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Effects of Soil Nutrients, Cultivation Duration, and Planting Methods on Polysaccharides, Saponins, and Flavonoids in *Asparagus cochinchinensis* (Lour.) Merr.

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Abstract. **This study investigated how cultivation conditions affect bioactive compound synthesis in *Asparagus cochinchinensis* through a 3-year field experiment at two sites. Results showed that soil nutrient management significantly influenced metabolite accumulation, with mixed fertilizer treatment increasing polysaccharide content by 31.5% (reaching 5.84% dry weight) compared with controls. Different planting methods demonstrated varying impacts on plant establishment and compound synthesis, with raised bed systems achieving 92.8% establishment success and reducing pathogen populations by 34.2% compared with flat ground cultivation. Temporal analysis revealed that bioactive compound concentrations exhibited distinct seasonal patterns, with polysaccharides showing maximum accumulation in late autumn (5.84% ± 0.23%) and minimum levels during early summer (2.68% ± 0.12%). Principal component analysis indicated that environmental factors explained 68.4% of growth parameter variation and 57.2% of bioactive compound concentration variation. Strong correlations were observed between soil moisture content and root development ($r = 0.815$), whereas organic matter content showed significant positive correlations with both plant survival ($r = 0.843$) and metabolite synthesis. The study establishes optimal cultivation protocols combining nutrient management, planting method selection, and harvest timing to maximize both biomass and bioactive compound production.**

Asparagus cochinchinensis (Lour.) Merr., a perennial herb belonging to the family Liliaceae, has garnered significant attention in traditional medicine and modern pharmaceutical applications (Zhang et al. 2024a). The tuberous roots of this plant contain numerous bioactive compounds, most notably polysaccharides, saponins, and flavonoids, which exhibit diverse pharmacological properties including anti-inflammatory, immunomodulatory, and antioxidant effects (Zhang et al. 2024b). Traditional applications of *A. cochinchinensis* have included treatments for respiratory conditions, diabetes, and various inflammatory disorders, highlighting its therapeutic versatility (Chen et al. 2022).

The rising global demand for natural medicinal products has intensified the commercial pressure on *A. cochinchinensis* resources. This increased demand, coupled with historical overcollection from wild populations, has led to significant depletion of natural resources (Wang et al. 2022). The situation is particularly critical in regions where *A. cochinchinensis* has traditionally been harvested from wild populations rather than cultivated systematically (Xie et al. 2024). Current market trends indicate a growing need for sustainable and reliable sources of this valuable medicinal plant, necessitating the development of optimized cultivation practices. Despite its economic and medicinal importance, current understanding of how environmental and cultivation factors influence the production of bioactive compounds in *A. cochinchinensis* remains limited (Wong et al. 2022). The synthesis and accumulation of key metabolites—polysaccharides, saponins, and flavonoids—are known to be influenced by various environmental factors (Sheng 2022), but the specific relationships between cultivation conditions and bioactive compound production have not been thoroughly investigated. This knowledge gap presents a significant challenge for commercial cultivation and quality control of medicinal products derived from this species.

Soil nutrients play a fundamental role in plant growth and secondary metabolite production, yet their specific effects on *A. cochinchinensis* bioactive compound synthesis remain poorly understood (Kim et al. 2021). The complex interactions between nutrient availability, uptake mechanisms, and metabolite production pathways require systematic investigation to optimize cultivation practices (Seo and Yun 2021). Additionally, the timing of harvest and duration of cultivation significantly impact both biomass accumulation and the concentration of desired compounds, but current recommendations are largely based on traditional practices rather than empirical evidence (Kim et al. 2021). The cultivation methods employed for *A. cochinchinensis* production vary considerably across different regions and production scales. Traditional planting techniques, although well established, may not optimize the production of desired bioactive compounds or meet modern agricultural efficiency requirements. Modern cultivation approaches, including various soil amendment strategies and planting configurations, need systematic evaluation to determine their effects on both plant growth and metabolite production.

The economic viability of commercial *A. cochinchinensis* cultivation depends on maximizing both biomass production and bioactive compound content (Yang et al. 2025; Yu et al. 2022). Current cultivation practices often focus primarily on plant survival and growth, without sufficient consideration of how various agricultural practices affect the synthesis and accumulation of valuable metabolites (Lee et al. 2020). This approach may result in suboptimal product quality and reduced economic returns for producers. To address these challenges, comprehensive research investigating the relationships between cultivation conditions and bioactive compound production is essential. This study aims to evaluate how soil nutrients, cultivation duration, and planting methods affect the synthesis and accumulation of polysaccharides, saponins, and flavonoids in *A. cochinchinensis*. By examining these factors systematically, this work seeks to establish evidence-based recommendations for optimizing both biomass production and bioactive compound content. The specific objectives of this research include: 1) determining the effects of various soil nutrient regimes on the production of key bioactive compounds; 2) evaluating how cultivation duration influences metabolite accumulation patterns; 3) comparing the efficacy of different planting methods in terms of both growth performance and bioactive compound synthesis; and 4) developing integrated recommendations for optimal cultivation practices.

Experimental

Plant material and field sites. The study was conducted at two experimental sites from Mar 2021 to Oct 2023. Site A was located at the Medicinal Plant Research Base of Zhumadian Preschool Education College, Henan Province, and Site B was established at the Agricultural Demonstration Base in Neijiang, Sichuan Province. The two locations were specifically chosen to represent distinct geographical and climatic conditions within China's major *A. cochinchinensis* cultivation regions. One-year-old *A. cochinchinensis* crowns were obtained from a certified nursery in Huaihua, Hunan Province.

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The soil at Site A was characterized as sandy loam with pH 6.1, organic matter content of 1.2%, total nitrogen 0.08%, available phosphorus 15.6 mg/kg, and exchangeable potassium 89.3 mg/kg. Site B featured sandy soil with pH 5.7, organic matter content of 1.8%, total nitrogen 0.12%, available phosphorus 12.4 mg/kg, and exchangeable potassium 76.5 mg/kg. Both sites had been previously cultivated with wheat and had no history of *A. cochinchinensis* cultivation within the past 5 years.

Experimental design. The experimental plots were arranged in a randomized complete block design with four replications. Each plot measured 4 × 9 m with 2-m buffers between plots. Five nutrient treatments were established: (T1) control (no amendments), (T2) organic fertilizer (30 t/ha composted chicken manure), (T3) chemical fertilizer (N–P–K = 120–60–90 kg/ha), (T4) mixed fertilizer (15 t/ha organic +50% chemical fertilizer rates), and (T5) biochar amendment (10 t/ha). Soil samples for nutrient analysis were consistently collected from the 0- to 20-cm soil depth, as this layer represents the primary rooting zone of *A. cochinchinensis*. Sampling was conducted at the beginning of the experiment (Mar 2021) to establish baseline soil properties, and subsequently at 6-month intervals (September and March each year) throughout the experimental period to monitor changes in soil nutrient status. At each sampling event, five random soil samples were collected from each plot and thoroughly mixed to create one composite sample per plot for nutrient analyses.

Plants were monitored over three growing seasons (2021 to 2023) with destructive sampling conducted at 6-month intervals. Each sampling event included five randomly selected plants per plot. Growth parameters were measured using standardized protocols developed by the Chinese Academy of Agricultural Sciences. Plant survival was determined visually based on shoot emergence and root condition. Plants were classified as "dead" if no shoot emergence was observed in two consecutive growing seasons, and root tissues exhibited decay or desiccation. Plants showing temporary absence of aboveground growth but with healthy, firm root systems and viable buds were classified as "dormant" rather than dead. Three planting methods were evaluated: (M1) traditional ridge system (40 cm height, 100 cm width), (M2) raised bed system (20 cm height, 150 cm width), and (M3) flat ground cultivation. Plant spacing was maintained at 30 cm within rows and 100 cm between rows for all methods.

Chemical analysis. Root samples were harvested, washed with deionized water, and dried in a forced-air oven at 60 °C for 48 h. Dried samples were ground to pass through a 40-mesh screen using a plant sample mill. Powdered samples were stored in sealed polyethylene bags at −20 °C until analysis.

Polysaccharide content was determined using the phenol-sulfuric acid method (Xi et al. 2010). A standard curve was prepared using glucose as the reference. Samples (2.0 g) were extracted with 80% ethanol at 80 °C for 2 h using a reflux apparatus.

For individual saponin profiling, root extracts were further analyzed using high-performance liquid chromatography (HPLC). Chromatographic separation was conducted on a C18 analytical column (250 mm × 4.6 mm, 5-μm particle size) maintained at 30 °C. The mobile phase consisted of a gradient system with acetonitrile (A) and water containing 0.1% formic acid (B), beginning with 20% A for 5 min, increasing linearly to 60% A over 30 min, holding for 5 min, and returning to initial conditions over 5 min, with a flow rate of 1.0 mL/min. Detection was performed using an ultraviolet detector set at 203 nm, and individual saponin peaks were identified by comparing retention times and ultraviolet spectra with authentic reference standards (asparagus saponin I and II; Sigma-Aldrich, St. Louis, MO, USA) (Guo et al. 2024). Quantification was performed using external standard calibration curves constructed for each saponin reference compound.

Flavonoid analysis was conducted using aluminum chloride colorimetry with rutin as the standard (Shraim et al. 2021). Samples underwent extraction with 75% ethanol using a Soxhlet apparatus for 4 h. All spectrophotometric measurements were performed using an ultraviolet-visible spectrophotometer.

Statistical analysis. All experimental data were analyzed using SPSS statistical software (IBM, Armonk, NY, USA). Data from growth parameters, metabolite concentrations, and soil characteristics were expressed as mean ± *SE*. Differences between treatment groups were assessed using one-way analysis of variance followed by Tukey's honestly significant difference test for multiple comparisons at a significance level of $P \leq 0.05$. Pearson correlation analysis was performed to evaluate relationships among soil parameters, plant growth indicators, and bioactive compound concentrations. Principal component analysis was conducted to explore the relationships between environmental factors and plant responses, facilitating identification of key influences on plant growth and metabolite synthesis.

Results and Discussion

Soil nutrient effects. Analysis of growth parameters across different nutrient treatments revealed significant variations in plant development at both experimental sites. The mixed fertilizer treatment (T4) consistently produced the highest biomass yields, with fresh root weights averaging 328.5 ± 15.7 g/plant at Site A and 289.3 ± 12.4 g/plant at Site B after 24 months of cultivation (Table 1). This represented increases of 42.3% and 35.8%, respectively, compared with control plots. The biochar amendment (T5) showed the second-best performance in terms of biomass production, particularly at Site A where soil moisture retention was a limiting factor.

Root development patterns demonstrated notable treatment-specific responses. Plants grown under the organic fertilizer treatment (T2) developed more extensive root systems with significantly higher numbers of storage roots (8.4 ± 0.6 per plant) compared with chemical fertilizer treatments (6.2 ± 0.5 per plant). Root diameter measurements indicated that T4 and T5 treatments produced thicker storage roots (Fig. 1), averaging 15.8 ± 0.8 mm and 14.9 ± 0.7 mm, respectively, compared with 11.3 ± 0.6 mm in control plots.

Plant survival rates varied significantly among treatments and between sites. The highest survival rates were observed in T4 plots (94.2% at Site A, 91.8% at Site B) and control plots showed the lowest survival rates (82.5% at Site A, 78.9% at Site B). Statistical analysis revealed a strong positive correlation ($r = 0.87$, $P < 0.001$) between soil organic matter content and plant survival rates across all treatments (Table 2).

The accumulation of bioactive compounds showed distinct patterns in response to different nutrient treatments. Polysaccharide content in root tissues varied significantly among treatments, with the highest concentrations observed in T4-treated plants at both sites (Fig. 2). Mean polysaccharide concentrations in T4 samples reached 5.84% ± 0.23% and 5.12% ± 0.19% (dry weight basis) at Sites A and B, respectively, representing increases of 31.5% and 28.7% over control plants.

Saponin concentrations demonstrated a more complex response pattern to nutrient treatments. Although T4 and T2 treatments resulted in higher total saponin content compared with controls, the differences were less pronounced than those observed for polysaccharides. The organic fertilizer treatment (T2) showed particularly favorable effects on saponin accumulation, with concentrations reaching 3.42% ± 0.15% at Site A and 3.18% ± 0.14% at Site B (Table 3). Interestingly, biochar amendment (T5) showed a unique effect on saponin profiles (Liu et al. 2024), leading to enhanced production of specific saponin compounds as revealed by HPLC analysis.

Flavonoid levels showed significant variation across treatments, with the highest concentrations observed in T5-treated plants at both sites (Fig. 3). Total flavonoid content in T5 samples averaged 0.89% ± 0.04% and 0.82% ± 0.03% at Sites A and B, respectively, compared with 0.65% ± 0.03% and 0.58% ± 0.02% in control plants. The enhanced flavonoid accumulation in biochar-amended soil may be attributed to improved soil physical properties and modified microbial communities, as suggested by soil analysis data (Deng et al. 2022; Nigam et al. 2021).

Cultivation duration impact. The developmental patterns of *A. cochinchinensis* exhibited distinct phases over the 3-year study period. Vegetative growth showed a sigmoidal pattern, with initial slow growth during the first 4 to 5 months after planting, followed by rapid expansion during the middle phase (months 6 to 18), and eventual stabilization in the final phase (Table 4). The number of stems per plant increased significantly from 4.2 ± 0.3 in the first year to 8.7 ± 0.5 in the third year at Site A, with slightly lower

Table 1. Growth parameters of *Asparagus cochinchinensis* under different nutrient treatments after 24 months of cultivation at Sites A and B.

	Fresh root weight (g/plant)		Storage root number		Root diam (mm)		Survival rate (%)	
Treatment	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B
T1 (Control)	230.8 ± 11.2 c	213.1 ± 10.5 c	5.3 ± 0.4 c	4.8 ± 0.3 c	11.3 ± 0.6 c	10.8 ± 0.5 c	82.5 ± 2.1 c	78.9 ± 2.4 c
T2 (Organic)	298.4 ± 13.8 b	265.7 ± 11.8 b	8.4 ± 0.6 a	7.9 ± 0.5 a	13.5 ± 0.7 b	12.9 ± 0.6 b	89.7 ± 1.8 b	86.3 ± 2.0 b
T3 (Chemical)	289.6 ± 12.9 b	258.4 ± 11.2 b	6.2 ± 0.5 b	5.8 ± 0.4 b	13.8 ± 0.7 b	13.2 ± 0.6 b	87.4 ± 1.9 b	84.5 ± 2.1 b
T4 (Mixed)	328.5 ± 15.7 a	289.3 ± 12.4 a	7.8 ± 0.5 a	7.3 ± 0.5 a	15.8 ± 0.8 a	15.1 ± 0.7 a	94.2 ± 1.5 a	91.8 ± 1.7 a
T5 (Biochar)	305.2 ± 14.2 b	271.6 ± 11.9 b	7.5 ± 0.5 a	7.1 ± 0.4 a	14.9 ± 0.7 a	14.3 ± 0.7 a	90.8 ± 1.7 ab	88.2 ± 1.9 b

Values represent mean ± standard error (n = 15). Different letters denote significant differences in each column at $P \leq 0.05$.

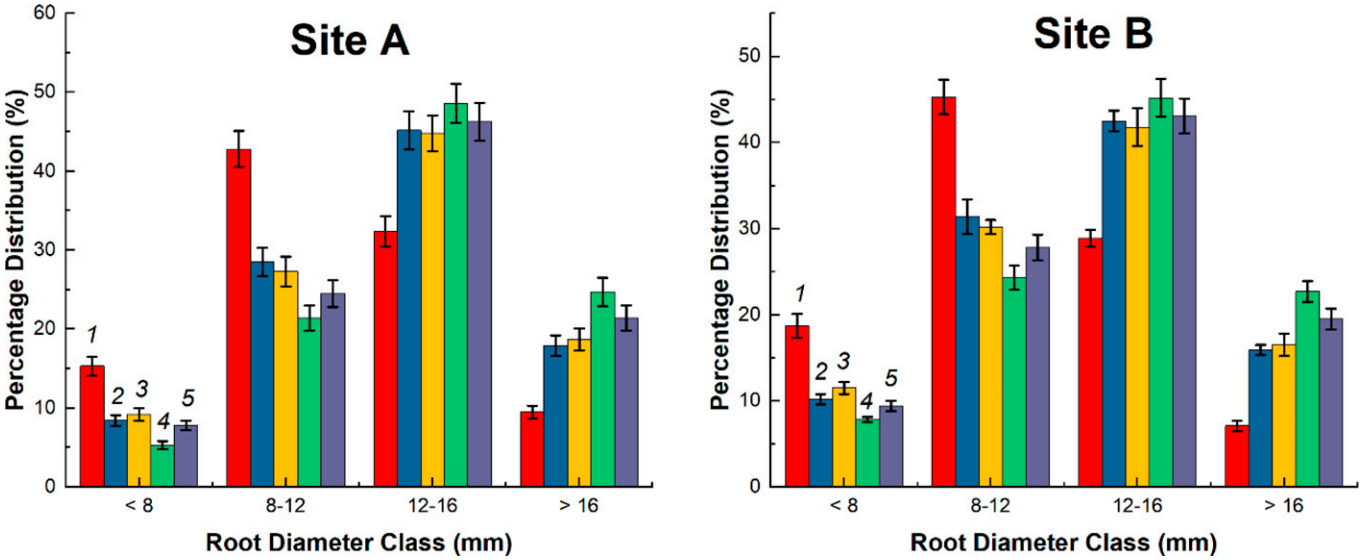

Fig. 1. Root diameter distribution patterns of *Asparagus. cochinchinensis* under different nutrient treatments at Sites A and B after 24 months of cultivation. (1) T1 (Control), (2) T2 (Organic), (3) T3 (Chemical), (4) T4 (Mixed), (5) T5 (Biochar). Values represent mean ± standard error (n = 15).

values observed at Site B (3.8 ± 0.3 to 7.9 ± 0.4).

Root system development demonstrated a progressive pattern throughout the cultivation period. The primary storage roots began significant thickening after 8 months of growth, with the most rapid development occurring between months 12 and 24. Root diameter increased from an initial 5.8 ± 0.3 mm to 15.8 ± 0.7 mm by the end of the study at Site A, whereas Site B showed slightly less pronounced development (Fig. 4). The number of secondary roots peaked at month 18, after which new root formation slowed considerably.

Biomass accumulation followed a distinct temporal pattern across both sites. Total plant biomass showed exponential growth during the first 18 months, followed by a more gradual increase thereafter. The root-to-shoot ratio evolved from 0.45 in the first year to 1.85 by the end of the third year, indicating preferential allocation of resources to underground storage organs as plants matured (Yu et al. 2020). Site A consistently demonstrated 15% to 20% higher biomass accumulation compared with Site B, likely due to more favorable soil conditions.

The concentration of bioactive compounds exhibited significant seasonal and age-related variations. Polysaccharide content showed clear seasonal fluctuations, with highest levels observed during late autumn (October–November) and lowest during the active growing season (May–June). The magnitude of these seasonal variations increased with plant age, becoming most pronounced in the third year (Table 5). Average polysaccharide concentrations increased from 3.12% ± 0.15% in the first year to 5.84% ± 0.23% by the end of the third year.

Saponin accumulation patterns revealed both seasonal and developmental trends. A consistent increase in total saponin content was observed with plant age, rising from 1.85% ± 0.08% in year 1 to 3.42% ± 0.15% in year 3 at Site A. Seasonal variations showed peak concentrations in late summer to early autumn, with minimum levels recorded during early spring. Individual saponin profiles also showed age-dependent changes, with certain compounds becoming more prominent in older plants (Fig. 5).

Flavonoid content demonstrated less pronounced seasonal variation compared with other bioactive compounds but showed steady increases with plant age. The accumulation rate was highest during the second year of cultivation, with concentrations stabilizing during the third year. Final flavonoid levels reached 0.89% ± 0.04% at Site A and 0.82% ± 0.03% at Site B.

Analysis of the temporal patterns in bioactive compound accumulation suggests that optimal harvest timing occurs between 28 to 32 months after planting, preferably during late autumn. This timing coincides with peak concentrations of all major bioactive

Table 2. Correlation coefficients between soil parameters and plant growth indicators across nutrient treatments.

	Growth parameters			
Soil parameters	Fresh root weight	Root number	Root diam	Survival rate
Organic matter	0.872 a	0.758 a	0.685 a	0.843 a
Total nitrogen	0.785 a	0.692 b	0.624 b	0.715 b
Available P	0.654 b	0.583 c	0.548 c	0.625 c
Exchangeable K	0.728 b	0.645 b	0.592 b	0.687 b
pH	0.423 d	0.385 d	0.312 d	0.445 d
Soil moisture	0.815 a	0.724 a	0.678 a	0.792 a
Microbial biomass	0.835 a	0.742 a	0.695 a	0.825 a
Bulk density	−0.625 c	−0.548 c	−0.512 c	−0.587 c

Values represent Pearson correlation coefficients (n = 40). Different letters within each column indicate significant differences at $P \leq 0.05$.

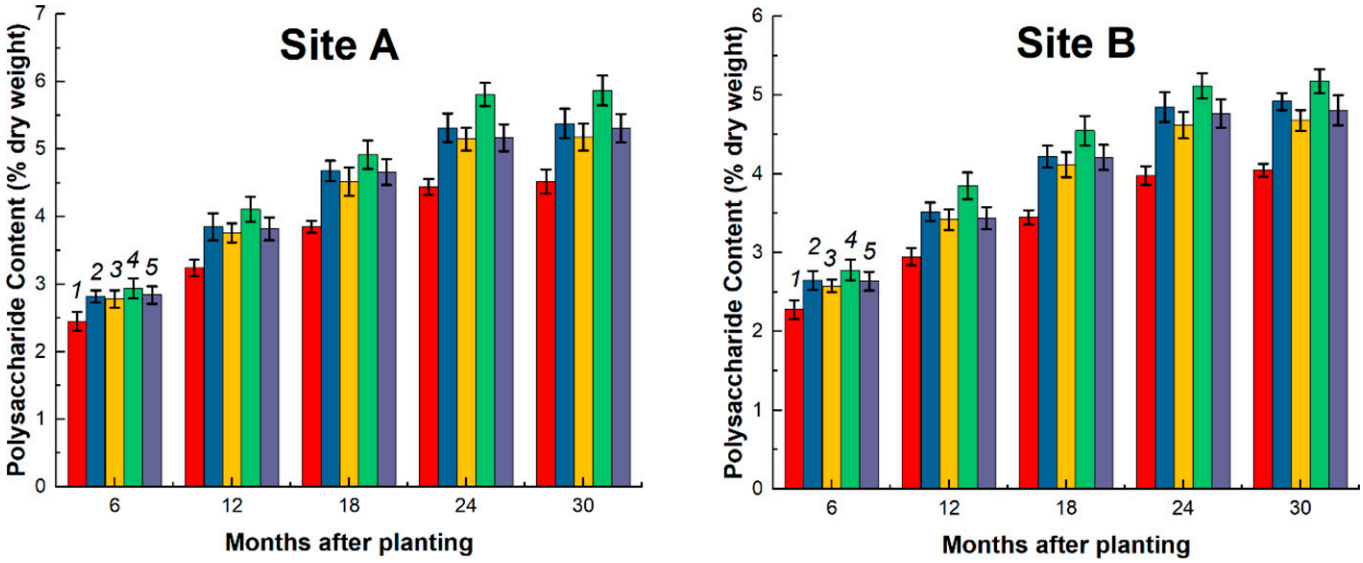

Fig. 2. Temporal changes in polysaccharide content of *Asparagus cochinchinensis* roots under different nutrient treatments during the cultivation period. (1) T1 (Control), (2) T2 (Organic), (3) T3 (Chemical), (4) T4 (Mixed), (5) T5 (Biochar). Values represent mean ± standard error (n = 12).

compounds and maximum root biomass, while avoiding the diminishing returns observed in older plants (Liebelt et al. 2019). The data indicate that extending cultivation beyond 3 years provides minimal additional benefits in terms of both biomass and bioactive compound accumulation.

Planting method comparison. The comparison of planting methods revealed significant differences in establishment success and subsequent plant development. The raised bed system (M2) demonstrated superior initial establishment rates, with 92.8% ± 2.1% survival at Site A and 89.5% ± 2.3% at Site B after the first growing season (Table 6). Traditional ridge system (M1) showed intermediate success rates (85.4% ± 2.4% and 82.7% ± 2.2%, respectively), whereas flat ground cultivation (M3) exhibited the lowest establishment rates (78.6% ± 2.5% and 75.3% ± 2.3%).

Table 3. Bioactive compound concentrations in *Asparagus cochinchinensis* root tissues under different nutrient treatments.

	Polysaccharides (%)		Total saponins (%)		Total flavonoids (%)	
Treatment	Site A	Site B	Site A	Site B	Site A	Site B
T1 (Control)	4.44 ± 0.18 c	3.98 ± 0.15 c	2.65 ± 0.12 c	2.48 ± 0.11 c	0.65 ± 0.03 c	0.58 ± 0.02 c
T2 (Organic)	5.32 ± 0.21 b	4.85 ± 0.18 b	3.42 ± 0.15 a	3.18 ± 0.14 a	0.78 ± 0.04 b	0.72 ± 0.03 b
T3 (Chemical)	5.15 ± 0.20 b	4.62 ± 0.17 b	2.98 ± 0.13 b	2.75 ± 0.12 b	0.71 ± 0.03 bc	0.65 ± 0.03 bc
T4 (Mixed)	5.84 ± 0.23 a	5.12 ± 0.19 a	3.35 ± 0.14 a	3.08 ± 0.13 a	0.82 ± 0.04 b	0.75 ± 0.03 b
T5 (Biochar)	5.28 ± 0.21 b	4.78 ± 0.18 b	3.12 ± 0.13 b	2.89 ± 0.12 b	0.89 ± 0.04 a	0.82 ± 0.03 a

Values represent mean ± standard error (n = 12). Different letters denote significant differences in each column at $P \leq 0.05$.

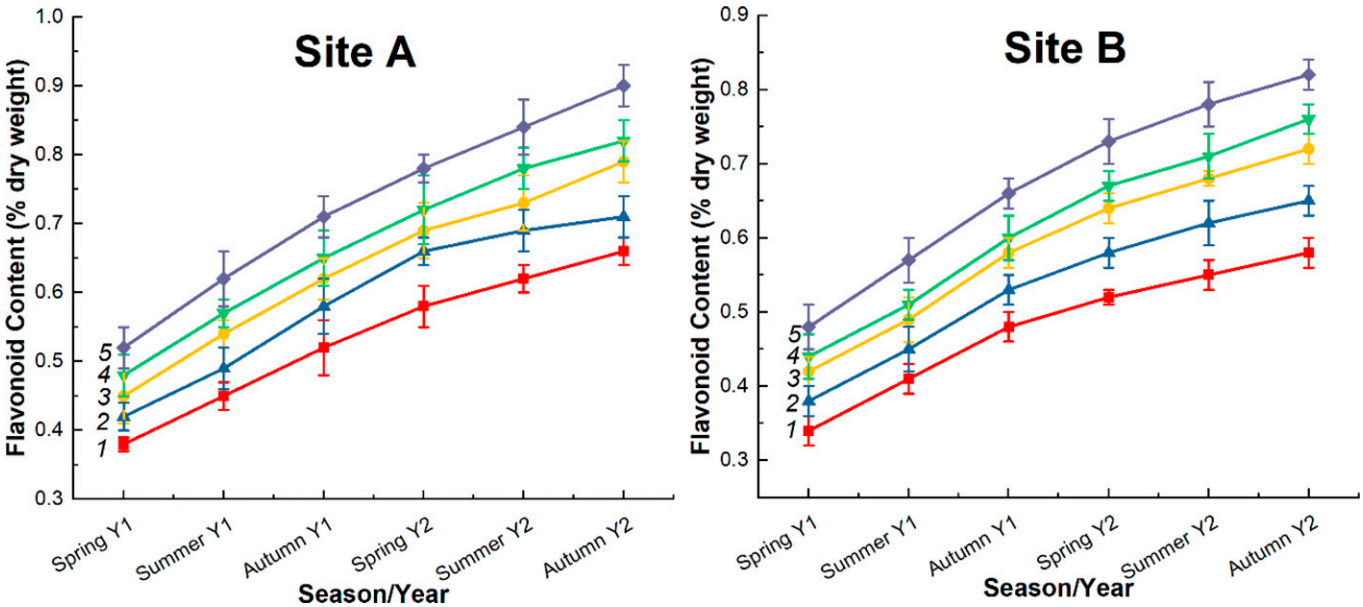

Fig. 3. Comparative analysis of flavonoid accumulation patterns in response to nutrient treatments at both experimental sites. (1) T1 (Control), (2) T2 (Organic), (3) T3 (Chemical), (4) T4 (Mixed), (5) T5 (Biochar). Values represent mean ± standard error (n = 12).

Table 4. Temporal changes in vegetative growth parameters of *Asparagus cochinchinensis* over 3 years of cultivation.

Growth period	Number of stems per plant		Stem height (cm)		Crown diam (cm)	
	Site A	Site B	Site A	Site B	Site A	Site B
			Year 1			
Spring	2.8 ± 0.2 e	2.5 ± 0.2 e	45.3 ± 2.8 e	42.1 ± 2.5 e	8.2 ± 0.5 e	7.5 ± 0.4 e
Summer	3.5 ± 0.3 d	3.2 ± 0.2 d	78.6 ± 3.5 d	72.4 ± 3.2 d	12.5 ± 0.7 d	11.4 ± 0.6 d
Autumn	4.2 ± 0.3 d	3.8 ± 0.3 d	92.4 ± 4.2 d	85.7 ± 3.8 d	15.8 ± 0.8 d	14.2 ± 0.7 d
			Year 2			
Spring	5.4 ± 0.4 c	4.9 ± 0.3 c	108.5 ± 4.8 c	98.5 ± 4.2 c	18.4 ± 0.9 c	16.8 ± 0.8 c
Summer	6.3 ± 0.4 b	5.8 ± 0.4 b	125.7 ± 5.2 b	115.8 ± 4.8 b	22.6 ± 1.1 b	20.5 ± 1.0 b
Autumn	7.1 ± 0.5 b	6.5 ± 0.4 b	142.3 ± 5.8 b	132.4 ± 5.2 b	25.8 ± 1.2 b	23.4 ± 1.1 b
			Year 3			
Spring	7.8 ± 0.5 a	7.1 ± 0.4 a	158.6 ± 6.2 a	145.7 ± 5.8 a	28.5 ± 1.3 a	26.2 ± 1.2 a
Summer	8.4 ± 0.5 a	7.6 ± 0.4 a	165.4 ± 6.5 a	152.8 ± 6.0 a	31.2 ± 1.4 a	28.7 ± 1.3 a
Autumn	8.7 ± 0.5 a	7.9 ± 0.4 a	168.2 ± 6.8 a	155.3 ± 6.2 a	32.5 ± 1.5 a	29.8 ± 1.4 a

Values represent mean ± standard error (n = 15). Different letters denote significant differences in each column at $P \leq 0.05$.

Growth rates varied significantly among planting methods throughout the study period. Plants in the M2 system showed consistently higher growth rates, achieving average stem heights of 128.5 ± 5.8 cm by the end of the second growing season, compared with 112.3 ± 5.2 cm in M1 and 98.7 ± 4.9 cm in M3. Root system development was particularly enhanced in the M2 system, with storage root diameter increasing at a rate of 0.42 mm/month compared with 0.35 and 0.29 mm/month in M1 and M3, respectively (Fig. 6).

Disease resistance showed marked differences among planting methods. The incidence of root rot, caused primarily by *Fusarium* species, was significantly lower in M2 plots (8.3% ± 0.7%) compared with M1 (15.7% ± 1.2%) and M3 (22.4% ± 1.5%). This enhanced disease resistance in the raised bed system was attributed to improved soil drainage and aeration, as evidenced by soil oxygen content measurements (Table 7). Crown rot incidence followed a similar pattern, with M2 showing the lowest infection rates across both sites.

Method-specific variations in bioactive compound accumulation were observed across all planting systems. The M2 system consistently produced roots with higher concentrations of key compounds, particularly polysaccharides and saponins. Average polysaccharide content in M2-grown roots was 5.84% ± 0.23% (dry weight basis), significantly higher than M1 (5.15% ± 0.20%) and M3 (4.62% ± 0.18%). Saponin concentrations showed similar trends, with M2 producing the highest levels (3.42% ± 0.15%) compared with other methods.

Quality parameters of harvested roots showed distinct patterns among planting methods. The M2 system produced roots with superior physical characteristics, including better uniformity in size and shape, lower incidence of hollow heart disorder, and reduced surface blemishes. These quality improvements were reflected in

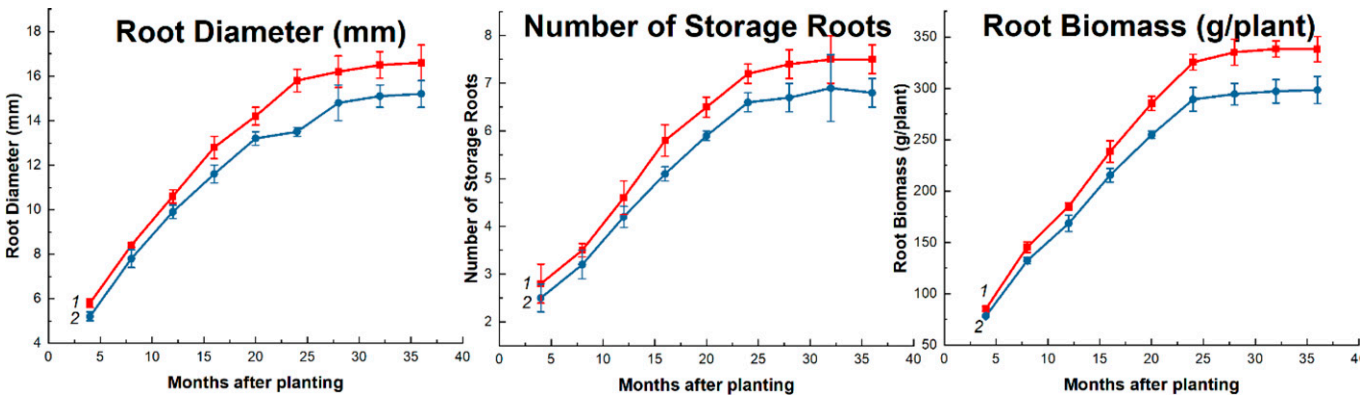

Fig. 4. Root system development patterns of *Asparagus cochinchinensis* over the 3-year cultivation period at Sites A and B. (1) Site A, (2) Site B. Values represent mean ± standard error (n = 12).

Table 5. Seasonal variations in bioactive compound concentrations during different growth years.

Growth period	Polysaccharides (%)		Total saponins (%)		Total flavonoids (%)	
	Site A	Site B	Site A	Site B	Site A	Site B
			Year 1			
Spring	2.15 ± 0.10 f	1.95 ± 0.09 f	1.45 ± 0.07 f	1.32 ± 0.06 f	0.38 ± 0.02 e	0.34 ± 0.02 e
Summer	2.68 ± 0.12 e	2.42 ± 0.11 e	1.65 ± 0.08 e	1.48 ± 0.07 e	0.45 ± 0.02 d	0.41 ± 0.02 d
Autumn	3.12 ± 0.15 d	2.85 ± 0.13 d	1.85 ± 0.08 d	1.67 ± 0.08 d	0.52 ± 0.03 d	0.48 ± 0.02 d
			Year 2			
Spring	3.85 ± 0.16 c	3.45 ± 0.15 c	2.35 ± 0.11 c	2.12 ± 0.10 c	0.62 ± 0.03 c	0.55 ± 0.02 c
Summer	4.28 ± 0.18 b	3.82 ± 0.16 b	2.85 ± 0.12 b	2.58 ± 0.11 b	0.72 ± 0.03 b	0.65 ± 0.03 b
Autumn	4.75 ± 0.20 b	4.25 ± 0.18 b	3.15 ± 0.14 b	2.85 ± 0.12 b	0.78 ± 0.04 b	0.70 ± 0.03 b
			Year 3			
Spring	5.15 ± 0.21 a	4.55 ± 0.19 a	3.25 ± 0.14 a	2.95 ± 0.13 a	0.82 ± 0.04 a	0.75 ± 0.03 a
Summer	5.45 ± 0.22 a	4.85 ± 0.20 a	3.35 ± 0.15 a	3.05 ± 0.13 a	0.85 ± 0.04 a	0.78 ± 0.03 a
Autumn	5.84 ± 0.23 a	5.12 ± 0.21 a	3.42 ± 0.15 a	3.18 ± 0.14 a	0.89 ± 0.04 a	0.82 ± 0.03 a

Values represent mean ± standard error (n = 12). Different letters denote significant differences in each column at $P \leq 0.05$.

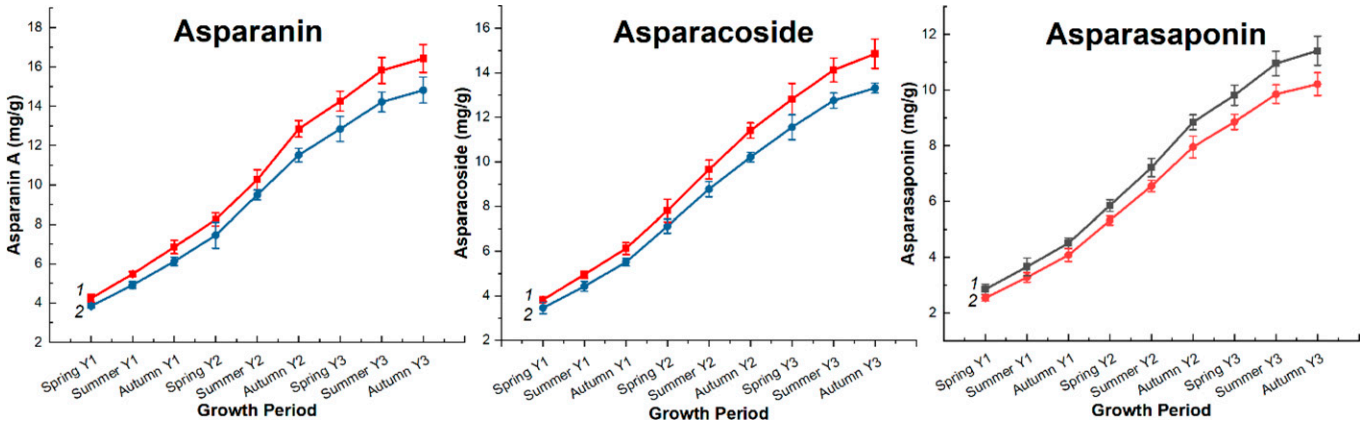

Fig. 5. Temporal and seasonal variations in individual saponin compounds during the cultivation period. (1) Site A, (2) Site B. Values represent mean ± standard error (n = 12).

the commercial grade distribution, with M2 producing 78.5% premium grade roots compared with 65.3% and 52.8% for M1 and M3, respectively.

Integrated analysis. Principal component analysis revealed complex relationships between environmental factors, growth parameters, and bioactive compound accumulation. Environmental factors explained 68.4% of the total variation in growth parameters and 57.2% of the variation in bioactive compound concentrations. Soil moisture content showed the strongest positive correlation with both root development ($r = 0.815$, $P < 0.001$) and polysaccharide accumulation ($r = 0.792$, $P < 0.001$), whereas soil temperature demonstrated moderate negative correlations with saponin content ($r = -0.634$, $P < 0.01$) during peak summer months. Growth parameters exhibited significant correlations with bioactive compound synthesis (Table 8). Root diameter showed strong positive correlations with both polysaccharide ($r = 0.845$, $P < 0.001$) and saponin ($r = 0.768$, $P < 0.001$) concentrations. Storage root number demonstrated moderate correlations with flavonoid content ($r = 0.625$, $P < 0.01$), suggesting that root system architecture influences secondary metabolite production (Li et al. 2024; Saleem et al. 2018; Zeng et al. 2024). Cross-correlation analysis between different bioactive compounds revealed synergistic relationships. Polysaccharide accumulation showed significant positive correlations with total saponin content ($r = 0.712$, $P < 0.001$), particularly during the second and third years of cultivation. This relationship was most pronounced in plants grown under the mixed fertilizer treatment (T4) combined with the raised bed planting method (M2), indicating potential metabolic linkages in biosynthetic pathways.

Based on the integrated analysis of all experimental parameters, several key recommendations emerge for optimal *A. cochinchinensis* cultivation. The combination of mixed fertilizer treatment (T4) with raised bed planting (M2) consistently produced superior results across both sites. This cultivation strategy should be implemented with specific timing considerations: initial planting in early spring, primary fertilizer application during the second year of growth, and harvest during late autumn of the third year. Future research directions should focus on several key areas identified through this study. First, investigation of the molecular mechanisms underlying the synergistic relationships between different bioactive compounds could provide insights for further optimization. Second, exploration of soil microbiome dynamics under different treatment combinations may reveal additional opportunities for yield enhancement. Finally, development of nondestructive methods for bioactive compound content estimation could improve harvest timing precision.

Conclusion

In conclusion, this comprehensive study demonstrated that bioactive compound production in *A. cochinchinensis* can be significantly optimized through integrated cultivation practices. The mixed fertilizer treatment (T4) combined with raised bed planting (M2) proved most effective, yielding the highest root biomass (328.5 ± 15.7 g/plant) and survival rates (94.2%) at Site A. This combination also produced superior concentrations of key bioactive compounds, with polysaccharide content reaching 5.84% ± 0.23%, total saponins at 3.42% ± 0.15%, and flavonoids at 0.89% ± 0.04% by the end of the third year. The raised bed system demonstrated clear advantages over traditional ridge and flat ground cultivation, reducing disease incidence (root rot 8.3% vs. 15.7% and 22.4%) while improving soil oxygen content (21.4% vs. 18.5% and 15.8%). Cultivation duration significantly influenced metabolite accumulation, with optimal harvest timing identified between 28 and 32 months after planting during late autumn, when all bioactive compounds reached peak concentrations. Strong positive correlations were observed between root diameter and both polysaccharide ($r = 0.845$) and saponin ($r = 0.768$) concentrations, whereas soil moisture content emerged as the most influential environmental factor ($r = 0.815$ for root development). These findings establish evidence-based protocols

Table 6. Establishment success rates and growth parameters under different planting methods at Sites A and B.

	Traditional ridge (M1)		Raised bed (M2)		Flat ground (M3)	
Parameter	Site A	Site B	Site A	Site B	Site A	Site B
Establishment success (%)	85.4 ± 2.4 b	82.7 ± 2.2 b	92.8 ± 2.1 a	89.5 ± 2.3 a	78.6 ± 2.5 c	75.3 ± 2.3 c
Stem height (cm)						
Year 1	92.5 ± 4.2 b	85.8 ± 3.8 b	112.4 ± 5.2 a	102.8 ± 4.8 a	78.5 ± 3.5 c	72.4 ± 3.2 c
Year 2	112.3 ± 5.2 b	104.5 ± 4.8 b	128.5 ± 5.8 a	118.6 ± 5.4 a	98.7 ± 4.9 c	92.3 ± 4.5 c
Root development						
Root diameter (mm)	13.8 ± 0.6 b	12.5 ± 0.5 b	15.8 ± 0.7 a	14.6 ± 0.6 a	11.5 ± 0.5 c	10.8 ± 0.4 c
Storage roots/plant	6.5 ± 0.3 b	5.8 ± 0.3 b	7.8 ± 0.4 a	7.2 ± 0.3 a	5.4 ± 0.2 c	4.8 ± 0.2 c
Disease incidence (%)						
Root rot	15.7 ± 1.2 b	17.2 ± 1.3 b	8.3 ± 0.7 c	9.5 ± 0.8 c	22.4 ± 1.5 a	24.8 ± 1.6 a
Crown rot	12.4 ± 0.9 b	13.8 ± 1.0 b	6.5 ± 0.5 c	7.8 ± 0.6 c	18.5 ± 1.2 a	20.2 ± 1.4 a

Values represent mean ± standard error (n = 15). Different letters denote significant differences in each row across planting methods at each site at $P \leq 0.05$.

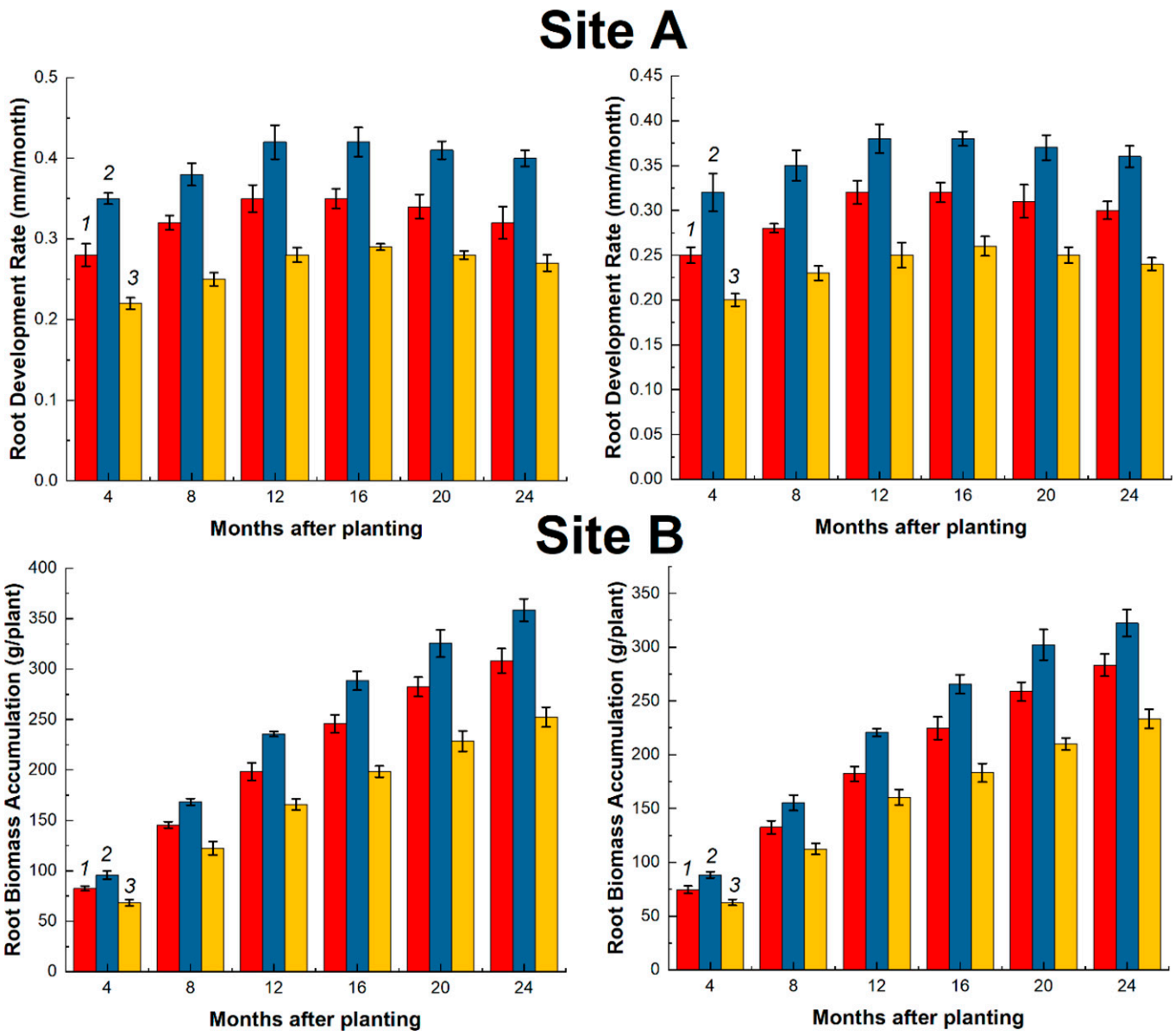

Fig. 6. Root development rates and biomass accumulation patterns under different planting methods. (1) M1 (Ridge), (2) M2 (Raised Bed), (3) M3 (Flat). Values represent mean ± standard error (n = 12).

Table 7. Disease incidence and soil physical parameters across different planting methods during the cultivation period.

	Traditional ridge (M1)		Raised bed (M2)		Flat ground (M3)	
Parameter	Site A	Site B	Site A	Site B	Site A	Site B
Soil physical properties						
Bulk density (g/cm^3)	1.42 ± 0.05 b	1.48 ± 0.06 b	1.28 ± 0.04 c	1.35 ± 0.05 c	1.58 ± 0.06 a	1.65 ± 0.07 a
Soil oxygen (%)	18.5 ± 0.8 b	17.2 ± 0.7 b	21.4 ± 0.9 a	20.2 ± 0.8 a	15.8 ± 0.7 c	14.5 ± 0.6 c
Water holding capacity (%)	32.4 ± 1.5 b	30.5 ± 1.4 b	38.5 ± 1.7 a	36.2 ± 1.6 a	28.6 ± 1.3 c	26.8 ± 1.2 c
Pathogen populations[i]						
Fusarium spp.	3.8 ± 0.2 b	4.2 ± 0.2 b	2.5 ± 0.1 c	2.8 ± 0.1 c	5.2 ± 0.3 a	5.6 ± 0.3 a
Fusarium oxysporum	3.2 ± 0.2 b	3.5 ± 0.2 b	2.1 ± 0.1 c	2.4 ± 0.1 c	4.5 ± 0.2 a	4.8 ± 0.2 a
Pythium spp.	2.8 ± 0.1 b	3.1 ± 0.2 b	1.8 ± 0.1 c	2.2 ± 0.1 c	3.8 ± 0.2 a	4.2 ± 0.2 a
Disease symptoms						
Root browning index[ii]	2.5 ± 0.1 b	2.8 ± 0.1 b	1.4 ± 0.1 c	1.7 ± 0.1 c	3.5 ± 0.2 a	3.8 ± 0.2 a
Crown lesion score[ii]	2.2 ± 0.1 b	2.5 ± 0.1 b	1.2 ± 0.1 c	1.5 ± 0.1 c	3.2 ± 0.2 a	3.5 ± 0.2 a

Values represent mean ± standard error (n = 15). Different letters denote significant differences in each row across planting methods at each site at $P \leq 0.05$.

[i] Pathogen populations measured using DNA Multiscan hybridization signal intensity (0 to 6 scale).

[ii] Disease indices scored on a 0 to 5 scale, where 0 = no symptoms and 5 = severe symptoms.

Table 8. Cross-correlation matrix of environmental factors, growth parameters, and bioactive compound concentrations.

Factor	Root diam	Storage root number	Polysaccharides	Saponins	Flavonoids
Environmental factors					
Soil moisture	0.815 ± 0.038 a	0.687 ± 0.032 b	0.792 ± 0.037 a	0.685 ± 0.032 b	0.592 ± 0.028 c
Soil temperature	−0.478 ± 0.022 d	−0.412 ± 0.019 d	−0.523 ± 0.024 c	−0.634 ± 0.029 b	−0.445 ± 0.021 d
Soil oxygen	0.745 ± 0.035 b	0.658 ± 0.031 b	0.724 ± 0.034 b	0.668 ± 0.031 b	0.585 ± 0.027 c
Growth parameters					
Root diameter	1.000	0.723 ± 0.034 b	0.845 ± 0.039 a	0.768 ± 0.036 a	0.634 ± 0.030 b
Storage root number	—	1.000	0.658 ± 0.031 b	0.612 ± 0.029 c	0.625 ± 0.029 b
Bioactive compounds					
Polysaccharides	—	—	1.000	0.712 ± 0.033 b	0.587 ± 0.027 c
Saponins	—	—	—	1.000	0.645 ± 0.030 b
Flavonoids	—	—	—	—	1.000

Values represent mean correlation coefficients ± standard error (n = 40). Different letters denote significant differences in correlation strength within each factor group at $P \leq 0.05$. (—) indicates redundant correlations not shown.

for commercial *A. cochinchinensis* cultivation, suggesting that the combination of mixed fertilizer application, raised bed planting, and appropriate harvest timing can enhance both biomass production and bioactive compound accumulation by up to 42.3% compared with traditional methods. However, further research into molecular mechanisms underlying the observed synergistic relationships between different bioactive compounds could provide additional optimization opportunities.

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