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# Optimizing Planting Date and Density for Enhanced Seed Yield and Quality in *Cyamopsis tetragonoloba* L.

**Running Title:** Guar Seed Yield & Quality Optimization

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## ABSTRACT

Considering the arid and low-rainfall climatic conditions of South Khorasan province and the necessity for cultivating low-water-demanding and industrial medicinal plants, guar was selected as a medicinal crop to evaluate the effects of planting date and density on its biochemical properties and quality traits of guar seeds. A split-plot design with three replications was employed in 2023 at two locations: Birjand and Sarayan, South Khorasan province, Iran. Treatments included three planting dates (May 12th, June 12th, and July 13th) as main plots and five plant densities (10, 20, 30, 40, and 50 plants/m²) as subplots. Analysis of variance revealed that planting date significantly affected seed yield, oil content, protein content, galactomannan content, carbohydrate content, total ash, and protein yield. Notably, delayed planting resulted in a substantial decrease in seed yield (by approximately 52.36%), carbohydrate content, galactomannan content, total ash, protein yield, and oil yield. Plant density also significantly influenced seed yield, oil content, protein content, galactomannan content, carbohydrate content, phosphorus content, total ash, protein yield, and oil yield. The highest seed yield (2125.3 kg/ha) was observed with a June 12th planting date at a density of 30 plants/m². Both planting date and density significantly affected yield and seed quality, with optimal results for protein, galactomannan, carbohydrate, and oil content achieved with the June 12th planting date and a density of 30 plants/m². This combination, likely benefiting from a longer growing season and favorable climatic conditions, yielded the highest seed yield. These findings demonstrate the potential of guar as a low-input crop for sustainable cropping systems in arid climates like South Khorasan, offering significant benefits for regional agriculture.
**Keywords:** Cluster bean, Galactomannan, Medicinal plants, Quantitative traits, South Khorasan



## INTRODUCTION

In hot and arid climates, the development of agricultural methods to maximize the use of available resources, especially water and soil, by eliminating water-intensive plants and replacing them with low-water-demanding in various regions, including medicinal plants with short growth cycles and low water requirements, is of particular importance. Cultivating medicinal plants, due to their high drought tolerance potential and ability to thrive on low-yield and marginal lands, can effectively boost the economic well-being of rural communities and supply various food and pharmaceutical industries. Furthermore, it can enhance the efficient use of water and soil resources while preventing the risk of species extinction. The feasibility of expanding the cultivation of these medicinal-industrial plants hinges on assessing their large-scale production capability and evaluating their resilience to adverse environmental conditions, notably limited irrigation water [1]. Legumes constitute a significant group of crops renowned for their ability to enhance soil fertility. Through nitrogen fixation, they enrich the soil, fostering the growth of soil organisms and benefiting other plants. Moreover, legume seeds are abundant in energy and protein, making them a valuable nutritional resource for both humans and livestock [2]. Guar (*Cyamopsis tetragonoloba* L. (Taub.)), a lesser-known legume, holds considerable industrial and medicinal value, particularly in the production of guar gum [3]. India and Pakistan are the leading guar producers globally, with India accounting for approximately 75-82% (2.5-3 million tons annually) and Pakistan contributing 10-12% (134,700 tons annually) of total global production. In Pakistan, guar cultivation exceeds 0.181 million hectares, while India boasts a cultivated area exceeding 3.2 million hectares, with Rajasthan alone cultivating 3 million hectares. The high industrial value of guar and the surging global demand have spurred increased cultivation efforts in other countries [4]. Guar gum holds therapeutic potential as a blood glucose regulator, antimicrobial agent, and appetite suppressant. Additionally, it serves as a laxative for treating diarrhea, irritable bowel syndrome (IBS), as well as obesity and diabetes. It's also used to lower cholesterol and prevent atherosclerosis [5-6, 7]. In the pharmaceutical industry, guar gum functions as a stabilizer, bulking agent, suspending agent, and binding agent. It is specifically used as a binding agent in tablets and a thickening agent in lotions and creams. Medicinal plants, including guar, are extensively used across developed and developing nations in various forms: as home remedies, over-the-counter (OTC) pharmaceuticals, and key inputs for pharmaceutical industries, securing a significant portion of the global drug market [8]. Recognizing the importance of medicinal plants, which form a cornerstone of traditional Iranian medicine and enjoy widespread public use, rigorous quality control is crucial. This ensures that diverse populations have access to consistently standardized, high-quality medicinal products [8].

Selecting the optimal planting date is paramount for maximizing crop yields. This optimal date can vary considerably across regions and even among different genotypes within a single region. The main cause of these variances is the impact of various environmental conditions on the phenological growth of the plant [9]. Temperature emerges as a key environmental factor significantly impacting plant physiological activities throughout all growth stages. In conjunction with other environmental factors, temperature is a major determinant of a crop's potential yield. At suboptimal temperatures, both germination rates and percentages decline due to diminished photosynthetic rates [10]. Considering the short growth cycle and potential for summer cultivation of guar highlighted by Meftakhizade et al. [11], we selected planting dates spanning from May 12 to July 13 to capture the effects of varying temperature and photoperiod regimes on guar development. These dates were chosen to represent early-, mid-, and late-season planting opportunities, allowing us to assess the crop's performance under different environmental conditions prevalent during the typical agricultural season in this region.

Optimizing plant density is critical for maximizing both crop yield and quality. The ideal plant density represents the arrangement that enables plants to most effectively utilize available resources, leading to maximum yield. This optimal density should maximize crop output per unit of land area [12]. While competition for light above ground rises at high plant densities, competition for underground resources like water and nutrients intensifies. This heightened competition often results in reduced pod production per individual plant. Consequently, in dense populations, the increase in overall biomass and yield per unit area may not proportionally match the increase in plant population [13].

The plant densities of 10, 20, 30, 40, and 50 plants/$m^2$ were chosen based on clearly state the rationale. Preliminary observations and local agricultural practices indicate a common density range of 10 to 50 plants/$m^2$ for guar. We hypothesized that the lower density (10 plants/$m^2$) might minimize competition but potentially underutilize resources, while the higher density (50 plants/$m^2$) could lead to excessive intraspecific competition, limiting individual plant growth and yield. The intermediate density (40 plants/$m^2$) was considered as a potential optimum for balancing resource utilization and minimizing competition. The findings of Zafaranieh [14], which reported optimal yields at 40 plants/$m^2$, further supported the inclusion of this density in our experimental design.

A study conducted in Jiroft investigating the impact of nitrogen and phosphorus levels on guar plant growth and yield across varying row spacings revealed that guar plants exhibit a strong canopy-filling capacity. Consequently, high plant densities (with 30 cm row spacing) may not be necessary, as they can lead to increased intraspecific competition among plants. Conversely, wide row spacing (60 cm) can also diminish seed yield due to reduced plant density and a lower plant population per unit area [15]. Abdelsalam and Adar [16], in their experimental findings, demonstrated that increasing plant density can significantly enhance forage quality. Haydarzadeh *et al.* [17] conducted a study in Gilan province to investigate the germination characteristics and seed quality of guar across varying planting times (May 22nd, June 5th, June 19th, and July 2nd) and planting densities (20, 40, and 60 plants per square meter). Their research revealed that a plant density of 40 plants per square meter exhibited a positive influence on the initial growth and germination of guar seeds. Furthermore, guar seeds sown on June 5th, coinciding with favorable climatic conditions for seed filling, demonstrated superior quality. Delayed planting, however, resulted in a decline in seed quality and germination characteristics, attributable to the overlap of critical reproductive growth stages with late-season rainfall.

To find out how planting date and density affected the morphological, physiological, and yield characteristics of guar plants, Zafaranieh [14] carried out a field experiment. The study included six planting dates (March 15, May 15, June 15, July 15, August 15, and September 15), three plant densities (20, 40, and 60 plants/$m^2$), and a randomized full block design with three replications across two growing seasons. Results demonstrated that the highest seed yield (3780 kg/ha), galactomannan content, oil percentage, and 100-seed weight were achieved with a planting date of May 15th and a density of 40 plants/$m^2$. This density facilitated optimal plant establishment, leading to increased seed production per unit area. Consequently, resource utilization was maximized, resulting in higher seed yields. Planting dates of May 15th and June 15th allowed for extended growth periods and enhanced photosynthetic activity, thereby promoting branch expansion and increasing the overall biological yield of guar plants. Pour Afra and colleagues [18] conducted a study to investigate the influence of planting date (July 1, July 15, June 1, and June 15) and plant density (130,000, 200,000, and 400,000 plants/ha) on the growth duration and seed yield of two guar ecotypes: Indian and Pakistani. Their findings revealed that the Pakistani ecotype exhibited superior performance compared to the Indian ecotype, likely attributed to its superior genetic potential. The highest overall yield and related yield components were obtained after planting on June 1st. The Pakistani ecotype also demonstrated a higher endosperm content (40.5%) compared to the Indian ecotype (38.8%). A plant density of 130,000 plants/ha yielded the highest seed production. Further increases in plant density led to diminished seed yields due to intensified intraspecific competition, resulting in weakened plant vigor. A study by Meftakhizade *et al.* [11] assessed the effects of different irrigation regimes (four levels) and planting dates (July 5th and August 5th) on the phytochemical characteristics of three guar genotypes (RGC1031, RGC936, and RGC1066). Their research revealed that the highest guar gum content (33.8%) was achieved with a planting date of August 5th in conjunction with the four-stage irrigation regime. The third genotype, RGC-1066, exhibited the highest protein content within the extracted gum. Based on these findings, the researchers proposed that guar, with its short growth cycle and inherent drought tolerance, possesses the potential for successful summer cultivation.

Building upon the existing knowledge regarding the influence of planting date and density on guar yield and quality [18, 14], this study was conducted to determine the optimal planting date and plant density for maximizing seed yield and enhancing the biochemical and quality attributes of guar seeds under the specific agro-climatic conditions of our study location. Understanding these physiological and ecological requirements is crucial for promoting the successful cultivation of guar in this region.

## MATERIAL AND METHODS

This experiment was conducted during the spring and summer of 2023 at two research farms: the Research Farm of the Faculty of Agriculture, University of Birjand (32°52′N, 59°08′E, 1425 meters above sea level, average annual rainfall of 171 mm) and the Research Farm of the Faculty of Agriculture, Sarayan (33°51′N, 58°27′E, 1395 meters above sea level, average annual rainfall of 147 mm). These locations were selected due to their semi-arid climates, which are representative of the typical guar cultivation regions in eastern Iran, allowing for the evaluation of planting dates and plant densities under relevant environmental conditions. A map illustrating the geographical locations of these research farms is provided in Figure 1.

The experiment employed a split-plot layout within a randomized complete block design with three replications at each location. Main plots consisted of three planting dates: May 12, June 12, and July 13. These dates were chosen to represent early-, mid-, and late-season planting windows commonly practiced in the region, allowing for the assessment of their impact on guar yield and quality under varying temperature and photoperiod conditions. Subplots were assigned to five plant densities: 10, 20, 30, 40, and 50 plants per square meter. These densities were selected to evaluate the effects of varying levels of interplant competition on growth, yield, and biochemical composition of guar. These plant densities were achieved by establishing five different intra-row spacings of 20, 10, 6.6, 5, and 4 centimeters, respectively, within rows that were consistently spaced at 50 centimeters apart. Prior to planting, composite soil samples were collected randomly from each experimental site at depths of 0–30 cm and 30–60 cm to characterize key physical and chemical properties. Soil analysis, conducted according to standard methods, included measurements of saturated paste extract (Sp), pH, electrical conductivity (EC), nitrogen percentage, phosphorus content (mg/kg), and potassium content (mg/kg) (Table 1). These soil properties are critical for guar growth and nutrient availability, influencing overall plant performance and seed quality.

The study utilized guar genotype RGC1031, which originated from Pakistan and is known for its high gum content and adaptability to semi-arid conditions. Before sowing, a germination test was conducted in a germinator at 25 °C to determine seed viability, ensuring a minimum germination rate of 90%. Based on the soil analysis results, triple super phosphate at a rate of 100 kg/ha, potash at a rate of 100 kg/ha and urea at a rate of 150 kg/ha fertilizers were applied to the soil as basal dressing before planting to ensure adequate nutrient supply for optimal growth. Following field preparation, seeds were manually sown at a depth of 5 cm with the intended intra-row spacing within 50-centimeter-wide rows using a furrow and ridge planting method. Each experimental plot consisted of five 6-meter-long rows. Throughout the growing season, uniform cultural practices were implemented across all experimental plots. These included thinning to achieve the desired plant densities, manual weeding as needed to control weeds, supplemental fertilization with urea at a rate of 50 kg/ha at the vegetative growth stage, and irrigation using a drip irrigation system. Irrigation was scheduled based on crop evapotranspiration and soil moisture monitoring, with an average of 340 liters of water applied per irrigation event at intervals of seven days, taking into account the soil texture at each location to ensure optimal water availability without causing water stress or saturation.

At the conclusion of the growth season, random samples of ten plants were taken from the center three rows of each plot, excluding the boundary rows to avoid edge effects. These samples were analyzed to determine gum (galactomannan) content, carbohydrate content, protein content, oil content, and total ash content. Gum extraction was performed by soaking 100 grams of seeds in 500 mL of distilled water for 7 hours at room temperature, followed by dehulling using a laboratory mill. The dehulled material was then dried in a forced-air oven at 50°C for 7 hours. The proportion of extracted gum to the embryo and endosperm was calculated by weighing the extracted gum [19]. The oil contented of a 25-gram sample of dehulled seeds was determined by Soxhlet extraction using 300 mL of hexane for 6 hours according to the AOAC method [20]. The total nitrogen content was determined using the Kjeldahl method, and the protein content was calculated by multiplying the nitrogen percentage by a conversion factor of 6.25 [21]. Carbohydrate content was quantified by extracting powdered samples with 13 mL of 80% ethanol in centrifuge tubes at 80°C for 30 minutes. The extraction process was repeated once, and the combined supernatants were analyzed using the phenol-sulfuric acid method. One milliliter of the combined supernatant was mixed with 5 mL of concentrated sulfuric acid and 1 mL of 5% phenol solution, and the absorbance of the resulting solution was measured at 490 nm using a spectrophotometer [22]. Total ash content was determined by incinerating 5 grams of ground seed sample in muffle furnace at 550 °C until a constant weight was achieved, following the AOAC method [20]. At the conclusion of the growing season, the seed yield was determined by harvesting the plants from a one-square-meter area in the center of each plot and adjusting the yield to 12% moisture content. Homogeneity of variances across the two locations for each measured variable was assessed using Bartlett's test to ensure the appropriateness of combining data for subsequent analysis. Bartlett's test is suitable for checking the equality of variances of several samples from a normal distribution. Data analysis was performed using SAS software (version 9.2) and Microsoft Excel. Means were compared using the Least Significant Difference (LSD) test at the 0.05 significance level. The LSD test was chosen for pairwise comparisons of treatment means because it is a commonly used and relatively straightforward method for identifying significant differences following a significant F-test in the analysis of variance (ANOVA). No, transformations were applied to the data prior to analysis, as the assumptions of normality and homogeneity of variances were generally met after checking with Shapiro-Wilk and Bartlett's tests, respectively.

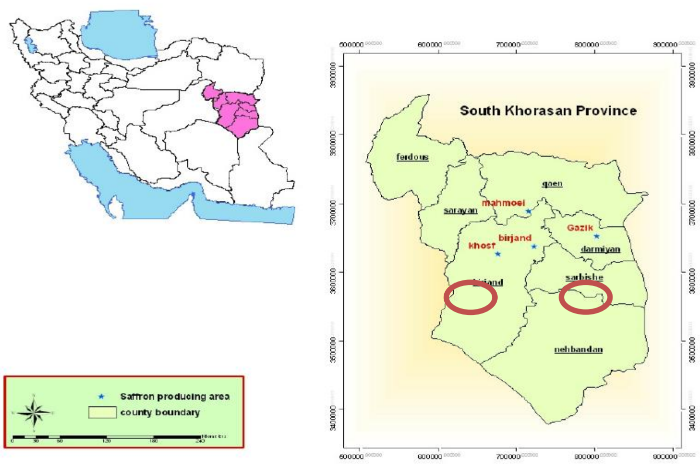


**Fig.1** Map showing the geographical locations of the research farms in Birjand and Sarayan, Iran.

**Table 1** Chemical and physical characteristics of the soil in the area under study (0–30 cm and 30–60 cm depth).

| Region | Depth (cm) | Sp (%) | pH | EC (ds/m) | N (%) | P (mg/kg) | K (mg/kg) |
|---|---|---|---|---|---|---|---|
| Birjand | 0-30 | 40 | 8 | 5.67 | 0.031 | 8.9 | 240 |
| | 30-60 | 44 | 8 | 9.5 | 0.025 | 7.9 | 220 |
| Sarayan | 0-30 | 23 | 8.4 | 1.1 | 0.047 | 8.4 | 210 |
| | 30-60 | 25 | 8.2 | 1.2 | 0.039 | 7.2 | 200 |

## RESULTS AND DISCUSSION

### Effect of Planting Date and Plant Density on Seed Yield

The objective of this study was to evaluate the individual and interactive effects of planting date and plant density on the seed yield and quality parameters of guar (*Cyamopsis tetragonoloba* L.) in different locations. Our findings indicate that both factors significantly influence these crucial traits, as detailed below.

Bartlett's test confirmed the homogeneity of variances for all traits, allowing for a combined analysis of variance assuming a fixed location effect. The combined ANOVA revealed that location, planting date, and plant density significantly ($P < 0.01$) affected seed yield (Table 2).

Correlation analysis indicated that seed yield was positively correlated with galactomannan content and seed protein percentage. Conversely, seed yield showed negative correlations with total ash content, phosphorus content, and carbohydrate content. Furthermore, significant positive correlations were observed between seed yield and both protein yield and oil yield (Table 3). The strongest positive correlation was found between seed yield and protein yield, suggesting that increased seed yield is associated with increased dry matter and, consequently, higher protein yield.

Significant interactions ($P < 0.01$) were observed for planting date × density, location × density, and location × planting date × density (Table 2). Mean comparisons showed that the highest seed yield (2125.3 Kg/ha) was achieved with the June 12th planting date at a density of 30 plants/m² (Figure 2). This result can be attributed to optimal growth conditions and favorable temperatures during the seed-filling and maturation stages. In contrast, later planting dates experienced sub optimal conditions, such as cooler temperatures and shorter day lengths, which likely reduced photosynthetic activity and decreased seed yields. These results are consistent with previous findings [23]. Across the May 12th and July 13th planting dates, the highest yield per square meter was obtained with a plant density of 50 plants per square meter. The increase yields with increasing plant density is likely due to better plant establishment at a density of 30 plants per square meter, leading to a higher number of plants per unit area and consequently, more seeds. This also allows for more efficient utilization of resources. Some studies have shown that both seed yield and biomass of beans decrease with increasing row spacing (from 7 to 10 cm) [24, 25]. As row spacing increases, the plant population per hectare decreases, leading to lower yields. Farmers generally believe that increasing plant density (by reducing row spacing) can increase yield, but it is important to note that excessively high densities can negate this effect due to decreased plant weight [26]. The lowest seed yield (1012.6 Kg/ha) was obtained from the July 13th planting date with a density of 10 plants/m² (Figure 2). The data revealed a consistent trend of decreasing seed yield with delayed planting at all plant densities. Delayed planting resulted in reduced yield (by approximately 52.36%), likely due to poor growth and a shortened seed-filling and maturation period. In the latest planting date (July 13th), the onset of flowering coincided with decreasing temperatures, suggesting that cooler temperatures during flowering may have contributed to the reduced yield. Previous studies have shown that early planting dates can lead to increased quinoa seed yield with optimal plant densities [27]. Zafaranieh [14] reported that delayed planting in guar reduces plant growth duration, vegetative and reproductive structures, and consequently, yield and yield components. Having examined the individual effects of planting date and plant density on seed yield, the subsequent section will focus on their impact on seed quality parameters. The results of this research offer significant practical implications for guar growers and the industry. Identifying the optimal planting date of June 12th and a plant density of 30 plants/m² for maximum seed yield provides a clear guideline for enhancing productivity. Furthermore, understanding the trade-offs, such as increased protein content with later planting dates despite reduced yield, allows growers to make informed decisions based on their primary objectives—whether it be maximizing yield, protein content, or galactomannan. These findings can guide the development of improved agronomic practices for sustainable guar cultivation in similar agro-ecological zones.

**Table 2** Combined analysis of variance (mean squares) for the effects of planting date and plant density on the biochemical and quality characteristics of guar seeds.

| S.O.V | Df | Seed yield (Kg/ha) | Oil (%) | Protein (%) | Galactomannan content (g) | Carbohyd rate (%) | Phosphorous (mg) | Total Ash (%) | Protein yield (%) | Oil yield (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Location | 1 | 1091421.34 $^{**}$ | 12.00 $^{**}$ | 827.36 $^{**}$ | 0.0000 $^{ns}$ | 2480.73 $^{**}$ | 0.086 $^{*}$ | 99.02 $^{**}$ | 6735.73 $^{**}$ | 131.38 $^{**}$ |
| Block (Location) | 4 | 99904.01 $^{ns}$ | 0.47 $^{ns}$ | 3.26 $^{ns}$ | 0.0003 $^{*}$ | 4.05 $^{ns}$ | 0.074 $^{**}$ | 0.26 $^{ns}$ | 73.65 $^{ns}$ | 4.03 $^{ns}$ |
| Planting Date | 2 | 2736064.67 $^{**}$ | 17.90 $^{**}$ | 93.78 $^{**}$ | 0.0013 $^{*}$ | 44.38 $^{*}$ | 0.034 $^{ns}$ | 4.93 $^{**}$ | 1189.09 $^{**}$ | 1.02 $^{ns}$ |
| Location x Planting Date | 2 | 187047.38 $^{ns}$ | 3.06 $^{*}$ | 4.02 $^{ns}$ | 0.0013 $^{*}$ | 6.71 $^{ns}$ | 0.178 $^{*}$ | 0.80 $^{ns}$ | 6.34 $^{ns}$ | 26.85 $^{*}$ |
| Error Planting Date | 8 | 150117.41 $^{ns}$ | 0.39 $^{ns}$ | 1.65 $^{ns}$ | 0.0002 $^{ns}$ | 8.36 $^{ns}$ | 0.022 $^{ns}$ | 0.36 $^{ns}$ | 91.13 $^{ns}$ | 5.61 $^{ns}$ |
| Plant Density | 4 | 515302.97 $^{**}$ | 22.47 $^{**}$ | 79.85 $^{**}$ | 0.0049 $^{**}$ | 33.50 $^{*}$ | 0.582 $^{**}$ | 13.08 $^{**}$ | 1389.40 $^{**}$ | 52.92 $^{**}$ |
| Location x Plant Density | 4 | 3406838.15 $^{**}$ | 8.73 $^{**}$ | 70.42 $^{**}$ | 0.0003 $^{ns}$ | 44.69 $^{**}$ | 0.427 $^{**}$ | 12.65 $^{**}$ | 3392.83 $^{**}$ | 114.81 $^{**}$ |
| Planting Date x Plant Density | 8 | 364391.55 $^{**}$ | 12.53 $^{**}$ | 61.82 $^{**}$ | 0.0005 $^{**}$ | 290.70 $^{**}$ | 0.208 $^{**}$ | 6.32 $^{**}$ | 410.80 $^{**}$ | 43.04 $^{**}$ |
| Location x Planting Date x Plant Density | 8 | 389400.19 $^{**}$ | 4.55 $^{**}$ | 35.58 $^{**}$ | 0.0002 $^{ns}$ | 98.12 $^{**}$ | 0.386 $^{**}$ | 1.67 $^{**}$ | 212.41 $^{*}$ | 28.63 $^{**}$ |
| Residual | 48 | 81552.27 | 0.54 | 3.76 | 0.0001 | 10.30 | 0.019 | 0.50 | 80.50 | 3.96 |
| Total | 89 | 383950.65 | 3.89 | 29.32 | 0.0005 | 73.97 | 0.120 | 3.43 | 428.41 | 18.90 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| C.V.% | 16.58 | 18.79 | 7.58 | 3.42 | 12.14 | 5.847 | 14.79 | 20.06 | 29.78 |

ns* and ** are non-significant, significant at 5 and 1% probability levels, respectively.

df: degrees of freedom, S.O.V: Source of Variation, CV: Coefficient of Variation

**Table 3** Pearson correlation coefficients for the effects of planting date and plant density on the biochemical and quality characteristics of guar seeds.

| | Seed yield (Kg/ha) | Oil (%) | Protein (%) | Galactomannan content (g) | Carbohydrate (%) | Phosphorous (mg) | Total Ash (%) | Protein yield (%) | Oil yield (%) |
|---|---|---|---|---|---|---|---|---|---|
| Seed yield | 1 | | | | | | | | |
| Oil Percent | -0.040 ns | 1.000 | | | | | | | |
| Protein Percent | 0.195 ns | 0.325 * | 1.000 | | | | | | |
| Galactomannan content | 0.046 ns | -0.503 ** | -0.253 ns | 1.000 | | | | | |
| Carbohydrate | -0.245 ns | -0.250 ns | -0.419 ** | 0.059 ns | 1.000 | | | | |
| Phosphorous | -0.072 ns | -0.216 ns | -0.415 ** | 0.378 ** | 0.057 ns | 1.000 | | | |
| Total Ash | -0.307 ns | -0.127 ns | -0.440 ** | -0.162 ns | 0.333 ns | 0.198 ns | 1.000 | | |
| Protein yield | 0.871 ** | 0.158 ns | 0.618 ** | -0.112 ns | -0.386 ns | -0.241 ns | -0.456 ** | 1.000 | |
| Oil yield | 0.537 ** | 0.779 ** | 0.436 ** | -0.395 * | -0.383 ns | -0.246 ns | -0.312 ns | 0.668 ** | 1.000 |

ns* and ** are non-significant, significant at 5 and 1% probability levels, respectively.

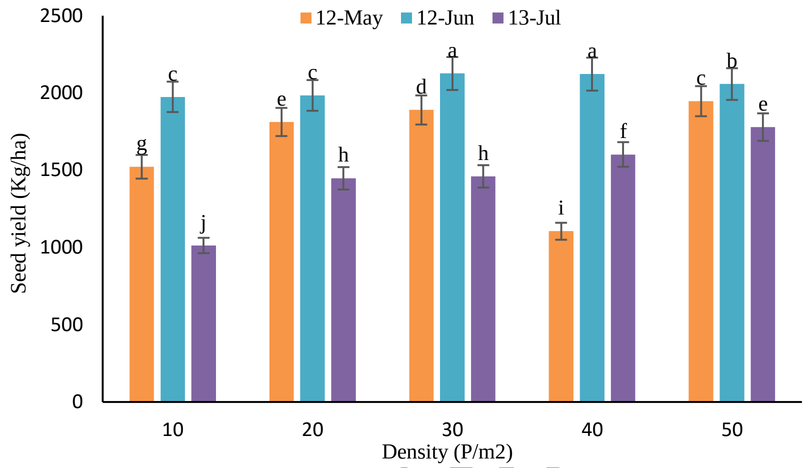


**Fig. 2** Interaction effect of planting date and plant density on guar seed yield. (Error bars represent standard deviation)

### Effect of Planting Date and Plant Density on Seed Quality

### Seed Oil Content

Location, planting date, plant density, planting date × plant density, location × plant density, location × planting date × plant density, and location × planting date × plant density all had a significant ($P < 0.01$) effect on seed oil percentage (Table 2). The interaction between location and planting date also had a significant effect ($P < 0.05$). The maximum proportion of seed oil was observed on July 13th across all density levels (Figure 3). The highest seed oil percentage (3.6%) was found at a density of 40 plants/m², which was not significantly different from the oil percentage at 10 plants/m². These results are consistent with studies on soybeans, where increased plant density reduced seed oil percentage and increased seed protein content [28].

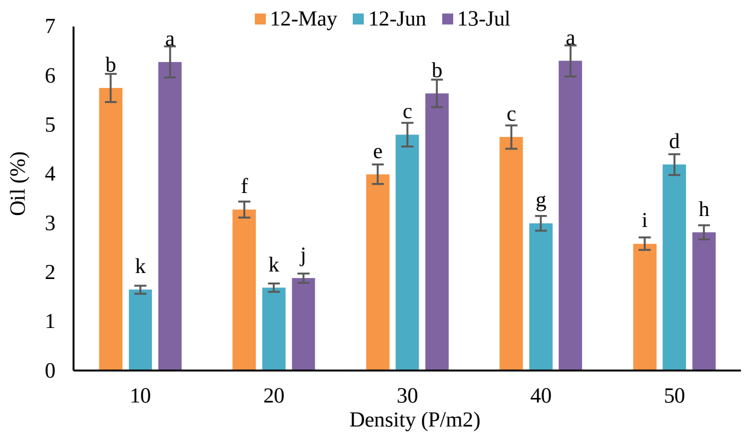


**Fig. 3** Interaction effect of planting date and plant density on oil content of guar seeds. (Error bars represent standard deviation)

### Seed Protein Content

Location, planting date, and plant density, as well as their interactions (location × plant density, planting date × density, and location × planting date × density), were found to have significant ($P < 0.05$) effects on the protein content of guar seeds by analysis of variance (Table 2). No significant relationship was found between planting date and location. The highest seed protein content (30.94%) was obtained with a July 13th planting date at a density of 40 plants/m² (Figure 4). Delayed planting in the soybean has also been shown to increase protein content [29]. While increased plant density can initially increase seed protein content, excessive density can hinder this effect [30]. Protein content is influenced by genotype, photosynthesis, nutrient availability, and climatic conditions, and factors that shorten the plant growth period can increase protein content [31].

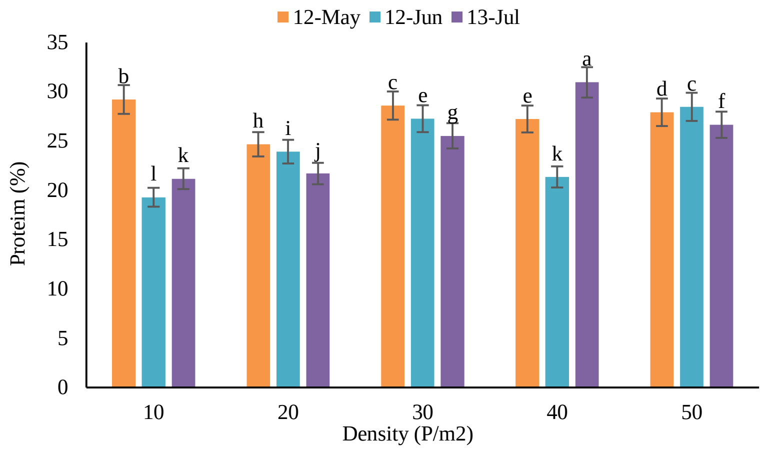


**Fig. 4** Interaction effect of planting date and plant density on protein content of guar seeds. (Error bars represent standard deviation)

### Seed Gum Content (Galactomannan)

Analysis of variance revealed significant ($P \leq 0.05$) effects of planting date, plant density, and their interactions (including location × planting date) on seed galactomannan content, an economically important trait in guar. According to correlation analysis, there was a substantial positive correlation with oil yield ($P < 0.05$) and a significant positive association with galactomannan and phosphorus content ($P \leq 0.01$) (Table 3). Planting on June 12th at a density of 20 plants/m² produced the highest galactomannan content (0.35 g), whereas planting on July 13th at a density of 50 plants/m² produced the lowest (0.29 g) (Figure 5). No significant difference in galactomannan content was found between densities of 50 and 30 plants/m².

Galactomannan content in guar plants is enhanced by favorable climatic conditions during seed filling and maturation. Delayed planting, likely resulting in lower temperatures and fewer growing degree days, has been shown to decrease galactomannan content. Kumar and Rodge [32] reported that longer day lengths during the reproductive stage, coupled with favorable climatic conditions during seed filling and maturation, contribute to increased galactomannan accumulation in guar plants. Early planting dates typically result in higher galactomannan content, which can be attributed to increased seed yield. Findings from Zafaranieh [14] corroborate these observations, reporting a 68% reduction in galactomannan content with delayed planting.

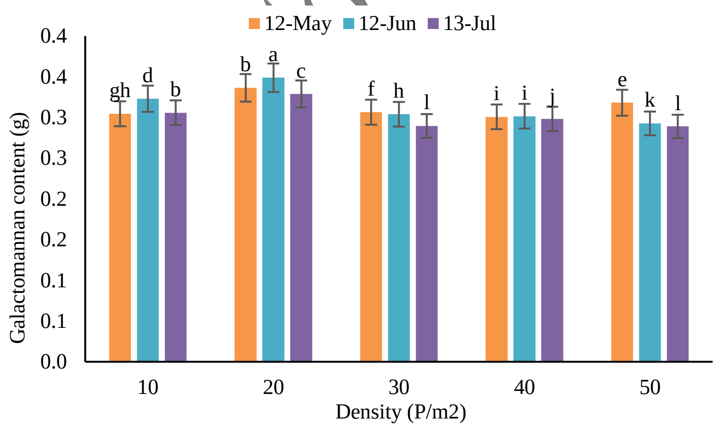


**Fig. 5** Interaction effect of planting date and plant density on galactomannan content of guar seeds. (Error bars represent standard deviation)

### Seed Carbohydrate Content

According to a combined ANOVA, planting date, plant density, and location all had a significant effect on the proportion of carbohydrates ($P < 0.05$), as did the interactions between planting date and density, location and density, and all three factors ($P < 0.01$) (Table 2). The 10 plants/m² density treatment at the June 12th planting date had the highest carbohydrate content (34.51%), according to analyses of means for the interaction of planting date and density. Conversely, the lowest carbohydrate content (14.76%) was observed in the 10 plants/m² density treatment at the May 12th planting date (Figure 6). Previous studies have demonstrated an increase in lignin and structural carbohydrate content with increasing age in forage plants, while non-structural carbohydrate content decreases [33]. Consequently, plants with shorter growth periods tend to be more succulent and retain higher levels of non-structural carbohydrates, which aligns with the findings of this study.

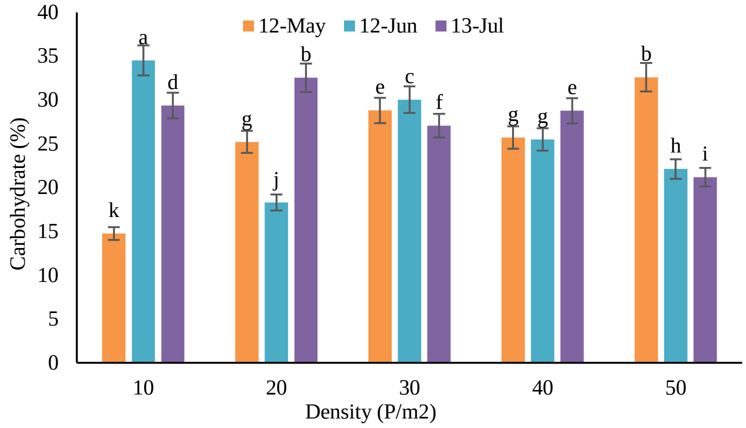


**Fig. 6** Interaction effect of planting date and plant density on carbohydrate content of guar seeds. (Error bars represent standard deviation)

### Seed Phosphorus Content

Plant density had a significant main effect on seed phosphorus content (P < 0.01), according to analysis of variance. Furthermore, the effects of location and location × planting date interaction were significant at the 5% level (P ≤ 0.05), whereas the interactions of planting date × density, location × density, and location × planting date × density were highly significant (P < 0.01) (Table 2). The highest seed phosphorus content (2.65 mg) was observed in the 10 plants/m² density treatment at the July 13th planting date (Figure 7).

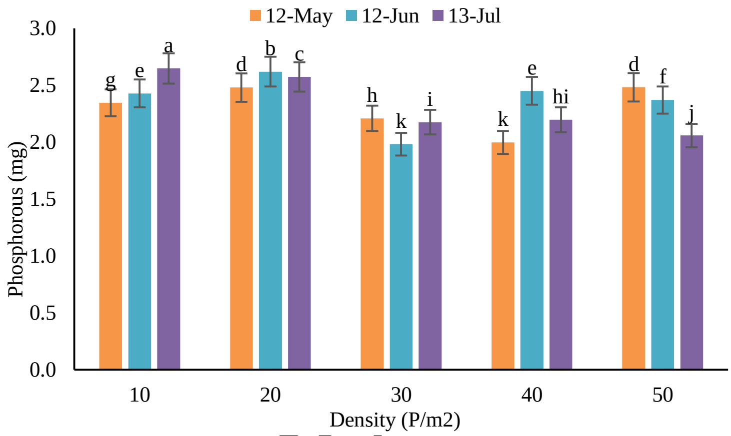


**Fig. 7** Interaction effect of planting date and plant density on phosphorus content of guar seeds. (Error bars represent standard deviation)

### Total Seed Ash Content

The results obtained showed that the total seed ash content was significantly (P < 0.01) affected by location, planting date, plant density, and their interactions (planting date × plant density, location × plant density, and location × planting date × plant density) (Table 2). Protein yield and total seed ash content were shown to be significantly and positively correlated (P < 0.01) by correlation analysis (Table 3). The highest total seed ash content (7.25%) was observed with a planting date of June 12 and a plant density of 40 plants per square meter (Figure 8). Ash content serves as an indicator of the mineral content within seeds. The ash content in the guar seeds ranged from 2.2 to 7.25%. Vaseghi and Davazdahemami [34] reported that variations in ash content within a species can be attributed to geographical variations, climatic conditions, plant nutritional status, and plant genetic and botanical characteristics.

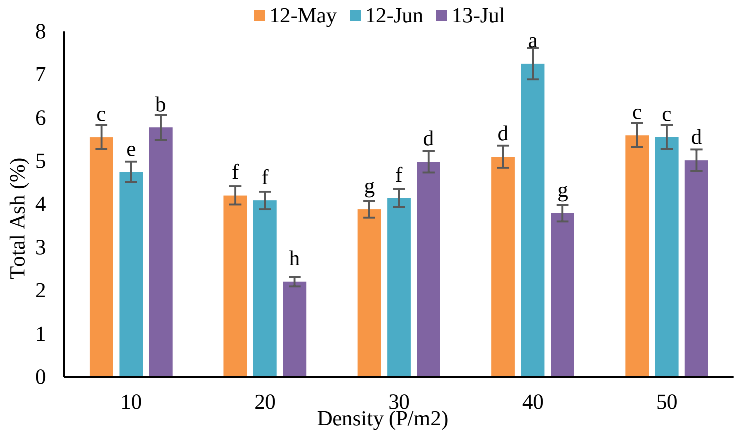


**Fig. 8** Interaction effect of planting date and plant density on total ash content of guar seeds. (Error bars represent standard deviation)

### Seed Protein Yield

Significant differences (P ≤ 0.01) in seed protein yield were found by analysis of variance between plant densities, planting dates, and locations. The location × density × planting date interaction was significant at the 5% level (P ≤ 0.05), but the planting date × density and location × density interactions were highly significant (P < 0.01) (Table 2). At a density of 50 plants/m2, the June 12th planting produced the highest seed protein yield (62.75%) (Figure 9). Seed yield and protein yield showed a substantial positive association (Table 3), indicating that seed yield has an important effect on protein content. This finding aligns with the study by Sadeghi and Noorhosseini Niyaki [35], which demonstrated significant effects of both planting date and plant density on seed protein content. The interactive effects of these factors, particularly in conjunction with varying temperature regimes, can significantly impact protein content outcomes.

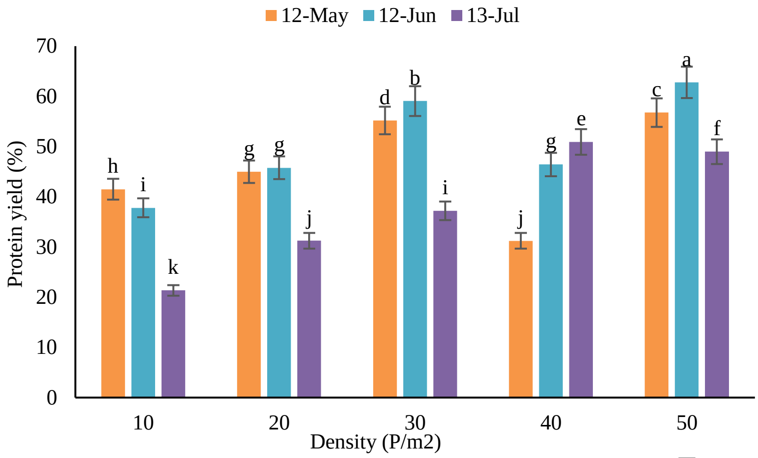


**Fig. 9** Interaction effect of planting date and plant density on protein yield of guar seeds. (Error bars represent standard deviation)

### Seed Oil Yield

Analysis of variance showed that location and plant density had a highly significant main effect (P ≤ 0.01) on seed oil yield, whereas planting date had no significant main effect. Additionally, the location × planting date interaction was significant at the 5% level (P < 0.05), and the planting date × density, location × density, and location × planting date × density interactions were highly significant (P ≤ 0.01) (Table 2). The 30 plants/m² density treatment at the June 12th planting date had the highest seed oil yield (10.67%) (Figure 10). The lowest oil yield (2.76%) was observed with a plant density of 20 plants/m² and a planting date of July 13 (Fig. 9). Delayed planting has been shown to negatively impact oil yield, likely due to reduced seed yield, as reported by Karimi *et al.* [36]. Similarly, Amiri Ghanat Saman *et al.* [37] found that delaying planting significantly reduced sesame oil yield per hectare. As oil yield is a function of both seed yield and seed oil content, an increase in either factor can positively contribute to overall oil yield [30].

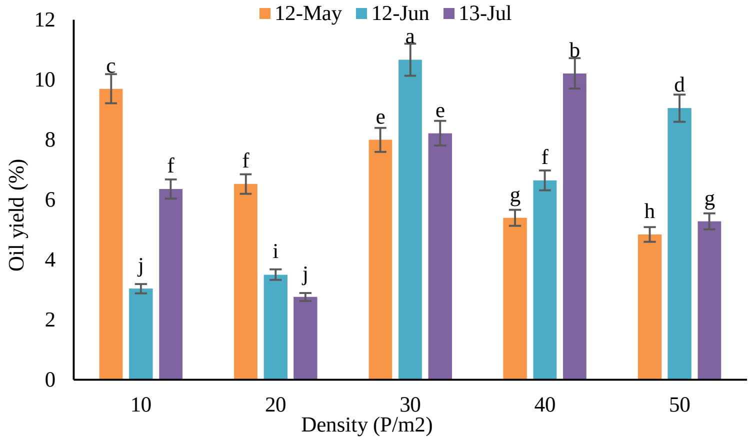


**Fig. 10** Interaction effect of planting date and plant density on oil yield of guar seeds. (Error bars represent standard deviation)

### Results of the Comparison of the Interaction effect of Planting Date and Density on Seed Quality

The comparison of the average interaction effects of planting date, density, and location data indicates that the highest seed yield was achieved on June 12th with a density of 10 plants per square meter in Birjand. The highest oil percentage was observed with the planting date of May 12th and a density of 10 plants per square meter in Birjand. The highest protein percentage was also found under the same planting date and density but in Sarayan. The highest carbohydrate percentage was noted with the planting date of May 12th and a density of 50 plants per square meter in Birjand (Table 4). The highest phosphorus content was recorded for the planting date of July 13th and a density of 10 plants per square meter in Sarayan. The highest percentage of total ash was found on the planting date of June 12th, with a density of 40 plants per square meter in Birjand. The highest protein yield was obtained on the planting date of June 12th, with a density of 50 plants per square meter in Sarayan. The highest oil yield was also observed on the planting date of June 12th, with a density of 30 plants per square meter in Sarayan (Table 4). Furthermore, our study revealed that delayed planting resulted in decreased seed yield, carbohydrate content, galactomannan content, total ash, protein yield, and oil yield. This observation is consistent with the findings of

Haydarzadeh et al. [17], who reported that delayed planting led to a decline in seed quality and germination characteristics due to the overlap of critical reproductive growth stages with late-season rainfall. In contrast, Ramazani and Moshtaghi [38] focused on callus induction in guar and found that hormonal compositions and ex plant types significantly influenced callus formation. While their study did not directly address planting date or density, it emphasizes the importance of understanding the physiological responses of guar to different environmental and hormonal cues.

Our study's findings provide a comprehensive understanding of how planting date and plant density interact to influence both the quantity and quality of guar seeds across different locations. The consistently significant effects observed for these factors highlight their critical role in guar production.

**Table 4** Results of the comparison of the interaction effect of planting date and density on grain quality characteristics of guar plant in the location

| Planting Date | Plant Density | Seed yield ($g/m^2$) | | Oil (%) | | Protein (%) | | Carbohydrate (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Birjand | Sarayan | Birjand | Sarayan | Birjand | Sarayan | Birjand | Sarayan |
| May 12nd | 10 | 205.15 h | 99.31s | 7.55 a | 3.94 i | 23.75 l | 34.69 a | 14.06 v | 15.46 u |
| | 20 | 194.97 i | 167.45 j | 3.54 k | 3.01 l | 25.88 i | 23.48 lm | 30.37 h | 20.05 q |
| | 30 | 163.05 jk | 215.06 g | 2.17 p | 5.82 e | 24.36 k | 32.80 b | 31.60 g | 26.03 lm |
| | 40 | 85.51 t | 135.44 pq | 3.89 ij | 5.61 f | 23.29 mn | 31.17 de | 32.65 f | 18.79 r |
| | 50 | 114.82 r | 274.60 ab | 2.69 m | 2.47 n | 24.89 j | 30.93 e | 44.33 a | 20.86 p |
| June 12nd | 10 | 278.03 a | 116.84 r | 1.40 r | 1.89 q | 18.92 r | 19.66 q | 40.08 b | 28.95 i |
| | 20 | 243.53 d | 153.15 lm | 2.08 p | 1.29 r | 20.30 p | 27.54 g | 20.35 pq | 16.27 t |
| | 30 | 192.94 i | 232.26 e | 2.47 n | 7.13 b | 22.93 n | 31.57 d | 37.65 d | 22.44 o |
| | 40 | 159.11 kl | 265.31 c | 2.24 op | 3.76 j | 19.23 qr | 23.47 lm | 29.29 i | 21.71 o |
| | 50 | 140.97 op | 270.67 bc | 3.53 k | 4.85 h | 22.06 o | 34.86 a | 27.20 jk | 17.05 s |
| July 13nd | 10 | 103.54 s | 98.98 s | 6.24 d | 6.31 d | 19.08 r | 23.25 mn | 36.56 e | 22.16 o |
| | 20 | 138.57 p | 151.04 mn | 1.39 r | 2.37 no | 24.47 jk | 18.93 r | 39.73 bc | 25.32 mn |
| | 30 | 146.16 no | 145.77 no | 5.85 e | 5.43 g | 18.93 r | 32.12 c | 27.51 j | 26.64 kl |
| | 40 | 121.56 r | 198.69 hi | 5.87 e | 6.73 c | 26.89 h | 35.00 a | 39.02 c | 18.51 r |
| | 50 | 131.10 q | 224.80 f | 2.19 p | 3.44 k | 23.41 lm | 29.88 f | 24.86 n | 17.51 s |

Means within each column and within each section followed by the same letter(s) are not significantly different ($P<0.05$).

**Table 4 (Continued).** Results of the comparison of the interaction effect of planting date and density on grain quality characteristics of guar plant in the location

| Planting Date | Plant Density | Phosphorous (mg) | | Total Ash (%) | | Protein yield (%) | | Oil yield (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Birjand | Sarayan | Birjand | Sarayan | Birjand | Sarayan | Birjand | Sarayan |
| May 12nd | 10 | 2.47 i | 2.23 l | 5.07 j | 6.04 f | 48.72 hi | 34.27 m | 15.48 b | 3.91 l |
| | 20 | 2.51 gh | 2.45 i | 5.21 hij | 3.20 n | 50.70 gh | 39.21 l | 7.97 g | 5.07 k |
| | 30 | 2.46 i | 1.96 p | 5.17 ij | 2.59 p | 39.75 l | 70.59 d | 3.55 lm | 12.44 d |
| | 40 | 1.95 p | 2.05 o | 6.93 c | 3.27 mn | 20.38 s | 42.03 k | 3.17 mn | 7.62 g |
| | 50 | 2.58 f | 2.38 jk | 8.08 a | 3.12 n | 29.33 opq | 84.19 b | 2.88 n | 6.81 hi |
| June 12nd | 10 | 2.10 n | 2.76 b | 4.16 k | 5.34 hi | 52.61 g | 22.98 r | 3.90 l | 2.17 o |
| | 20 | 2.73 c | 2.51 g | 5.30 hi | 2.88 o | 49.46 h | 42.07 k | 5.02 k | 1.99 o |
| | 30 | 2.19 m | 1.78 r | 5.95 f | 2.33 qr | 44.24 j | 73.85 c | 4.77 k | 16.56 a |
| | 40 | 2.51 gh | 2.40 j | 8.15 a | 6.35 e | 30.45 op | 62.37 f | 3.38 m | 9.90 e |
| | 50 | 2.63 e | 2.11 n | 7.33 b | 3.79 l | 31.12 no | 94.38 a | 4.97 k | 13.14 c |
| July 13nd | 10 | 2.48 hi | 2.82 a | 5.91 f | 5.65 g | 19.65 s | 23.04 r | 6.48 ij | 6.24 j |
| | 20 | 2.57 f | 2.58 f | 2.45 pq | 1.97 s | 33.88 m | 28.61 pq | 1.93 o | 3.59 lm |
| | 30 | 2.52 g | 1.83 q | 6.53 d | 3.43 m | 27.65 q | 46.73 i | 8.59 f | 7.84 g |
| | 40 | 2.36 k | 2.04 o | 5.37 h | 2.22 r | 32.60 mn | 69.22 de | 7.13 h | 13.29 c |
| | 50 | 1.45 s | 2.67 d | 6.03 f | 4.01 k | 30.70 nop | 67.22 e | 2.82 n | 7.74 g |

Means within each column and within each section followed by the same letter(s) are not significantly different ($P<0.05$).

## CONCLUSION

This study rigorously investigated the impact of varying planting dates and plant densities on guar seed yield and key quality characteristics. Our findings unequivocally demonstrate that planting guar in early June, specifically around June 22nd, under favorable early-season climatic conditions, significantly enhances seed yield compared to later planting dates. This increased yield is primarily attributed to the extended vegetative growth period afforded by earlier planting. Conversely, delayed plantings, such as on July 22nd, resulted in diminished yields due to shortened vegetative phases and reduced plant canopy development.

Regarding seed quality, the June 22nd planting date consistently yielded the highest values for crucial parameters, including seed yield, carbohydrate, galactomannan, ash, protein, and oil content. Notably, while the optimal plant density varied for each trait, a density of 30 plants/m² in the June 22nd planting emerged as the most favorable combination for maximizing both seed yield and galactomannan content – a critical factor for industrial applications of guar. It is worth noting that for specific quality traits like oil and protein content in the July 22nd planting, a higher density of 40 plants/m² proved optimal, and for seed phosphorus, a density of 10 plants/m² on the same date yielded the best results. Furthermore, in Birjand, the highest seed yield, the highest oil percentage, the highest carbohydrate percentage, and the highest total ash percentage were obtained. However, Sarayan also shows superiority in producing seeds with specific quality characteristics, as the highest protein percentage, highest phosphorus content, highest protein yield, and highest oil yield were achieved in this location.

The practical implications of this research are significant for guar cultivation in the investigated regions. Our study strongly recommends planting guar around June 22nd with a plant density of 30 plants/m² to achieve optimal seed yield and galactomannan content. However, for growers prioritizing specific quality traits like oil or protein, adjusting plant density based on our findings for later planting dates may be beneficial.

It is important to acknowledge a potential limitation of this study: the findings are specific to the climatic conditions and soil types of the investigated regions. Future research could explore the generalizability of these results across a wider range of geographical locations and environmental conditions. Furthermore, investigating the physiological mechanisms underlying the observed relationships between planting date, plant density, and seed yield and quality would provide a deeper understanding and potentially lead to further optimization strategies.

In conclusion, this study provides valuable insights into optimizing guar production by identifying the critical role of planting date and plant density. The recommendation of a June 22nd planting with 30 plants/m² offers a practical and evidence-based strategy for maximizing both seed yield and the industrially important galactomannan content in the studied regions, contributing significantly to efficient guar cultivation practices.

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