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Research Article

Effect of Different Nitrogen Nutrition on Growth, Yield and Quality of *Senna tora* in Northern Vietnam

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Abstract

Background and Objective: *Senna tora* is a valuable medicinal plant and an important natural source of aurantioobtusin, a compound with strong anti-inflammatory activity. However, knowledge of how nitrogen (N) nutrition influences its growth, seed yield and pharmaceutical quality is limited. This study aimed to determine the optimal N application rate for maximizing both seed yield and aurantioobtusin content in *S. tora*. **Materials and Methods:** A field experiment was conducted in Thanh Tri (Hanoi, Vietnam) using six N application rates: N0 (0 kg/ha), N1 (35 kg/ha), N2 (70 kg/ha), N3 (105 kg/ha), N4 (140 kg/ha) and N5 (175 kg/ha). Plant growth, seed yield and aurantioobtusin content were measured at harvest. Statistical analysis was performed using CropStat 7.2, Microsoft Excel 2016 and RStudio, with treatment effects tested at $p < 0.05$ and regression used to evaluate relationships between N rates and measured parameter. **Results:** Plants receiving N3 (105 kg/ha) exhibited the highest growth performance, with a seed yield of 2.02 t/ha and aurantioobtusin content of 0.24%. While higher N rates increased foliage biomass, they significantly reduced seed yield ($p < 0.05$). The N3 treatment effectively balanced both seed productivity and medicinal quality. **Conclusion:** Application of 105 kg/ha N optimizes seed yield and aurantioobtusin content in *S. tora*, providing practical guidance for sustainable cultivation and medicinal compound production. Excessive N application increases vegetative growth but compromises seed yield, highlighting the need for balanced nutrient management. Future studies could explore long-term environmental impacts and interactions with other nutrients.

Key words: *Senna tora*, nitrogen fertilizer, aurantioobtusin, growth, yield

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cassia L. is a large genus of the family Fabaceae. Members of this genus are predominantly found in monsoon-influenced regions, where they grow naturally under humid conditions and are commonly observed on marginal or uncultivated lands in countries such as Bangladesh, India, Pakistan and western China^{1,2}. Taxonomists have divided the genus into three genera: *Cassia* L., *Senna* Miller and *Chamaecrista* (L.) Moench. *Senna tora* (L.) Roxb or *Cassia senna* (L.) Roxb classified in *Senna* Miller, is the most abundant species in tropical America, followed by Africa and Australia. *S. tora* is a perennial undershrub legume, reaching a height of 60-100 cm. In Vietnam, *S. tora* is widely distributed in localities with altitudes <1,000 m asl³.

Senna tora has been used since ancient times for both traditional herbal medicine and food⁴. Different parts of *S. tora* have been reported to contain bioactive compounds, including aurantioobtusin, tannins, saponins, steroids and alkaloids, which contribute to its *in vitro* antioxidant and antibacterial activities as well as its *in vivo* anti-inflammatory and analgesic effects^{1,5,6}. Aurantioobtusin, an anthraquinone compound isolated from dried *S. tora* seeds, is a key active ingredient with potential as a therapeutic agent against various inflammatory diseases⁷. The demand for *S. tora* seed is consequently increasing in both national and international markets.

In Vietnam, the supply of aurantioobtusin, a key anthraquinone compound derived from *S. tora* still depends largely on wild collection and imported raw materials. The growing demand for herbal ingredients in recent years has emphasized the importance of improving the cultivation techniques and productivity of this species. Among the agronomic factors affecting crop performance, fertilizer management plays a crucial role in regulating plant growth, development and yield. Balanced fertilizer application enhances soil fertility, promotes vigorous root and shoot growth and stimulates photosynthetic efficiency, thereby increasing biomass accumulation and seed productivity⁸. However, excessive or imbalanced fertilization can decrease nutrient use efficiency and negatively affect the concentration and quality of medicinal compounds, particularly anthraquinones, the major bioactive constituents in *S. tora* seeds⁹.

As a non-nitrogen-fixing member of the Caesalpinaceae family¹⁰, *S. tora* responds positively to nitrogen (N) fertilization^{8,11}. Nevertheless, information on the optimal N requirement for this species remains limited. While adverse effects of excessive N have been observed in legumes such as

mung bean (*Vigna radiata*)¹² and French bean (*Phaseolus vulgaris*)¹³, studies on *S. tora* have reported significant improvements in growth, yield and seed quality at N rates up to 120 kg/ha in the Sudan Savanna Ecological Zone, Nigeria⁸. In semi-arid environments, N fertilization exerts a more pronounced effect on *S. tora* compared to phosphorus (P) or potassium (K) application^{14,15}. Nitrogen is one of the most essential macronutrients for plant metabolism, serving as a structural component of amino acids, proteins, enzymes and nucleic acids¹⁶. When N availability is limited, plants tend to allocate more resources to root development, whereas higher N supply favors vegetative and leaf growth¹⁷. Although *S. tora* exhibits a high N demand for both biomass accumulation and aurantioobtusin biosynthesis¹⁴, systematic research on the optimal N fertilization rate under Vietnamese conditions remains scarce. Therefore, optimizing fertilizer type and dosage is essential to ensure sustainable production and consistent quality of *C. tora* under different ecological conditions.

This study comprises a field experiment with varying N applications on *S. tora*. Biomass and aurantioobtusin concentration were measured in the seeds to determine the optimal N supply.

MATERIALS AND METHODS

Plant material and experimental site: This field study was performed from February to November 2023 at the Research Center for Medicinal Plants-National Institute of Medicinal Materials (20°53 N, 105°44E, 20 m asl) in Ha Noi, Vietnam. We evaluated the effect of N application rate on the growth parameters, yield and aurantioobtusin content of *S. tora*. Seeds of *S. tora* were provided in house. They were planted in plots with an area of 10 m². The within-row plant spacing was 40×40 cm, giving a population density of ~ 62,500 plants/ha. Soil analyses were conducted in the laboratory of the Soils and Fertilizers Institute, Vietnam. The key soil properties and the sources of the analytical methods are in Table 1.

Experimental methods and treatments: A randomized block design with three replications was used to evaluate the N treatment effects. Nitrogen was added at six rates (0, 35, 70, 105, 140 and 175 kg N/ha) in the form of urea fertilizer. Other nutrients were applied at sufficient rates to optimize *S. tora* growth: 95 kg/ha P₂O₅ and 180 kg/ha K₂O in the superphosphate and potassium chloride, respectively. Phosphorus (100%) was applied at planting. Nitrogen fertilizer was applied in four split doses: 10% prior to the development

Table 1: The initial nutrient analysis of the soil

Soil properties	Value	Method/Reference
Soil pH _{KCl}	6.81	1 mol/L KCl
Cation exchange capacity-CEC (cmol/kg)	102.0	Walkley and Black ¹⁸
Organic matter-OM (%)	13.9	Walkley and Black ¹⁸
Organic C-OC (%)	0.59	Walkley and Black ¹⁸
Total N (N%)	0.07	Subbiah and Asija ¹⁹
Total P (P%)	3.5	Olsen <i>et al.</i> ²⁰
Total K (K%)	0.89	Hunter and Pratt ²¹
Available N (g/kg)	0.63	Subbiah and Asija ¹⁹
Available P (g/kg)	17.77	Olsen <i>et al.</i> ²⁰
Available K (g/kg)	6.27	Rayment and Lyons ²²

of primary branches, 25% before the emergence of secondary branches, 40% before flowering and the remaining 25% before pod setting. Potassium was applied in two equal splits: 50% before flowering and 50% before pod setting.

Observations: Measurements of plant height, stem diameter, number of branches and leaf area index (LAI) were recorded from ten randomly selected plants per plot. Plant height was measured from the ground to the base of the topmost fully expanded leaf. Stem diameter was measured at 3 cm above the stem base using slide calipers. The number of branches in *S. tora* was recorded by counting all primary branches emerging directly from the main stem immediately before harvesting. A destructive harvest was conducted 210 d after sowing. Yield components recorded per sampled plant included number of pods per plant, number of seeds per pod, number of filled seeds per pod and 1000-seed weight. Seed yield was calculated from plot yield.

The Leaf Area Index (LAI) was calculated as:

$$\text{Leaf area index} = \frac{\text{Leaf area (m}^2\text{)}}{\text{Ground area (m}^2\text{)}}$$

Aurantioobtusin content was quantified as described by Mao *et al.*²³ using High Performance Liquid Chromatography (HPLC), following the extraction and processing procedures outlined in the Vietnam Pharmacopoeia (2017 Edition)⁴. Briefly, 0.5 g of powdered *S. tora* was washed in 70% ethanol and filtered through a 710 µm 200 mm-sieve. The retentate was dried, weighed, mixed with 50 mL of mobile phase and soaked for 2 hrs. The mixture was then ultrasonicated for 30 min, cooled and any weight loss was compensated by adding mobile phase. The resulting suspension was filtered through a 0.45 µm membrane filter and the filtrate was analyzed.

The main analytical equipment used was a Shimadzu HPLC system (Shimadzu, Japan) comprising an LC-20AD pump, a SIL-20AHT autosampler and a diode-array detector. Data acquisition and processing were performed with

LabSolutions software (Shimadzu). Chromatographic separation was achieved on an Agilent Eclipse Plus C18 column (250×4.6 mm, 5 µm). The aurantioobtusin standard compound was from Bio purify Phytochemicals Ltd., batch numbers 111,900-201,504 (Chengdu, People's Republic of China). A linear regression between the logarithms of peak responses and the logarithms of aurantioobtusin concentrations (mg/mL) from standard solutions was used to determine the concentration of aurantioobtusin (C, mg/mL) in the sample extracts as follows:

$$X(\%) = \frac{C \times V \times P \times 100}{1000 \times m (100 - B)} \times 100$$

where:

- X = Aurantioobtusin content (%)
- C = Concentration of the aurantioobtusin in the sample solution (mg/mL)
- V = Volume of the test solution (mL)
- P = Purity of the standard substance (%)
- M = Weight of *S. tora* seed used to prepare the sample solution (g)
- B = Moisture content of the sample (%)

Agronomic efficiency (AE) of N-fertilizer was calculated using the formula²⁴:

$$AE = \frac{Y_n - Y_0}{F}$$

where, Y_n is the yield of *S. tora* (kg/ha) under fertilizer application, Y_0 is the yield of *S. tora* (kg/ha) without N-fertilizer and F (kg/ha) is the amount of N-fertilizer.

Statistical analysis: Statistical data analysis was performed using CropStat 7.2 (International Rice Research Institute, Manila) and Microsoft Excel 2016 (Microsoft Corporation, Washington USA) to evaluate *S. tora* parameters. The significance of treatment effects on aurantioobtusin content

and yield were assessed at $p < 0.05$. Regression analyses to explore relationships between N rates and measured parameters were conducted using R-Studio software (Systat Software Inc., California, USA).

RESULTS

Effect of nitrogen fertilizer on growth parameters of *S. tora*:

Application of increasing N rates resulted in significant improvements in plant height, stem diameter and number of branches of *S. tora* ($p < 0.05$, Table 2). Under the N3, N4 and N5 treatments, average plant heights were 188.50, 189.63 and 190.43 cm and stem diameters reached 3.47, 3.63 và 3.77 cm. However, these differences were not significant ($p > 0.05$, Table 2).

The average number of branches per plant differed significantly among treatments, with values ranging from 13 to 17 ($p < 0.05$). The N3 treatment (16 branches) was significantly higher than the lower treatments but was statistically comparable to N4 and N5, indicating no additional significant increase in branching when N was increased from 105 to 175 kg N/ha.

The leaf area index of *S. tora* was significantly influenced by N application throughout the growth period ($p < 0.05$, Fig. 1). The LAI values increased steadily from 60 DAP (0.13-0.33) to a peak at 180 DAP (10.51-12.97), with all N treatments consistently outperforming the control. Although the highest LAI was recorded under N5, no significant differences were observed among N3, N4 and

N5 during the later stages (150-210 DAP). Considering both statistical significance and resource efficiency, N3 (105 kg N/ha) is the most favorable treatment. It produced a high LAI comparable to higher N rates while avoiding excessive N input, thereby supporting improved seed yield and quality with lower fertiliser use.

Effect of nitrogen application rates on *S. tora* yield components:

The number of pods per plant, number of filled pods per plant, seeds per pod and 1000 seed weight of *S. tora* were significantly affected by N application ($p < 0.05$, Table 3). The control (N0) produced the lowest values for all traits, while higher N rates generally increased pod and seed attributes. The N3 treatment (105 kg N/ha) gave the best overall combination of characteristics: 470 pods per plant, 432 filled pods per plant, 24 seeds per pod and the highest 1000-seed weight (21.40 g). For most yield components (pods per plant, filled pods per plant and seeds per pod) the higher rates (N3, N4, N5) did not differ significantly from each other, indicating no further significant gain beyond 105 kg N/ha. Considering both performance and input efficiency, N3 is therefore the most favorable treatment.

Effect of nitrogen application rates on *S. tora* seed and foliage yield:

Increasing the N application rate from 0 to 170 kg/ha significantly decreased the yield in both the root and foliage *S. tora* (Fig. 2) ($p < 0.05$). The curvilinear regression

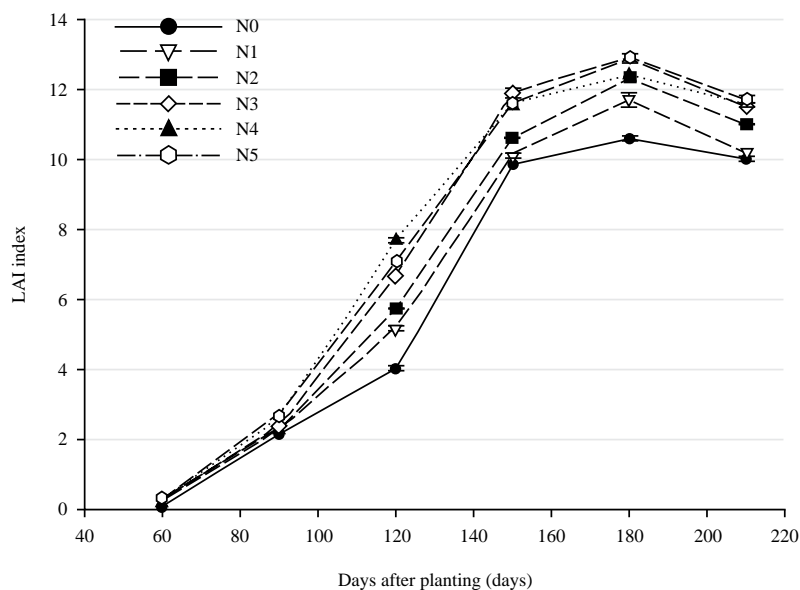


Fig. 1: Effect of nitrogen rates on LAI of *S. tora*

N0 (0 kg N/ha), N1 (35 kg N/ha), N2 (70 kg N/ha), N3 (105 kg N/ha), N4 (140 kg N/ha) and N5 (175 kg N/ha)

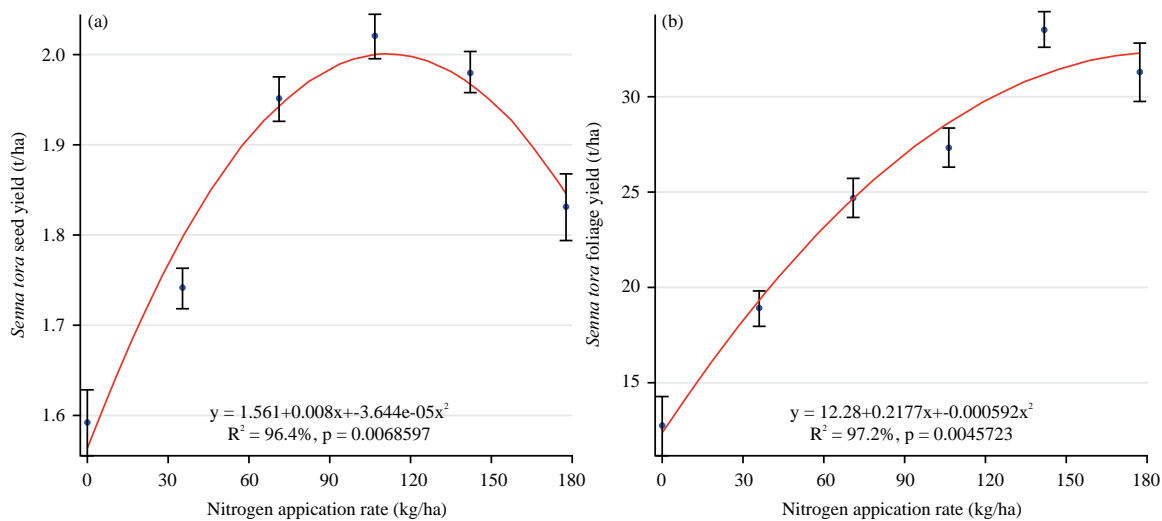


Fig. 2(a-b): Effect of (a) Nitrogen application rates on seed yield and (b) Foliage yield of *S. tora*
 N0 (0 kg N/ha), N1 (35 kg N/ha), N2 (70 kg N/ha), N3 (105 kg N/ha), N4 (140 kg N/ha) and N5 (175 kg N/ha), The red line represents the fitted quadratic regression model. Error bars indicate the standard error

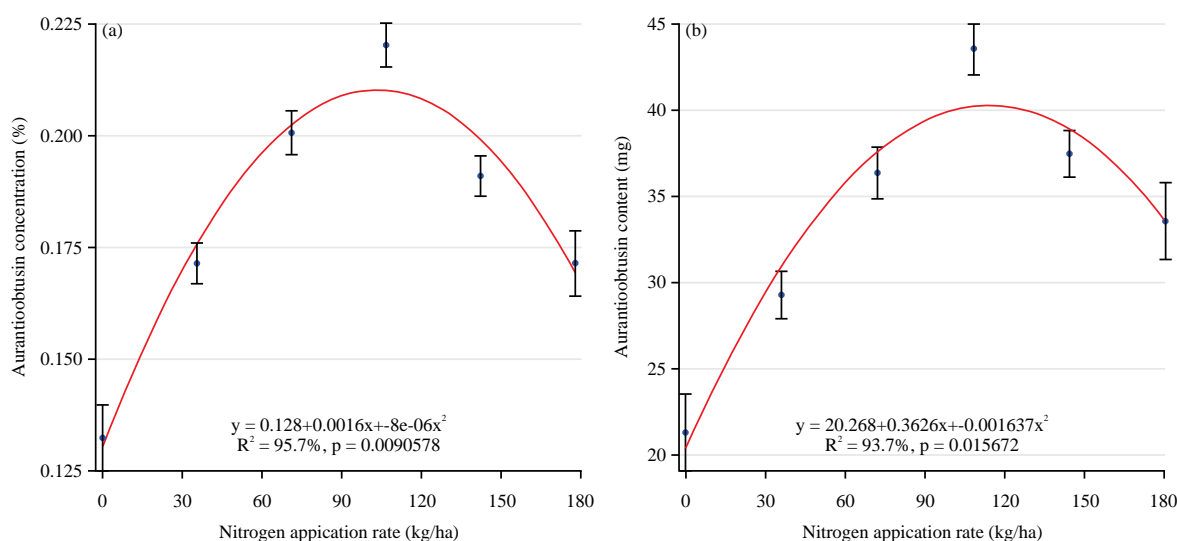


Fig. 3(a-b): Relationship between (a) N application rates and aurantioobtusin concentration and (b) Aurantioobtusin concentration in *S. tora* seeds
 N0 (0 kg N/ha), N1 (35 kg N/ha), N2 (70 kg N/ha), N3 (105 kg N/ha), N4 (140 kg N/ha) and N5 (175 kg N/ha), The red line represents the fitted quadratic regression model. Error bars indicate the standard error

Table 2: Effect of nitrogen rates on growth parameters of *S. tora*

Treatment	Plant height (cm)	Stem diameter (cm)	Number of branches
N0	171.10 ^c	1.87 ^c	13 ^c
N1	181.30 ^b	2.43 ^b	15 ^b
N2	185.70 ^{ab}	2.83 ^b	15 ^b
N3	188.50 ^a	3.47 ^a	16 ^a
N4	189.63 ^a	3.63 ^a	17 ^a
N5	190.43 ^a	3.77 ^a	17 ^a
CV (%)	2.6	9.4	4.0
LSD _{0.05}	4.86	0.51	0.92

N0 (0 kg N/ha), N1 (35 kg N/ha), N2 (70 kg N/ha), N3 (105 kg N/ha), N4 (140 kg N/ha) and N5 (175 kg N/ha), a, b, c: Different letters denote significant differences among treatments, Means within a column followed by the same letter are not significantly different and p<0.05

Table 3: Effect of nitrogen rates on yield components of *S. tora*

Treatment	Number of pods per plant	Number of filled pods per plant	Number of seeds per pod	1000 seed weight (g)
N0	423 ^b	382 ^c	21 ^b	19.27 ^b
N1	428 ^b	399 ^{bc}	21 ^b	19.17 ^b
N2	444 ^b	409 ^b	22 ^b	19.87 ^{ab}
N3	470 ^a	432 ^a	24 ^a	21.40 ^a
N4	470 ^a	436 ^a	23 ^a	21.10 ^a
N5	470 ^a	439 ^a	23 ^a	20.80 ^{ab}
CV (%)	5.1	3.3	8.2	5.0
LSD _{0.05}	24.56	22.55	1.32	1.83

N0 (0 kg N/ha), N1 (35 kg N/ha), N2 (70 kg N/ha), N3 (105 kg N/ha), N4 (140 kg N/ha) and N5 (175 kg N/ha), a, b, c: Different letters denote significant differences among treatments. Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 4: Effect of nitrogen application rate on nitrogen use efficiency of *S. tora*

Treatment	Yield increase over control (no N) (kg/ha)	Agronomic efficiency (kg/N)
N0	0	-
N1	100	2.86
N2	140	2.00
N3	460	4.38
N4	440	3.14
N5	410	2.34

N0 (0 kg N/ha), N1 (35 kg N/ha), N2 (70 kg N/ha), N3 (105 kg N/ha), N4 (140 kg N/ha) and N5 (175 kg N/ha)

model identified a maximum N rate for *S. tora* of 105 kg/ha. As N application increased from 0 to 175 kg/ha, *S. tora* seed yield increased by approximately 27%, from 1.59 to 2.02 t/ha ($p < 0.05$). However, when N application rate increased from 140 to 175 kg/ha the dry seed yield decreased by about 12% from 2.02 to 1.80 t/ha (Fig. 2a). At the optimal rate, N3 (105 kg/ha), seed yields were ~2.02 t/ha.

In contrast to seed yield, foliage yield showed a marked increase of about 70%, rising from 18.81 to 33.5 t/ha as N application increased from 0 to 140 kg/ha (Fig. 2b) and then decreased at 175 N but not significantly. That is, while higher N application maximized foliar biomass, it concurrently leads to a reduction in seed yield.

Effect of nitrogen application on aurantioobtusin concentration and content in *S. tora* seeds:

Nitrogen application rates had positive linear correlations with both aurantioobtusin concentration and total content in *S. tora* seeds (Fig. 3). In this experiment, aurantioobtusin concentration and content in *S. tora* seeds were significantly affected ($p < 0.05$) across six N application rates. Aurantioobtusin concentration almost doubled from 0.13% as the N application rose from 0 to 105 kg/ha, then decreased at the N4 and N5 rates. Similarly, aurantioobtusin content increased by approximately 27% when N application increased from 0 to N3 (105 kg/ha), but no significant effect was observed at N4 and N5 rates. The highest aurantioobtusin content was recorded at the N3 rate (105 kg/ha).

Effect of nitrogen application rate on nitrogen use efficiency of *S. tora*:

Nitrogen use efficiency can be assessed using various indicators; however, this study focuses on evaluating agronomic efficiency (AE) (Table 4).

Agronomic efficiency varied significantly among treatments, reflecting differences in N utilization at different application rates. At 35 kg N/ha, plants used N efficiently (2.86 kg product per kg N), indicating suitability during the early growth stage. Increasing the rate to 70 kg/ha reduced efficiency to 2.00. The highest agronomic efficiency (4.38) was recorded at 105 kg/ha, demonstrating that this level was optimal for maximizing yield response to N. At higher rates (140 and 175 kg/ha), agronomic efficiency declined (3.14 and 2.34, respectively).

DISCUSSION

All N treatments increased growth parameters and leaf area index (LAI) compared to the control (0 kg/ha). The highest plant height was observed at 140 kg/ha, while the maximum stem diameter was recorded at 105 kg/ha. Although no prior studies specifically address N effects on *S. tora* plant growth under field conditions, related research on *C. senna* (syn. *Senna tora*) reported positive responses to N, P and K fertilization¹⁴. Nitrogen stimulates leaf production and plant growth by enhancing cell division and tissue development²⁵⁻²⁷. Pratibha *et al.*²⁷ noted a 10.1% increase in *S. tora* growth index with N rates increasing from

50 to 475 kg/ha under rainfed conditions. Recent studies also confirm that N and P applications influence the growth of *Cassia angustifolia* L. plant height²⁸, with sufficient N promoting new cell formation and stem growth²⁹.

The leaf area index (LAI) quantifies the leaf area within an ecosystem and is a key variable in processes such as photosynthesis, respiration and precipitation interception³⁰. The LAI results aligned with established plant growth patterns. In this study, the LAI of *S. tora* was unaffected by N application. However, there was a positive correlation between leaf number and LAI, indicating that leaf number is a critical determinant of *S. tora* growth and biomass yield. This is evidenced by *S. tora* plants under N treatment exhibiting significantly greater plant height, stem development and yield compared to the control. The LAI results aligned with established plant growth principles, reinforcing the observed developmental trends.

Increased branch number and leaf area expanded the canopy, which likely enhanced canopy photosynthetic capacity and the supply of assimilates for flower and pod formation. As a result, N fertilization significantly increased pod number, filled pods, seeds per pod and 1000 seed weight ($p < 0.05$). These findings are consistent with reports for other legumes in which appropriate N fertilization improved effective flowers, pod set and seed filling^{31,32}. However, high N rates stimulated further vegetative growth without commensurate gains in reproductive performance. Although the highest pod counts were observed at the largest N rate, the best combination of seeds per pod and 1000 seed weight occurred at 105 kg/ha (24 seeds per pod, 1000 seed weight = 21.40 g). This pattern suggests a trade-off at excessive N: Greater pod initiation but reduced assimilate partitioning per seed, limiting seed filling. Similar trade-offs have been documented in other legumes where overapplication of N promotes vegetative sinks at the expense of reproductive sinks^{31,32}.

Field responses in this and other studies also reflect broader soil-plant interactions: Improved soil conditions (organic matter, microbial activity, growth regulators) can amplify the benefits of fertilization beyond immediate nutrient supply²⁶. Nonetheless, excessive fertilization can reduce crop quality and cause environmental harm such as nitrate accumulation and leaching³³. Agronomic gains must be balanced against environmental and economic costs^{34,35}. For example, Khammari *et al.*³⁶ reported a roughly 30% increase in seed yield of *Cassia angustifolia* L. with N rates up to 150 kg/ha, but peak responses and absolute yields vary by species, environment and management.

The N use efficiency provide a basis for determining the optimal N rate to avoid waste, environmental pollution and nitrate residues in medicinal materials. This also supports the establishment of balanced and sustainable fertilization practices. In this study, the 105 kg/ha treatment produced both the highest yield and the greatest agronomic efficiency, confirming it as the optimal N rate for *S. tora* seed production. This finding underscores the importance of identifying the N threshold that maximizes yield while minimizing costs and environmental impacts.

The present study demonstrated that aurantioobtusin concentration and content in *S. tora* seeds increased with N application up to the N3 rate (105 kg N/ha) and declined at higher levels, indicating a typical dose-response relationship in secondary metabolite synthesis. This suggests that moderate N enhances the biosynthetic capacity and precursor supply for aurantioobtusin accumulation, whereas excessive N may cause dilution effects or redirect assimilates toward vegetative growth or alternative metabolic pathways. Similar patterns have been reported in other crops, where optimal fertilization promoted bioactive compound accumulation but further N increases reduced their levels³⁷. In *Senna obtusifolia*, aurantioobtusin content also varied among genotypes and environmental conditions, highlighting the role of both genetics and nutrient management in regulating secondary metabolism²³. The significant positive correlations observed in this study ($p < 0.05$) support the conclusion that N is a limiting factor for aurantioobtusin biosynthesis. Therefore, N3 can be recommended as the most suitable N rate for maximizing aurantioobtusin yield while avoiding unnecessary fertilizer input.

CONCLUSION

This study demonstrates that 105 kg N ha⁻¹ N optimized the yield of *S. tora* seed and aurantioobtusin yield. These results underline the importance of carefully calibrated N fertilization in optimizing the cultivation of *S. tora* as a medicinal plant in northern Vietnam. Optimizing N fertilization will promote consistent yields of *S. tora* and increase the supply of the therapeutic compound aurantioobtusin.

SIGNIFICANCE STATEMENT

This study provides new insight into how nitrogen nutrition regulates both productivity and bioactive compound accumulation in *Senna tora*. It highlights the critical balance between vegetative growth and reproductive performance,

offering evidence-based guidance for improving medicinal plant quality. The findings support efficient fertilizer management strategies to enhance crop value and ensure sustainable production systems.

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