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EFFECT OF PLANTS SPACE AND FOLIAR APPLICATION OF HUMIC ACID IN SEED YIELD AND BIOCHEMICAL COMPOSITION OF GOAT PEA (*SECURIGERA SECURIDACA* L.)

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SUMMARY

A field experiment ensued during the winter crop season 2018–2019 at the District Baqubah, Diyala Governorate, Iraq, to study the effects of three plant spacing (20, 40, and 60 cm) and foliar application of humic acid with four concentrations (0, 10, 20, and 40 L ha⁻¹) on seed yield-related traits and active ingredients of goat pea (*Securigera securidaca* L.). The recorded data underwent statistical analysis using a randomized complete block design (RCBD) with factorial arrangement. The results showed that the plant spacing of 60 cm was superior in the percentage of oil and the total content of glycosides in the seeds, amounting to 3.84% and 27.86 mg g⁻¹, respectively, and the plant distance of 40 cm was superior in the seeds' total phenol content (1.23 mg g⁻¹). As for 20 cm, it was excellent in the oil yield (15.56 kg ha⁻¹). The spraying with humic acid at the rate of 20 L ha⁻¹ provided the highest percentage and harvest of oil and total phenol content, reaching 3.69%, 9.30 kg ha⁻¹, and 1.21 mg g⁻¹, respectively. The interaction between the plant distance (60 cm) and foliar application of humic acid (10 L ha⁻¹) showed a significant increase in the percentage of oil, amounting to 3.92%. The interaction between the plant distance (40 cm) and foliar application of humic acid (20 L ha⁻¹) provided a substantial increase in the total phenol content of seeds (1.25 mg g⁻¹).

Keywords: goat pea (*Securigera securidaca* L.), plants distances, humic acid, oil percentage, yield, phenols, glycosides

Key findings: Increased plants distance to 60 cm led to an increase in the percentage of oil content. Foliar application of humic acid (20 L ha⁻¹) gave a significant superiority in seed yield in the plant, oil content ratio and harvest, and total phenol content.

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INTRODUCTION

Goat pea (*Securigera securidaca* L.) is one of the winter annual plants of the family Fabaceae, phenotypically characterized by dense vegetative branches that spread close to the soil surface. In several pharmaceutical products, goat pea is one of the most important medicinal plants. The goat pea (*S. securidaca*) seeds are rich in unsaturated fatty acids, such as, oleic and linoleic acids, which mainly help in treating arterial diseases and reducing blood fats on a large scale (Rajaei *et al.*, 2015; Jamshidzadeh *et al.*, 2018), as well as, treating blood diseases, blood vessels and the heart, and diabetes (Onwubiko, 2020; Alizadeh-Fanalou, 2020). The *S. securidaca* seed is also a good source of phenolic compounds with high antioxidant properties. In addition, the plant seeds contain many phytochemicals primarily composed of aromatics and oxygenated hydrocarbons, where Dodecanedioic acid and its derivatives and sitosterol are pharmacologically active components. Therefore, it gives importance to the ethnopharmacological use of this plant to treat diabetes and hyperlipidemia (Behnamnik *et al.*, 2019; Aldain *et al.*, 2020). Several studies also indicated that the seeds of the *S. securidaca* plant are like drugs, especially the current anti-diabetic medicines, with the minimum side effects (Nasehi *et al.*, 2023). In addition, the seeds of the *S. securidaca* plant serve in cancer treatment (Abdelbagi *et al.*, 2023).

The plants' space is one of the agricultural tools determining the nature of plant growth. Plants space provides a great opportunity and helps enable the process of photosynthesis and the production of necessary metabolic compounds for the growth and development of plants per unit area. The density of suitable crops reduces disturbances in photosynthesis due to competition between plants, which maintains the balance between primary and secondary metabolism and increases the synthesis and accumulation of active ingredients (Liu *et al.*, 2021). Enhanced plant density in the fenugreek crop led to an increase in the seed weight, the number of pods, and the seed yield per plant and

provided an opportunity to absorb nutrients with high efficiency, especially when exposed to environmental stress conditions (Ahmad and Abdulla, 2016; Tiwari *et al.*, 2016).

The vital importance of humic acid effects on cell membranes leads to enhanced transport of minerals, improved protein synthesis, and plant hormone-like activity, promoted photosynthesis, and modified enzyme activities (Hamideh *et al.*, 2013). It also enhances the relative growth rates of shoots and roots under abiotic stress conditions, which was considered a promising soil amendment to overcome the adverse effects of salinity stress on the absorption of elements (Meganid *et al.*, 2015). Thus, it stimulates the crucial pathways for secondary metabolites' synthesis in medicinal plants. No relevant study exists in the past on the *S. securidaca* plant in Iraq; hence, the presented study planned to assess the effects of plants' distance and foliar application of humic acid on the seeds' yield and its oil content and active ingredients.

MATERIALS AND METHODS

The experiment proceeded during the winter crop season of 2018–2019 in the field in a semi-arid environment at the District Baqubah, Diyala Governorate, Iraq. The trials, laid out in a randomized complete block design (RCBD), had a factorial arrangement and three replications. The study included two factors, with the first having three plant spaces (20, 40, and 60 cm), and the second factor was a humic foliar application with four different levels (0, 10, 20, and 40 L ha⁻¹). Humic acid was the "Liquid Organic Fertilizer" manufactured by a Turkish Company called Al-Jawhara Humic (Table 1). The humic acid use comprised applying three times; the first was on 15 December 2018, with the other two foliar applications made had 20 days interval.

Crop husbandry

The experimental soil underwent plowing and leveling orthogonally, then divided into experimental units with dimensions of 4 m ×

Table 1. The chemical components of the Al-Jawhara Humic produced by the Jewel of Spring Company.

The material	The ratio %
Humic acid	12%
Fulvic acid	3%
Mn	200 ppm
Zn	200 ppm
Fe	300 ppm
B	100 ppm
N	3%

Table 2. The physical and chemical properties of the soil before planting.

Measurements	Value	Unit of measurement
Organic matter	0.57	g kg ⁻¹
CO ₃	0.30	%
Ec	2.12	ds m ⁻¹
Ph	6.21	-
N	10.6	Ppm
P	35.1	Ppm
K	187.3	Ppm
Clay	45.2	g kg ⁻¹
Silt	25.92	g kg ⁻¹
Sand	28.88	g kg ⁻¹
Texture of soil	Clay	-
HCO ₃	85	mEq L ⁻¹
SO ₃	0.4	%

3.5 m. Each experimental section included six lines, with a distance between the lines of 50 cm and between the trial units and replicates of one meter. Planting goat pea seeds were in rows. The experimental soil received fertilization of phosphate (P) and nitrogen (N) fertilizers at a rate of 80:100 kg NP ha⁻¹ (Datta *et al.*, 2017). The phosphate fertilization was after plowing and just before planting, while nitrogen fertilization was in two split doses, with the first dose used in the first week after germination and the second dose after one month.

The collection of soil samples was random from the field at a depth of 15–30 cm before planting, and its analysis ran in the central laboratory, College of Science, University of Baghdad, Iraq, to study their physical and chemical properties (Table 2). The crop service and irrigation processes also happened when needed. The goat pea seeds came from the National Center for Agricultural Research and Extension, Hashemite Kingdom

of Jordan. Testing the seeds' vitality at 95% ensued before the sowing. Crop harvest was on 25 May 2019 after their legumes' color became light brown and ripened.

Studied traits

Seed yield

Ten plants randomly selected from each experimental unit attained recording for data on the following traits: number of pods per plant, 500-seed weight (g), and seed yield per plant (g).

Biochemcial composition

Oil percentage

Oil extraction from the goat pea seeds used the Soxhlet method, with the oil percentage estimated according to the standard method (AOAC, 1984). The extracts continued drying at a temperature of 60 °C until the weight of

the oil became stable. The percentage of oil calculation had the following equation.

$$\text{Percentage of oil} = \frac{\text{oil weight}}{\text{seed sample weight}} \times 100$$

Oil yield (Kg h⁻¹)

The oil yield calculation used the following equation:

$$\text{Oil yield} = \text{percentage of oil (\%)} \times \text{seed yield per hectare (kg h}^{-1}\text{)}.$$

Total glycosides in seeds (mg g⁻¹)

A 20 g dried and ground seeds of goat pea proceeded to soak in methanol (80%) at a rate of 3 × 300 ml for 48 h, filtered and concentrated by adopting the method of Solich *et al.* (1992). The cardiac glycoside content of the extract estimation ran by taking an extract of 10% concentration and then mixed with 0.16 ml of freshly prepared Baljet reagent. After an hour, the mixture dilution used 10 ml of distilled water. Measuring the absorbance at 495 nm employed a spectrophotometer for UV/VIS. A standard curve's preparation progressed by taking 10 ml of different concentrations (12.5–100 ppm) of the standard, according to Tofghi (1992). In each sample, the expression of the total glycosides was as mg g⁻¹ securidaside in dried seed extract.

Total phenols in seeds (mg g⁻¹)

For total phenol content, 1 g of dried and ground seeds added with 20 ml of ethanol (80%) remained at storage for 24 h while preparation of the Folin-Ciocalteu reagent progressed in determining the phenolic compounds (Slinkard and Singleton, 1977; Jeffery *et al.*, 1989). Afterward, dilution followed (1 ml reagent: 4 ml distilled water), with 1 ml added to 50 microliters of the above extract and shaken for 3 min. Next, 1 ml of Na₂CO₃ solution at a concentration of 10% added to the mixture occurred before leaving it an hour in the dark. The absorbance of phenolic compounds' measurement engaged a

spectrophotometer at a wavelength of 760 nm. The total phenolic compounds estimated as gallic acid equivalents followed the method according to Masum-Akond *et al.* (2010).

Statistical analysis

All the recorded data on various variables' analysis used the SAS Statistical Program (2001), with the averages compared and separated by using Duncan's Multiple Range (DMR) test at a 5% level of probability (Al-Rawi and Khalaf-Allah, 1980).

RESULTS

Seed yield

The plants' space of 60 cm resulted in a significant effect, providing the highest average in two traits, the number of pods per plant (69.6 pods) and 500-seed weight (4.45 g). The 40-cm spacing followed by the highest average seed yield per plant (4.81 g) compared with the lowest averages obtained from the plant distance of 20 cm (61.9 pods, 4.12, and 4.40 g, respectively) in goat peas (Table 3). The foliar application of humic acid also showed significant differences in various levels for the above traits. The humic acid 20 L ha⁻¹ demonstrated superiority in two characteristics: number of pods per plant (70.7) and seed yield per plant (5.36 g), while at the rate of 40 L ha⁻¹, it gave the maximum average of the 500-seed weight (4.41 g) compared with the control (treatments with no humic acid spray), which reached 61.9, 4.14, and 4.28 g, respectively.

The interaction of plants' distance and humic acid foliar application significantly impacted seeds' yield and biochemical traits (Table 3). Plant distance (60 cm) with organic fertilizer (20 L ha⁻¹) led in two parameters, i.e., the number of pods per plant (75.1) and 500-seed weight (4.62 g), followed by plant distance (40 cm) and the humic acid (20 L ha⁻¹) with the highest seed yield per plant (5.96 g) compared with the plant distance of 20 cm and no humic acid application, giving the

Table 3. Effect of plants space and foliar application of humic acid in the pods per plant, 500-seed weight, and seed yield of goat pea.

Plants' distance (cm)	Spraying with humic acid (L ha ⁻¹)				Means
	0	10	20	40	
Pods per plant (
20	58.4 G	62.9 EF	67.3 CD	59.0 G	61.9 C
40	60.7 GF	65.5 ED	69.9 BC	61.6 GF	64.4 B
60	66.7 CD	70.7 B	75.1 A	65.9 ED	69.6 A
Means	61.9 C	66.3 B	70.7 A	62.1 C	
500-seeds weight (g)					
20	4.10 F	3.91 H	4.20 E	4.27 D	4.12 C
40	4.06 G	4.29 D	4.26 D	4.42 C	4.25 B
60	4.26 D	4.40 C	4.62 A	4.54 B	4.45 A
Means	4.14 D	4.20 C	4.36 B	4.41 A	
seed yield per plant (g)					
20	4.07 H	4.45 EF	4.95 C	4.13 GH	4.40 C
40	4.32 FG	4.62 DE	5.96 A	4.35 F	4.81 A
60	4.46 EF	4.71 D	5.19 B	4.44 EF	4.70 B
Means	4.28 C	4.59 B	5.36 A	4.30 C	

lowest values for the number of pods per plant (58.4) and seed yield per plant (4.07 g) and interaction of the plants' distance (20 cm) with humic acid (10 L ha⁻¹), also providing with the lowest 500-seed weight (3.91 g).

Biochemical traits

The various plants' space also caused significant differences in varied biochemical traits. The plant distance of 60 cm gave the highest average in the oil percentage (3.84%) in seeds, compared with the 20 cm spacing (3.53%) (Table 4). Meanwhile, the 20 cm plant distance indicated the maximum average in the oil yield (15.56 kg h⁻¹) versus the plant distance of 60 cm (6.02 kg h⁻¹). The humic acid foliar application also caused a significant impact on the above trait. Spraying with humic acid at 20 L ha⁻¹ provided the utmost average oil percentage (3.69%) and oil yield (9.30 kg ha⁻¹) in the goat peas' seeds compared with the control treatment (3.60% and 7.73 kg ha⁻¹, respectively).

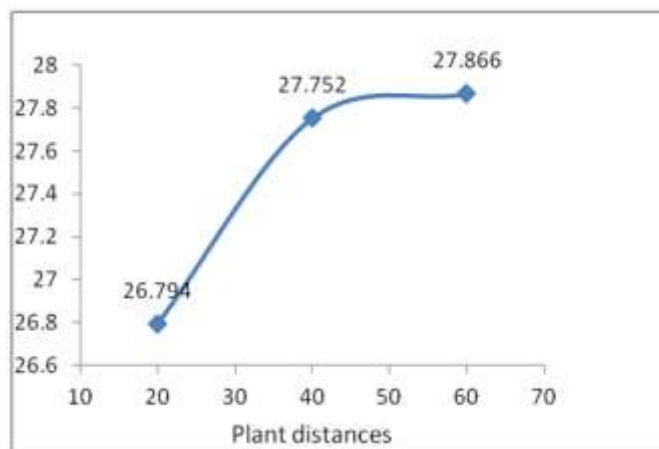
The results in (Table 4) indicated noteworthy differences among the plant distances for total phenols and glycosides. The plant distance of 40 cm was superior in total phenols and glycosides content, reaching 1.23 mg g⁻¹ and 27.86 mg g⁻¹, respectively, compared with the lowest averages of 20 cm

spacing (1.15 and 26.79 mg g⁻¹, respectively). The results further revealed in (Table 4). that humic acid application with different levels had a notable effect on the total phenol content in seeds, and the humic acid at 20 L ha⁻¹ gave the highest average of total phenols (1.21 mg g⁻¹), compared with the control treatment (1.18 mg g⁻¹). However, the data did not indicate a significant effect of humic acid application on the total glycoside content in the goat pea seeds.

On the interaction of plants' distance and humic acid application, the plant's distance of 60 cm and humic acid at 20 L ha⁻¹ were superior in oil percentage (3.92%), compared with the plant spacing of 20 cm and the control, which gave the lowest value for the above oil trait (3.48%). Further, the plant distance of 20 cm and humic acid at 20 L ha⁻¹ were superior in the oil yield (17.77 kg ha⁻¹) versus the 60 cm spacing and humic acid at 40 L ha⁻¹, which gave the lowest value for the above oil feature (5.65 kg ha⁻¹). The plant distance of 40 cm and humic acid at 20 L ha⁻¹ also showed superior, providing the highest rate of total phenols (1.25 mg g⁻¹) compared with the plant distance of 20 cm with the control treatment (1.13 mg g⁻¹). The presented results had no significant interaction between the treatments in terms of the total glycoside content in the goat pea seeds (Table 4).

Table 4. Effect of plants space and foliar application of humic acid in the oil percentage and yield, total phenols, and total glycosides of goat pea seeds.

Plants' distance (cm)	Humic acid Application (L ha ⁻¹)				Means
	0	10	20	40	
OIL PERCENTAGE (%)					
20	3.48 F	3.51 EF	3.59 C	3.56 CD	3.53 C
40	3.52 E	3.54 DE	3.58 C	3.57 CD	3.55 B
60	3.82 B	3.83 B	3.92 A	3.82 B	3.84 A
Means	3.60 D	3.62 C	3.69 A	3.65 B	
oil yield (kg ha⁻¹)					
20	14.16 B	15.61 B	17.77 A	14.70 B	15.56 A
40	7.60 DE	8.17 D	10.66 C	7.76 DE	8.54 B
60	5.67 E	6.01 E	6.78 DE	5.65 E	6.02 C
Means	9.14 B	9.93 B	11.73 A	9.37 B	
total phenols (mg g⁻¹)					
20	1.135 L	1.153 K	1.163 I	1.158 J	1.152 C
40	1.200 H	1.243 C	1.253 A	1.248 B	1.236 A
60	1.209 G	1.227 F	1.237 D	1.232 E	1.226 B
Means	1.181 D	1.207 C	1.217 A	1.212 B	
total glycosides (mg g⁻¹)					
20	26.191 A	26.976 A	27.148 A	26.861 A	26.794 B
40	27.148 A	27.940 A	28.105 A	27.818 A	27.752 AB
60	27.263 A	28.048 A	28.221 A	27.933 A	27.866 A
Means	26.867 A	27.654 A	27.824 A	27.537 A	

**Figure 1.** Effect of plants' distance on total glycoside content.

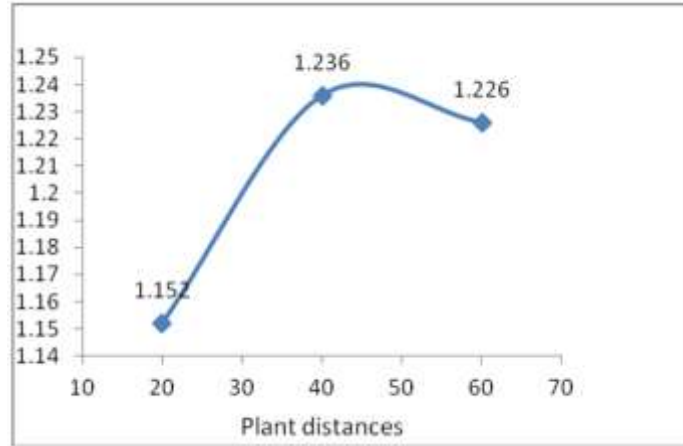


Figure 2. Effect of plants' distance on total phenol content.

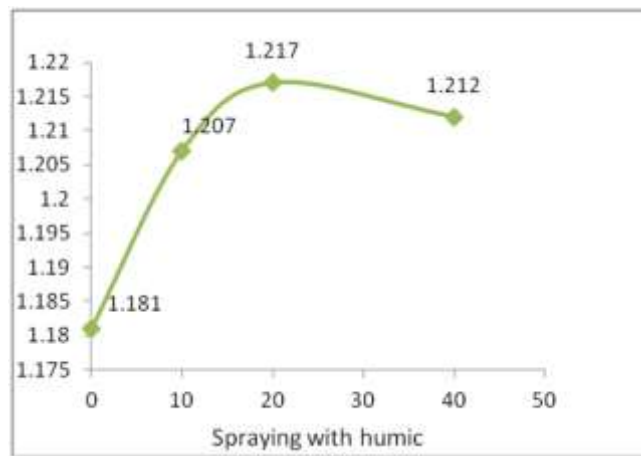


Figure 3. Effect of foliar application of humic acid on total phenol content.

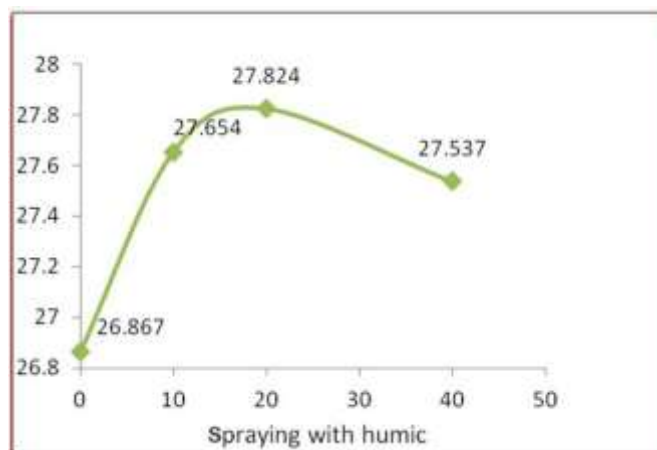


Figure 4. Effect of foliar application of humic acid on total glycoside content.

DISCUSSION

The superiority of the number of pods per plant and 500-seed weight at the plant spaces of 60 cm may be due to the reduced competition between plants for environmental factors, such as, light, carbon dioxide, water, and nutrients (Tiwari *et al.*, 2016). The optimum intensity balances the interception of the sunlight with the shading volume of the lower leaves and efficiency in the process of converting light energy into chemical energy that contributes to the rate of completing the photosynthesis process, increasing the number of pods per plant, the seeds' formation, and an enhancement in their weight. Also, the reduced plant distance (40 cm) provided an opportunity to increase other yield components, thus, boosting the plant yield of seeds. These results are consistent with the findings of Ahmad and Abdulla (2016) and Tiwari *et al.* (2016), who reported that reducing plant density per unit area of fenugreek plants led to a significant increase in seed weight, number of pods, and seed yield per plant.

The superiority in the number of pods per plant and 500-seed weight with humic acid foliar application (at 20 and 40 L ha⁻¹) is attributable to the vital role of the nutrient in forming chlorophyll in leaves and its efficiency in photosynthesis production, which eventually increased the number of pods in the plant and seed weight (Abd-El-Aal and Eid, 2017). El-Nwehy *et al.* (2020) also reported that foliar spray of humic acid on soybean crops caused an increase in the number of pods per plant. The presented results also revealed that an increase in the seed yield per plant might also be due to the surge in the number of pods per plant. These results were in analogy with the past findings of Mafakheri and Asghari (2018), who concluded that humic acid application significantly affected seed yield and its components in fenugreek crops.

The buildup in the goat pea seed's oil content with plants' space of 60 cm may refer to the lessening of competition factor between plants, the expansion of the vegetative area exposed to sunlight, and the improvement in the efficiency of carbon metabolism production

and its transfer rate to the seeds, which leads to an enhanced seed oil content. El-Saady *et al.* (2013) also reported that expanding the plant distance in the *Lallemantia iberica* crop caused an increase in the seed oil percentage. The relevant results revealed that the low oil yield per hectare was due to a decreased harvest of seeds with the increase in the plants' space between them. The surge in the oil content in seeds with foliar application of 20 L ha⁻¹ may be due to the role of the humic acid in increasing the activity of vital enzymes, including those involved in forming chlorophyll molecules. Mafakheri and Asghari (2018) also disclosed that humic acid application on the fenugreek crop led to a significant rise in the oil content of the seeds.

The superiority of the glycosides in the seeds at the plants' distance of 40 cm may be because of the expansion of the vegetative area of the plants and the efficiency of photosynthesis, contributing to the production of vital compounds by stimulating the enzymes of an uridyl transferase and glycosyl transferase. It also facilitates forming uridine diphosphate, the basic building formation of the aglycone compound in plants.

Reducing the plants' space to 40 cm provided a better opportunity to decrease biological stress among plants, forming intermediates for the synthesis of phenols (Lombardo *et al.*, 2009) because the active substances work as secondary products for the photosynthesis process (Al-Mohammadi, 2009). The superiority of the total phenols in the seeds after spraying with humic acid at 20 L ha⁻¹ may be attributable to creating a state of nutritional balance in the goat pea plants that encourages photosynthesis, which contributes to producing primary metabolic compounds and catalyzes enzymatic and vital reactions toward creating secondary metabolic compounds, including phenol content in plant seeds. Adding organic compounds that contain humic to goat pea (*S. securidaca* L.) plants increased the oil content and phenolic compounds in seeds (Hassan, 2023). These results were also consistent with the findings of Mohammed *et al.* (2015), who reported that humic acid foliar application on fenugreek

plants remarkably increased the content of the active substances, i.e., trigonelline and flavonoids in the seeds.

CONCLUSIONS

The results showed that cultivation of goat peas with a plant distance of 60 cm significantly affected the biochemical traits, such as, oil percentage and total glycoside content in seeds by maintaining the biological balance between plants and the environmental and nutritional requirements necessary for plant growth and management without competition in the unit area. The use of humic acid at a level of 20 L ha⁻¹ led to a notable effect in increasing the oil percentage and yield and total phenol content in goat pea seeds due to the vital role of the nutrient in securing plant growth requirements of ready-made elements. Therefore, more studies must implement advanced agricultural technologies to enhance goat pea seeds' harvest and active compounds.

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