, nutrients, light, and labor distribution. This method not only maximizes land use but also enhances crop yields. Fertilizers, essential for crop growth, play an irreplaceable role in ensuring food security. Nitrogen, phosphorus, and potassium, as major nutrient elements, are crucial for the normal growth and development of crops, making fertilizers an important material input for farmers during production. However, in a mixed cropping system, crops may compete for nutrients, hindering their ability to effectively utilize available nutrients (Nasar et al. 2021; Nyawade et al. 2021). *Ophiopogon japonicus* has distinct growth stages: seedling, storage root development, and storage root swelling. During the intercropping period with corn, *Ophiopogon japonicus* mainly focuses on leaf and nutritional root growth, with biomass accumulation primarily in these areas. Meanwhile, corn also enters a vigorous growth phase during this period, leading to high nutrient demand for both crops, particularly for nitrogen. Research indicates that both corn and *Ophiopogon japonicus* have significant nitrogen requirements, with corn, as a grass species, holding a competitive advantage for nitrogen. This dynamic further influences the nitrogen utilization efficiency of both crops (Li et al. 2001). Nitrogen is a key component for chlorophyll

Comentado [U32]: First present the intercropping and fertilization, in order to discuss the observed results. Otherwise, the discussion resembles a review.

content, enzyme levels, and enzyme activity in plants (Evans 1983; Pan et al. 2021).Supplementing with nitrogen can effectively mitigate the negative impacts of nutrient competition in the “Corn-*Ophiopogon japonicus”* intercropping system. Our study shows that the addition of nitrogen and other macronutrients significantly enhances the growth of both corn and *Ophiopogon japonicus*, leading to increased yields of silage corn and medicinal *Ophiopogon japonicus*, while also ensuring the quality of the medicinal product. Previous studies on corn-alfalfa and corn-soybean intercropping support these findings (Nasar et al. 2020; Shao et al. 2020). Results further indicate that the type of nitrogen fertilizer significantly affects corn plant height, particularly when ammonium nitrate is used, as it consists mainly of nitrate nitrogen, which dissolves in water for direct absorption by crops, requiring no conversion process. In contrast, the nitrogen in urea must be converted from ammonium to nitrate before plants can absorb it (Song et al. 2023; Waterhouse et al. 2017).

N, P, and K are essential elements for plant growth, and their application rates directly impact crop yield and quality. However, different intercropped plants exhibit variations in their nutrient demands and absorption patterns (Nasar et al. 2020; Zaeem et al. 2019). Relying solely on large quantities of chemical fertilizers can hinder yield and quality improvements, leading to nutrient overload in soils, reduced profitability, increased salinization, and water resource pollution (He et al. 2022; Shi et al. 2023). Micronutrients play a crucial role in plant growth; while they do not directly influence crop growth like macronutrients, they act as catalysts and participants in various physiological and biochemical processes within the plant. Magnesium (Mg) is a core element for chlorophyll formation and a cofactor for several carbon-fixation-related enzymes, and it is also vital for protein synthesis (Maathuis 2009). Furthermore, the nutritional status of magnesium fertilizer affects the transport of photosynthetic products from leaves to the growing roots, impacting the root system’s ability to absorb nutrients (Chen et al. 2017; Kiss et al. 2004). Specifically, magnesium can reduce manganese concentration in plants, not only due to biomass dilution effects but also by influencing root absorption capacity (Jezek et al. 2015; Kleiber et al. 2012). Our research shows that the application of magnesium and manganese fertilizers during “Corn-*Ophiopogon japonicus”* production significantly increases yields of silage corn and *Ophiopogon japonicus*, particularly through an increase in the number of storage roots, though this yield increase may lead to a decline in quality (Fan et al. 2023). Therefore, scientific fertilization should not only focus on supplementing nitrogen, phosphorus, and potassium but also consider the reasonable addition of essential micronutrients to improve both yield and medicinal quality. Improper fertilization can adversely affect the normal growth of medicinal materials, reducing both yield and active ingredient content. However, the photonic utilization efficiency of N, P, K, Mg, and Mn in the corn-*Ophiopogon japonicus* intercropping system warrants further investigation.

Different types of nitrogen fertilizers and topdressing significantly impact extracts, total polysaccharides, total saponins, total flavonoids, and other active components in *Ophiopogon japonicus*. The interactions among various factors affect these components differently, aligning with previous findings on fertilization in monocultures of *Ophiopogon japonicus*, which identified optimal organic and inorganic fertilizer combinations based on high activity and low heavy metal content. This may be attributed to the application of nitrogen and potassium fertilizers, which enhance the activity of carbon assimilation metabolic enzymes, promoting carbohydrate formation and thereby facilitating the synthesis of terpenes using non-structural carbohydrates as substratesDifferent types of nitrogen fertilizers and topdressing significantly impact extracts, total polysaccharides, total saponins, total flavonoids, and other active components in *Ophiopogon japonicus*. The interactions among various factors affect these components differently, aligning with previous findings on fertilization in monocultures of *Ophiopogon japonicus*, which identified optimal organic and inorganic fertilizer combinations based on high activity and low heavy metal content. This may be attributed to the application of nitrogen and potassium fertilizers, which enhance the activity of carbon assimilation metabolic enzymes, promoting carbohydrate formation and thereby facilitating the synthesis of terpenes using non-structural carbohydrates as substrates (Brooks & Szeto 2024; Waring et al. 2023; Xia et al. 2023).

Comentado [U33]: ????

In summary, for the production model of *Ophiopogon japonicus* in its growing regions, adopting a "Corn-*Ophiopogon japonicus*" intercropping model not only improves land utilization but also effectively addresses the issue of "competition for land" between medicinal and food crops. Given the nutrient competition between these two crops in the "Corn-*Ophiopogon japonicus*" intercropping model, nutrient supplementation during production should be tailored to the yield and quality requirements of both crops, considering not only the quantities and forms of nitrogen, phosphorus, and potassium but also the addition of micronutrients such as magnesium and manganese. Our findings indicate that an optimal fertilization scheme can: 1. Improve agronomic traits like corn plant height and stem thickness, promoting increased yields of silage corn; 2. Enhance the number of storage roots in *Ophiopogon japonicus,* boosting its medicinal yield; 3. Maintain or improve the quality of the medicinal *Ophiopogon japonicus* while ensuring the yields of both crops. Thus, under the best fertilization schemes identified in this study, "corn-*Ophiopogon japonicus*" intercropping can enhance the growth of both crops, increasing the yields of silage corn and medicinal *Ophiopogon japonicus* while ensuring the quality of the medicinal product.

Comentado [U34]: n the Discussion item, the authors basically presented a general review, lacking a discussion of the results obtained.

CONCLUSIONS

In the production areas of *Ophiopogon japonicus*, adopting a "Corn-*Ophiopogon japonicus*" intercropping model can significantly improve land utilization and effectively address the issue of competition for land between medicinal and food crops resulting from large-scale cultivation of *Ophiopogon japonicus*. The findings of this study provide new insights into nutrient supplementation for the "Corn-*Ophiopogon japonicus*" intercropping model.

Comentado [U35]: I suggest an evaluation of the intercropping's efficiency.

For the yield of silage corn and *Ophiopogon japonicus*, it is recommended to prioritize the nutrient supplementation scheme that includes "150 kg N/hm² of ammonium nitrate, 45 kg/ha of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) + 15 kg/ha of manganese sulfate ($MnSO_4 \cdot H_2O$), 75 kg P_2O_5/ha of superphosphate ($P_2O_5 \geq 12\%$), and 450 kg K_2O/ha of potassium sulfate ($K_2O \geq 52\%$)." This scheme can significantly enhance the yields of silage corn and medicinal *Ophiopogon japonicus*. Regarding the quality of medicinal *Ophiopogon japonicus*, it is advisable to prioritize the nutrient supplementation scheme that includes “150 kg N/hm² of urea, 75 kg P_2O_5/ha of superphosphate ($P_2O_5 \geq 12\%$), and 450 kg K_2O/ha of potassium sulfate ($K_2O \geq 52\%$).” This scheme not only ensures the yields of silage corn and medicinal *Ophiopogon japonicus* but also significantly improves the quality of the medicinal product.

Comentado [U36]: This text is best placed at the end of the discussion or results and what is at the end of the discussion is probably best placed in conclusions. Please evaluate this probability.

ADDITIONAL INFORMATION AND DECLARATIONS

Author Contributions: Experimental design and conduct of tests, as well as measurement of various indicators. X.C. H D and W.L.; Fund support and pilot guidance, M.L.; Responsibility for manuscript writing and chartmaking, X.C. and H.F.; Responsibility for language polish and text modification, H.W. and J.Z. All authors have read and agreed to the published version of the manuscript.

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

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