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Antimicrobial activity of essential oils from introduced varieties of *Dracocephalum moldavica* and *Hyssopus officinalis*

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ABSTRACT

Background: Aromatic and medicinal plants are highly valued for their potential influence on functional food systems due to their content of bioactive compounds and health-promoting properties. These plants have antimicrobial and antioxidant effects. Dracocephalum moldavica and Hyssopus officinalis essential oils have notable biological activity among aromatic and medicinal plants. As the demand for natural, plant-based alternatives to synthetic food preservatives has grown, these species have gained attention for their applications as functional foods. This study investigates the antimicrobial properties of their essential oils to evaluate their potential as natural, health-enhancing ingredients in functional foods.

Objective: This research aimed to evaluate the antimicrobial activity of essential oils extracted from *Dracocephalum moldavica* and *Hyssopus officinalis* while cultivating such plants under Armenian conditions. Furthermore, their potential as natural antimicrobial agents in functional food systems was assessed.

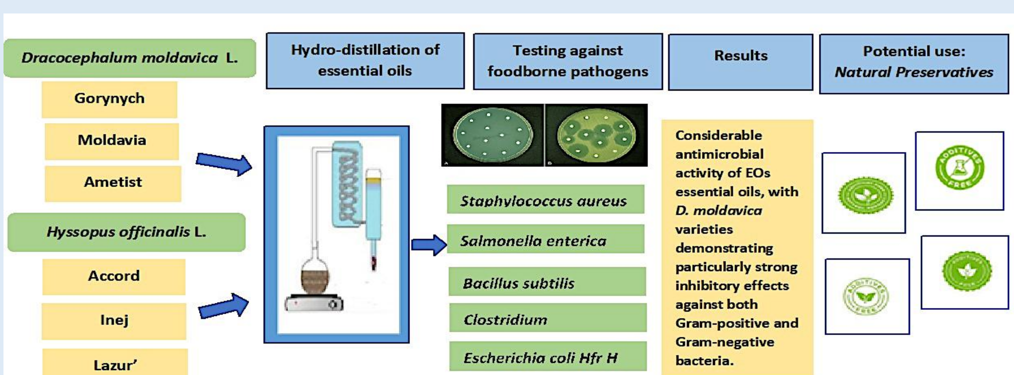
Methods: This study involved three varieties of *Dracocephalum moldavica* (Moldavia, Gorynych, and Ametist) and three varieties of *Hyssopus officinalis* (Accord, Inej, and Lazur). Essential oils were extracted through hydro-distillation. Their antibacterial properties were evaluated against five key sanitary indicator microorganisms: *Staphylococcus aureus*, *Salmonella enterica*, *Bacillus subtilis*, *Clostridium perfringens*, and *Escherichia coli* Hfr H. The antimicrobial activity was determined using the agar disk diffusion method, by which the inhibition zones around the oil-treated disks were measured.

Results: The essential oil *Dracocephalum moldavica* consistently demonstrated more substantial antimicrobial effects than *Hyssopus officinalis*. This was observed across all tested microorganisms and at both concentrations (5% and 0.5%). At 5%, the least effective D. moldavica variety (Ametist) produced inhibition zones between 18.1 mm (*E. coli*) and 33.0 mm (*B. subtilis*). The most active H. officinalis variety (Inej) ranged from 16.2 mm to 21.0 mm. Notably, *D. moldavica* (Gorynych) showed a significant effect against *S. aureus* with a 41.2 mm inhibition zone, nearly double the distance measured for *H. officinalis* (Accord: 18.3 mm). At 0.5%, *D. moldavica* oils maintained high activity (up to 36.0 mm), while *H. officinalis* rarely exceeded 16.7 mm. Both oils were effective against gram-positive bacteria, yet *D. moldavica* was more beneficial.

Novelty: This study uniquely compares the antimicrobial properties of essential oils derived from varieties of *Dracocephalum moldavica* and *Hyssopus officinalis* cultivated under Armenian conditions. The findings revealed that *D. moldavica* oils exhibit stronger and more consistent antimicrobial effects, especially against gram-positive bacteria, highlighting their promising application as natural preservatives in functional food systems.

Conclusion: The pronounced antimicrobial activity of essential oils from *D. moldavica* varieties, especially Gorynych, accentuates their potential to be used as natural preservatives. Their effectiveness at low concentrations ensures cost-efficiency and a lower likelihood of undesirable side effects. In contrast, while *H. officinalis* essential oils show more moderate activity; they may hold value in applications that prefer gentler antimicrobial action.

Keywords: Moldavian dragonhead, hyssop, *Staphylococcus aureus*, *Salmonella enterica*, *Bacillus subtilis*, *Clostridium perfringens*, *Escherichia coli* Hfr H.



Graphical Abstract: Antimicrobial activity of essential oils from introduced varieties of *Dracocephalum moldavica* and *Hyssopus officinalis.*

INTRODUCTION

Aromatic and medicinal plants are recognized as essential contributors to the development of functional food systems due to their rich content of bioactive compounds that may have functional food and nutraceutical applications [1-3]. These compounds enhance foods' nutritional and sensory properties while providing health-promoting effects such as antimicrobial, antioxidant, and anti-inflammatory activities [4,5]. As the global demand for plant-based, clean-label ingredients rises, essential oils derived from *Dracocephalum moldavica* (Moldavian dragonhead) and *Hyssopus officinalis* (hyssop) have gained attention as natural alternatives to synthetic preservatives. The components within these plants exhibit a wide range of biological activities [6,7] such as antimicrobial properties [8-10]. These properties allow aromatic and medicinal plants to be strong candidates for use as functional ingredients [11-13].

Dracocephalum moldavica L. (Moldavian dragonhead), a member of the Lamiaceae family, is valued for its aromatic and medicinal properties [14]. Traditionally used in herbal medicine across Eurasia, the plant has been associated with antioxidant, antimicrobial, and anti-inflammatory effects, primarily attributed to essential oil components. These components include geranyl acetate, geranial, and neral [15,16]. Similarly, *Hyssopus officinalis L.* (hyssop), another well-known aromatic species of the same family, has long been used in traditional medicine to treat respiratory disorders, digestive issues, and inflammation [17,18]. Its essential oil contains bioactive compounds including pinocamphone, isopinocamphone, and limonene, contributing to its pharmacological and antimicrobial effects [19,20]. These properties have made both species promising candidates for incorporation into functional foods, which enhances their health benefits and safety profiles.

Beyond their functional roles in food systems, the cultivation of these species holds relevance for climate-resilient agriculture. As climate change is a prominent issue, with excessive climate variability, using diverse and stress-tolerant crops such as *D. moldavica* and *H. officinalis* may contribute to the diversification of agricultural systems. This diversification maintains ecological stability, enhances resilience, and fosters sustainable and secure food production operations [21,22]. Since both species are found in the wild flora of Armenia [23], the agro-ecological conditions appear favorable for broader cultivation methods. Despite the presence of these species in Armenia, systematic breeding programs for hyssop and Moldavian dragonhead have not been established, limiting the availability of characterized varieties with standardized and optimized chemical compositions.

Given the global interest in replacing synthetic additives with plant-based, health-promoting alternatives, the functional potential of essential oils derived from these plants warrants deeper investigations. Their antimicrobial properties may enhance food safety and shelf life, which aligns with current trends in the development of functional foods [24].

This study assessed the antimicrobial activity of essential oils extracted from introduced Dracocephalum moldavica and Hyssopus officinalis varieties, cultivated under Armenian conditions. By evaluating their effectiveness against foodborne pathogens, this research supports the potential integration of these plants as natural preservatives, while contributing to the expansion of resilient crop options that promote sustainable agriculture. The results contribute to a stronger understanding of their potential application as natural antimicrobial agents, while supporting future efforts toward their integration in functional food systems.

MATERIALS AND METHODS

Plant material: The study was conducted using three introduced varieties of *Dracocephalum moldavica* (Moldavia, Gorynych, and Ametist) and three varieties of

Hyssopus officinalis (Accord, Inej, and 'Lazur'). These varieties were accessible from the Genebank of the Scientific Centre of Vegetable and Industrial Crops (SCVIC) [25]. The selected plant varieties were cultivated in the experimental plot of the SCVIC, in 2024, which is in Darakert village, Ararat province, Armenia. Seeds were sown in cells at the end of March and placed in a germination chamber. Once germinated, the seedlings were cultivated under greenhouse conditions before being transplanted to the open field in mid-May. Laboratory experiments assessing the antimicrobial activity of essential oils were conducted at the Laboratory of Plant Biotechnology, Phytopathology, and Biochemistry of SCVIC. Aerial parts of *H. officinalis* and *D. moldavica* were collected during the flowering stage (July-August, depending on variety). The timing of collection was based on previous studies, which indicated that the highest concentration of essential oils is found in the inflorescences during the peak flowering phase. The specific varieties evaluated in this study were informed by previous studies. These studies concluded that the agro-climatic conditions of the Ararat Valley promote the growth of varieties of Moldavian dragonhead and hyssop [26,27]. These varieties showed promising results in both agricultural productivity and environmental sustainability. Ultimately, this finding supports their potential for commercial cultivation in Armenia. Notably, the antimicrobial properties of the selected varieties have not been previously studied, emphasizing the novelty and relevance of this investigation.

Essential Oil Preparation: The essential oils (EOs) of *Hyssopus officinalis* L. and *Dracocephalum moldavica* L. were obtained through hydro-distillation [28] using a Ginsberg apparatus [29]. This method is commonly used for extracting essential oils from plant materials. It involves using steam as an extracting agent to vaporize and release volatile compounds from the raw plant material [30,31]. The obtained EOs were dehydrated over anhydrous sodium sulfate, filtered through 0.22 µm membrane filters, and stored at 4°C. The yield of essential oils was calculated by weighing the extracted oils and the result was expressed as a percentage of essential oil yield per 100 g of plant material [32].

Evaluation of Antibacterial Activity: The antibacterial activity of the EOs was tested against five important sanitary indicator microorganisms:

- ***Staphylococcus aureus*** (*ATCC 25923*) – A common foodborne pathogen producing heat-stable enterotoxins that can cause food poisoning. This microorganism is frequently found in improperly stored meat, dairy products, and salads.
- ***Salmonella enterica*** (*ATCC 14028*) – A major foodborne pathogen responsible for salmonellosis, typically transmitted through contaminated meat, poultry, eggs, and other animal-derived products.
- ***Bacillus subtilis*** (*ATCC 6633*) – A non-pathogenic, spore-forming bacterium frequently used as a model organism in food and pharmaceutical testing.
- **Clostridium perfringens** (*ATCC 13124*) – A toxin-producing bacterium that can cause serious foodborne illness and is often associated with improperly canned or stored food.
- ***Escherichia coli Hfr H*** (*ATCC 47030*) – A laboratory strain used in genetic studies, not typically linked to foodborne illness. In addition, the pathogenic strain *E. coli* O157:H7, a recognized foodborne pathogen, was included in the study.

Agar Disk Diffusion Assay: Antibacterial activity was assessed using the agar disk diffusion method. A 0.1 mL suspension of the test microorganism was evenly spread on the surface of a solidified nutrient medium in a Petri dish using a sterile spatula [33]. Sterile paper disks (5 mm diameter) were placed on the inoculated agar surface at equal distances, with a margin of 1.5–2.0 cm from the edge of the dish. EOs solutions in ethanol (10 µL per disk) were applied to the disks. The plates were stored at 4 °C for oil absorption, followed by incubation at 37 °C for 24 hours [34]. Antibacterial activity was evaluated by measuring the diameter of inhibition zones formed around the disks. The diameters were measured using a calliper.

RESULTS AND DISCUSSIONS

The EOs of *Dracocephalum moldavica* (Gorynych, Moldavia, Ametist) showed consistently more potent antimicrobial activity than *Hyssopus officinalis (*Accord, Inej, Lazur'*). This finding was sustained among* all tested microorganisms and at both concentrations. The Gorynych variety of *D. moldavica* displayed the largest inhibition zones, indicating it may be the most promising for antimicrobial applications (Tables 1,2).

Table 1. Antimicrobial activity of the essential oil of *Dracocephalum moldavica.*

Microorganism	Inhibition zone[2]					
	5%			0,5%		
	Gorynych	Moldavia	Ametist	Gorynych	Moldavia	Ametist
Staphylococcus aureus	41.2 ±0.3	38.7 ±0.4	32.3 ±0.2	36.0 ±0.4	31.9 ±0.6	28.7 ±0.3
Salmonella enterica	29.4 ±0.5	26.8 ±0.4	26.6 ±0.4	24.4 ±0.1	21.1 ±0.5	20.4 ±0.3
Bacillus subtilis	38.2 ±0.6	31.2 ±0.1	33.0 ±0.1	31.3 ±0.4	26.0 ±0.5	27.7 ±0.6
Clostridium perfringens	22.1 ±0.6	23.7 ±0.2	18.7 ±0.5	18.7 ±0.6	18.2 ±0.4	12.0 ±0.6
Escherichia coli Hfr H	28.1 ±0.4	27.0 ±0.2	18.1 ±0.2	23.3 ±0.1	22.7 ±0.4	13.8 ±0.5

[2] including diameter of disc (6 mm), values are given as mean ± SD (3 replicates)

Table 2. Antimicrobial activity of the essential oil of *Hyssopus officinalis*

Microorganism	Inhibition zone[1]					
	5%			0,5%		
	Accord	Inej	Lazur'	Accord	Inej	Lazur'
Staphylococcus aureus	18.3 ±0.2	17.7 ±0.6	15.8 ±0.3	12.0 ±0.5	11.9 ±0.4	11.2 ±0.1
Salmonella enterica	17.6 ±0.3	18.8 ±0.5	15.4 ±0.5	12.4 ±0.6	13.6 ±0.4	10.1 ±0.3
Bacillus subtilis	15.6 ±0.6	16.2 ±0.6	15.0 ±0.4	10.1 ±0.4	12.0 ±0.3	11.7 ±0.4
Clostridium perfringens	16.1 ±0.6	20.7 ±0.3	18.7 ±0.3	11.7 ±0.2	16.2 ±0.2	14.0 ±0.1
Escherichia coli Hfr H	18.1 ±0.4	21.0 ±0.2	17.2 ±0.4	12.3 ±0.1	16.7 ±0.4	12.8 ±0.2

[1] including diameter of disc (6 mm), values are given as mean ± SD (3 replicates)

At a 5% concentration, *H. officinalis* essential oils exhibited moderate antimicrobial activity, with inhibition zones ranging from 15.0 mm to 20.7 mm. The highest activity was observed against *Clostridium perfringens* (20.7 mm, Inej variety) and *Escherichia coli* Hfr H (21.0 mm, Inej variety). At a 0.5% concentration, the inhibition zones decreased, ranging from 10.1 mm to 16.7 mm, indicating a dose-dependent response. In contrast, *D. moldavica* essential oils demonstrated significantly higher antimicrobial activity. At a 5% concentration, inhibition zones ranged from 18.1 mm to 41.2 mm, with the Gorynych variety showing the highest activity against *Staphylococcus aureus* (41.2 mm). Substantial inhibition zones were observed at a 0.5% concentration, ranging from 12.0 mm to 36.0 mm. This indicates potent antimicrobial properties even at low concentrations (Table 1,2).

Across both concentrations and all tested microorganisms, *Dracocephalum moldavica* consistently outperformed *Hyssopus officinalis*. At 5% concentration, the weakest D. moldavica variety (Ametist) showed inhibition zones ranging from 18.1 mm (*E. coli*) to 33.0 mm (*B. subtilis*). In contrast, the most substantial *H. officinalis* variety (Inej) ranged from 16.2 mm (*B. subtilis*) to 21.0 mm (*E. coli*). This pattern is particularly notable against *Staphylococcus aureus*, where *D. moldavica* (Gorynych) reached 41.2 mm, nearly double the distance recorded from *H. officinalis* (Accord: 18.3 mm). Even at 0.5%, *D. moldavica* retained high efficacy with inhibition zones measuring up to 36.0 mm, while *H. officinalis* inhibition rarely exceeded 16.7 mm. This interspecies difference highlights the superior antimicrobial potential of *D. moldavica* oils, likely due to higher concentrations of bioactive monoterpenoids [35].

Comparison of Sensitivity: Gram-positive vs. Gram-negative Bacteria:

Dracocephalum moldavica

- At 5% concentration, gram-positive bacteria (particularly *Staphylococcus aureus* and *Bacillus subtilis*) exhibited larger inhibition zones than gram-negative strains. For example, *S. aureus* (41.2 mm in Gorynych) and *B. subtilis* (38.2 mm in Gorynych) vs. *E. coli* (28.1 mm) and *S. enterica* (29.4 mm).
- Although the same trend holds at 0.5, inhibition zones decreased across the board. gram-positive strains showed greater sensitivity, especially *S. aureus* and *B. subtilis*.
- *Consequence*: *D. moldavica* essential oil is more effective against gram-positive bacteria.

Hyssopus officinalis

- At 5% concentration, the differences are less pronounced, but still:
  - *S. aureus*, *C. perfringens*, and *B. subtilis* show inhibition zones in the 15.6–20.7 mm range.
  - Gram-negative bacteria (*S. enterica*, *E. coli*) range from 15.4 to 21 mm, with some overlap.
- At 0.5%, inhibition zones reduce sharply for Gram-negative strains, particularly in the *samples of S. enterica (as low as 10.1 mm) and E. coli (12.3 mm)*. However, gram-positive strains retain slightly larger zones.
- *Consequence*: *H. officinalis* oil shows greater efficacy against gram-positive bacteria, although the difference is less distinct than *D. moldavica*.

As summarized in Table 3, the EOs of *D. moldavica* demonstrated notably higher inhibition zones across all tested microorganisms, compared to those of *H. officinalis*. This was particularly observed against gram-positive bacteria such as *S. aureus* and *B. subtilis*. These findings are consistent with results reported in previous studies by other authors, which attribute the potent antimicrobial activity of *D. moldavica* to its rich content of geranial and neral, which was revealed through analyses of essential oil composition [36].

Table 3. Comparative Sensitivity of Tested Microorganisms to Essential Oils of *Dracocephalum moldavica* and *Hyssopus officinalis*

Microorganisms	**Observed Sensitivity to Essential Oils**
Gram-positive bacteria:	
Staphylococcus aureus	● *D. moldavica* (Gorynych): 41.2 mm (5%), 36.0 mm (0.5%) ● *H. officinalis* (Accord) varieties max out at: 18.3 mm (5%), 12.0 mm (0.5%)- much lower
Bacillus subtilis	● Highest: *D. moldavica* (Gorynych – 38.2 mm at 5%, 31.3 mm at 0.5%) ● *H. officinalis* (Inej) - maxes out around 16.2 mm (5%), 12.0 mm (0.5%)
Clostridium perfringens	● *D. moldavica* (Moldavia): 23.7 mm (5%); Gorynych (18.7 mm at 0.5%) ● *H. officinalis* (Inej): reaches 20.7 mm and 16.2 mm — close but still less active
Gram-negative bacteria:	
Salmonella enterica	● *D. moldavica* (Gorynych) shows again stronger results: 29.4 mm (5%), 24.4 mm (0.5%) ● *H. officinalis* (Inej): peaks at 18.8 mm (5%), 13.6 mm (0.5%)
Escherichia coli Hfr H	● *D. moldavica*: Gorynych: 28.1 mm (5%), 23.3 mm (0.5%) ● *H. officinalis* top value (Inej): 21.0 mm (5%), 16.7 mm (0.5%) –

The observed superior antimicrobial activity of *D. moldavica* essential oil aligns with previous research. For instance, Hashemi et al. observed inhibition zones of 40.0 mm against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella Typhimurium*. This highlights its broad-spectrum efficacy. The minimal inhibitory concentrations (MICs) reported in the study were low, such as 0.78% for *S. aureus* and *S. Typhimurium*, which indicates a high potency [37].

A study by Nazemisalman et al. demonstrated that *D. moldavica* essential oil exhibited significant antibacterial activity against *Lactobacillus acidophilus*, with inhibition zones measuring 36 mm [38].

While *H. officinalis* essential oils have been recognized for their antimicrobial properties, their efficacy appears lower. Studies have reported inhibition zones ranging from 12 mm to 20 mm against various bacterial strains, consistent with the current study's moderate activity [39].

This pattern is consistent with the general understanding that Gram-positive bacteria tend to be more susceptible to essential oils due to their less complex cell wall structures and lack of the outer membrane that characterizes Gram-negative bacteria, which acts as a barrier to many compounds [40].

Compared to previous research investigating different varieties of hyssop under conditions similar to those of Belarus [41], the inhibition zones observed in our study were slightly larger. This may be due to higher temperatures during the flowering stage, which may influence the chemical composition and enhance the antimicrobial activity of essential oils. A study by Heydari et al. examined the effect of heat stress on Mentha x piperita and Mentha arvensis. It was concluded that EOs extracted from plants subjected to higher temperatures during flowering exhibited increased antimicrobial activity against certain bacterial strains. This was attributed to changes in the chemical composition of the oils, particularly in monoterpene content [42]. Additionally, research on Thymus vulgaris L. demonstrated that the timing of harvest during different flowering phenophases significantly affected its essential oils' antibacterial and anti-biofilm activities. Essential oils obtained at the beginning of the flowering period exhibited the highest antibacterial activity, which was linked to variations in the concentration of bioactive compounds such as thymol and γ-terpinene [43]. These studies suggest that higher temperatures during the flowering stage can alter the composition of EOs, potentially enhancing their antimicrobial properties.

While the agar diffusion method used in this study is a widely accepted technique for preliminary screening of antimicrobial activity, it is essential to note that quantitative comparisons of inhibition zones across different studies or laboratories may be affected by methodological variations, such as inoculum density, diffusion rate, or agar composition.

This study presents the initial step to evaluate the antimicrobial potential of essential oils derived from *Dracocephalum moldavica* and *Hyssopus officinalis* varieties introduced in Armenia. Further investigations are necessary to determine the effective concentrations required for real food applications, considering matrix effects and possible impacts on sensory properties. Future research will assess the practicality of these essential oils as functional ingredients in food systems.

The antimicrobial efficacy of essential oils is closely linked to their chemical composition. *Dracocephalum moldavica* EOs contain high levels of geranyl acetate, geranial, and geraniol, which are known for their antimicrobial properties. Geraniol has been evaluated for safety, with studies indicating a favorable toxicological profile [44].

While this study did not include GC-MS analysis, it is acknowledged that compositional variations among plant varieties can influence antimicrobial activity. Future research should incorporate detailed chemical profiling to correlate specific constituents with bioactivity.

Scientific Innovation and Practical Implications: This study presents a novel comparison of essential oils from introduced varieties of *Dracocephalum moldavica* and *Hyssopus officinalis* cultivated under Armenian conditions. Unlike prior studies focusing on single cultivars, this research evaluates multiple varieties and their antimicrobial activity against a panel of foodborne pathogens. The results show that Moldavian dragonhead, especially the Gorynych variety, demonstrates more substantial antimicrobial effects than H. officinalis, even at low concentrations. These findings promote using *D. moldavica* EOs as effective natural preservatives, particularly against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. Their successful cultivation in Armenian conditions highlights their potential for local sourcing and integration into health-oriented food products. This work contributes to a better understanding of bioactive plant ingredients that enhance food safety and functionality.

CONCLUSION

The study's results highlight the considerable antimicrobial activity of essential oils obtained from introduced Dracocephalum moldavica and Hyssopus officinalis varieties. *D. moldavica* varieties demonstrate powerful inhibitory effects against Gram-positive and Gram-negative bacteria. These findings suggest that essential oils from such species hold a promising potential as natural bioactive ingredients. Integrating these essential oils into food offers a natural alternative to synthetic preservatives, while enhancing health benefits for consumers. These benefits are due to their known antioxidant, anti-inflammatory, and immunomodulatory properties. Using Eos may reduce the microbial load in food products, improving food safety and potentially reducing the risk of foodborne illnesses. Moreover, including these plant-derived compounds aligns with current consumer preferences for clean-label, health-promoting ingredients.

Given the growing demand for multifunctional ingredients in the food industry, the studied essential oils can be considered valuable candidates for enriching the functional profile of food products. These products can span from beverages and dairy to bakery and meat alternatives. However, further studies are necessary to evaluate their sensory impact, stability in various food matrices, and interactions with other ingredients under different processing and storage conditions.

List of abbreviations: SCVIC, Scientific Center of Vegetable and Industrial Crops; Eos, essential oils; µm, microns, also known as micrometers; equal to one millionth of a meter; g, gram; mg, milligram; mm, millimeter; SD, standard deviation.

Competing interests: The authors declare that they have no competing interests.

Authors' contributions: AA and LT designed the research. IV and ZH performed laboratory analysis. GM performed statistical analyses. MH selected and evaluated varieties, and RB analyzed the results and drew the graphs. MH contributed to writing the abstract and introduction. AA wrote the article. JB edited the article. All authors read and approved the final version of the manuscript.

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