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Research Article

Application of Chemical Fertilizers and Plant Spacing Improves Growth and Root Yield of *Rehmannia glutinosa* Libosch

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Abstract

Background and Objective: *Rehmannia glutinosa* Libosch. is an important medicinal plant, used widely in folk remedies for daily health care in Vietnam. This study aimed to evaluate the effect of nitrogen and phosphorus fertilizers and plant spacing on the growth, yield and quality of *R. glutinosa* roots. **Materials and Methods:** The experiment consisted of two factors: Factor A: Nitrogen (5 levels), phosphorus (5 levels) and Factor B: plant spacing (3 levels). Experiments were laid out in randomized complete block design (RCBD) with three replications. **Results:** The results show that nitrogen and plant spacing significantly affected the vegetative growth of *R. glutinosa* in which N application of 240 kg ha^{-1} and plants arranged in 20×30 cm spacing brought the highest growth characters while P application showed little difference in canopy diameter. N and P application and their interaction with spacing significantly influenced the yield and quality of roots in which N4 (180 kg N ha^{-1})×S3 (20×30 cm) and P4 (120 kg P ha^{-1})×S3 (20×30 cm) produced the highest yield and quality of roots. **Conclusion:** The current study provides further insights into the effectiveness of chemical fertilizer and plant spacing on the growth and development of *R. glutinosa*. Further evaluation of wider spacing and changes of bioactive compounds under various conditions would be necessary for medicinal plant utilization.

Key words: Agronomy, chemical fertilizers, plant spacing, medicinal plant, cultivation technique, commercial production, bioactive compounds

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

INTRODUCTION

Rehmannia glutinosa Libosch. is a traditional medicinal plant, which is also widely used as the main supplement in various folk remedies for daily health care in Vietnam and other Asian countries[1]. The herb can be used in different forms of medicinal materials such as fresh root, dried rhizome and prepared Rehmannia root with different purposes such as clearing away heat, promoting salivation and the production of body fluids and removing pathogenic heat from blood[2]. Recent studies have confirmed that *R. glutinosa* as varied pharmacological functions and chemical compositions rather than the initial knowledge in which this plant was used for supporting kidney performance. These reports described *R. glutinosa* as active principles on the blood system, immune system, endocrine system, cardiovascular system and nervous system and as anti-tumor, anti-senescence agents[3-9]. Chemical studies that established a foundation of pharmacological research have separated 70 monomeric compounds including catalpol, phenolic glycoside inone, flavonoid, amino acid, etc[1]. Of those, catalpol and glycoside are the main active principles of *R. glutinosa* which has the hypoglycaemic, diuretic and laxative effects, an anti-anoxia effect and immune regulative effect[10].

The study of Luo *et al.*[11] showed that there was a correlation between catalpol content and shape of root tuber, the larger the root, the higher the content of catalpol and vice versa. Few other studies have searched for methods to protect this species plantation from diseases[12,13]. Recent demand for this medicinal plant's materials has been increasing not only from traditional users but also from many pharmaceutical companies due to their strong bioactive compounds. However, research on promoting *R. glutinosa* cultivation to gain greater root yield through a better quality of fresh root was limited in the literature. Nutrients such as nitrogen and phosphorus fertilizers and optimal plant density are the most important cropping factors which determine the survival, growth and development of medicinal plants and decide the quality of root tuber, bioactive compound biosynthesis[14].

Nitrogen plays an important role in the synthesis of many organic compounds such as nucleic acids, enzymes, amino acids and proteins. The well-known function of nitrogen is to promote the leaf cell number and overall leaf production[15]. Phosphorus is integral to nearly all major metabolic processes as it constitutes of nucleic acid, phospholipids and coenzymes. Plants with insufficient phosphorus intake may result in a reduction of chloroplast carbon fixation and may be adversely affected by the photosynthetic process[15,16]. Plant spacing is an important factor in determining the micro-environment for the *R. glutinosa* and other medicinal plant species. The optimization of this factor can result in better growth as well as the quality and quantity of roots, which also determines the content of some bioactive compounds[17]. Due to poor commercial cultivation of *R. glutinosa* recently, little is known about cultivation techniques and mineral demand. Studies of these factors will provide scientific and practical benefits for farmers in agricultural production, particularly the cultivation of *R. glutinosa*.

The objectives of the present study were to examine the effects of nitrogen and phosphorus fertilizers, plant spacing and their interaction on growth characters, quality and quantity of roots of *R. glutinosa* in experimental fields in Phu Tho province, Vietnam.

MATERIALS AND METHODS

Site information: The field experiments were conducted at fields in Phu Tho Province, Vietnam during the Autumn-Winter season of 2018 and 2019 to study the effect of nitrogen (N), phosphorus (P) fertilization and plant spacing on the growth of herbage and yield of roots of *R. glutinosa*.

Experimental design: The influence of N and P concentration was examined with five concentration levels for each fertilizer in three different plant density arrangements according to the guidelines of Nguyen and Nguyen on the cultivation techniques, utilization and processing medicinal plants[18]. In the first experiment, fifteen treatments of each fertilization represented the combination of five levels of N including 0, 60, 120, 180, 240 kg ha^{-1} (in the form of ammonium nitrate); five levels of P including 0, 40, 80, 120, 160 kg ha^{-1} (in the form of super phosphate) were prepared. Every five fertilization treatments were assigned to plant spacing S1, S2, S3 with three density levels of 33, 25 and 16 plants m^{-2} planted with spacing of 15×20, 20×20 and 30×20 cm, respectively, in a plot size of 10 m^2 for each treatment. In total, 30 treatments were studied. All treatments were arranged in a completely randomized block design with three replicates, resulted in 90 studied plots. The fertilization of N was conducted at two equal portions: firstly, at the initial stage of vegetative growth (30 days) and the second portion was added after 20 days from the first one. While phosphorus fertilizers had been added before planting.

Field planting and collection of data: *R. glutinosa* roots were collected from Phu Tho province and identified by Dr. Pham Thanh Loan, Institute of Applied Research and Development, Hung Vuong University, Vietnam. Fresh root cuttings were planted at one cutting per stand in all plots treated with fertilizers and different plant spacing. Weeds were manually cleared as they grew to eliminate competition with the newly sprouted *R. glutinosa* plant.

The harvesting was conducted 170 days after planting. Field data other than the yield of roots were obtained by randomly sampling ten plants from each experimental plot before each harvest. The following pre-harvest and post-harvest growth and quality parameters such as shooting rate, number of shoot plant^{-1}, mortality, number of leaves plant^{-1}, plant height (cm), canopy diameter (cm), number of tuber plant^{-1}, average fresh weight of root (gram), fresh weight of roots plant^{-1}, yield of roots hectare^{-1} (kg) were recorded. The evaluation of these parameters was performed once, 170 days after planting.

Statistical analysis: Data collected were subjected to statistical analysis of variance by E test at the 0.05 probability level. Least significant difference (LSD) was used at 5% level of significance of treatments and significance means through the procedure described by Jan *et al.*[19]. The data were analyzed by using IRRISTAT 5.0 program.

RESULTS

Effect of nitrogen, phosphorus fertilizers and plant spacings on plant growth: There were insignificant interactions (p>0.05) between the nitrogen fertilization (NF) and plant spacing (PS) for all measured growth parameters (Table 1). In contrast, significant differences in the number of leaves, plant height and canopy diameter were observed as the influence of PS and NF in particular. The highest number of leaves plant^{-1}, plant height and canopy diameter were obtained at all sampling dates in 30×20 cm (S3) spacing due to spacing at 170 days after planting. Whereas, the lowest of these parameters were recorded when planting was in 15×20 cm spacing. Fertilization management also had a significant effect on plant growth when fertilized with the highest application of 240 N and the lowest of these values were observed in the none-nitrogen application.

High phosphorus (P) fertilizer generally brought a positive effect on plant growth. However, P fertilization and plant spacing interaction in this field study had no role in differentiating the growth of *R. glutinosa* as the sprouting rate, survival rate, number of leaves, plant height and canopy diameter, which were similar between plants at all plots (p>0.05) (Table 2). Nevertheless, there were significant differences in canopy diameter due to plant spacing or phosphorus fertilization separately and survival rate due to spacing in which planting with density of 16 plants m^{-2} (S3), *R. glutinosa* grew with the largest canopy of 38.7 cm on

Table 1: Growth characteristics in *R. glutinosa* subjected to different spacings and nitrogen fertilization

Plant spacing	Nitrogen fertilizer	Sprouting rate (%)	Survival rate (%)	No. of leaves plant	Plant height (cm)	Canopy diameter (cm)
S1	N1	95.6	92.8	21.4	30.2	35.4
	N2	95.2	92.4	21.9	30.8	36.4
	N3	94.6	92.2	21.4	31.6	37.2
	N4	95.1	92.8	21.6	32.4	37.8
	N5	95.6	92.6	21.9	32.8	38.5
S2	N1	94.5	91.8	22.5	32.2	36.6
	N2	95.1	92.2	22.6	32.6	37.4
	N3	95.6	92.4	22.4	32.8	38.5
	N4	95.4	90.8	22.6	33.1	39.3
	N5	96.7	91.6	23.5	33.4	39.8
S3	N1	94.5	92.8	22.1	33.2	38.3
	N2	95.2	92.2	22.9	33.5	38.8
	N3	95.6	93.4	23.4	34.1	39.2
	N4	95.1	93.8	23.6	34.4	39.4
	N5	94.2	93.6	23.2	34.8	39.5
P_N/$LSD_{0.5}$		>0.05/1.76	>0.05/2.60	<0.05/0.90	<0.05/0.82	<0.05/0.91
P_S/$LSD_{0.5}$		>0.05/1.94	>0.05/0.88	<0.05/0.74	<0.05/0.84	<0.05/0.36
$P_{N\&S}$/$LSD_{0.5}$		>0.05/3.05	>0.05/4.50	>0.05/1.56	>0.05/1.42	>0.05/1.57

N fertilization: N1: 0, N2: 60, N3: 120, N4: 180 and N5: 240 kg N ha^{-1}, Plant spacing: S1: 15×20 cm (33 plants m^{-2}), S2: 20×20 cm (25 plants m^{-2}), S3: 30×20 cm (16 plants m^{-2})

Table 2: Growth characteristics in *R. glutinosa* subjected to different spacings and phosphorus fertilization

Plant spacing	Phosphorus fertilizer	Sprouting rate (%)	Survival rate (%)	No. of leaves	Plant height (cm)	Canopy diameter (cm)
S1	P1	95.5	93.2	22.3	33.2	35.4
	P2	94.8	92.4	22.2	31.2	36.4
	P3	94.6	92.2	22.4	31.6	37.2
	P4	95.1	92.8	21.6	32.4	37.8
	P5	95.6	92.6	22.3	32.8	38.5
S2	P1	94.2	91.6	22.4	32.5	36.6
	P2	95.3	92.2	22.5	32.6	37.2
	P3	94.6	92.5	22.4	32.7	38.3
	P4	95.4	91.2	22.6	33.2	38.8
	P5	95.2	91.6	23.5	33.4	39.2
S3	P1	95.2	92.8	22.1	33.2	38.3
	P2	95.2	92.2	22.9	33.5	38.8
	P3	95.6	93.4	23.4	32.8	38.5
	P4	95.1	93.5	23.2	33.5	38.7
	P5	95.4	93.6	23.2	33.4	39.2
P_P /$LSD_{0.5}$		>0.05/1.74	>0.05/0.82	>0.05/0.97	>0.05/1.98	<0.05/1.01
P_S/$LSD_{0.5}$		>0.05/2.24	<0.05/2.67	>0.05/1.09	>0.05/3.48	<0.05/0.76
$P_{L\&S}$/$LSD_{0.5}$		>0.05/3.01	>0.05/4.62	>0.05/1.68	>0.05/3.43	>0.05/1.75

P fertilization: P1: 0, P2: 40, P3: 80, P4: 120 and P5: 180 kg P ha^{-1}, Plant spacing: S1: 15×20 cm (33 plants m^{-2}), S2: 20×20 cm (25 plants m^{-2}), S3: 30×20 cm (16 plants m^{-2})

average while the smallest of this value was 37.06 cm for the density of 33 plants m^{-2} (S1). Survival rate difference due to plant spacing appeared to have an insignificant impact on plant growth and development.

In short, there were no substantial changes in plant growth due to the interactions between plant spacings and chemical fertilization. However, nitrogen fertilization and spacings by themselves had a profound effect on the growth of leaves and plant height. In contrast, phosphorus fertilizer showed minor influence as it only affected the canopy diameter.

Effect of nitrogen fertilizer and plant spacings on fresh roots yield: Plant spacings had a significant effect on the number of roots $plant^{-1}$, average weight $root^{-1}$, total root weight $plant^{-1}$ and total yield ($p<0.05$). At the highest planting density (S1), the plant produced only 3.2 roots on average while this number was higher at the lighter density (S2 produced 3.5 roots/plant and S3 produced 3.9 roots/plant). Similarly, S3 spacings were significantly differentiated from S1 and S2 by an average weight $root^{-1}$, total root weight $plant^{-1}$ and total yield with the highest values of 46.4 g/root, 181.1 g root/plant and 208.7 kg of root ha^{-1}, respectively.

Different levels of nitrogen fertilization also significantly affected the yield of *R. glutinosa* root. The highest values of all yield parameters were observed in N5 fertilization. Plants fertilized with 240 N stimulated the development of the root systems with an average of 3.9 roots/plant, 39 g/root, 155.1 g of root/plant and a total yield of 251.8 kg ha^{-1} (the mean calculated by the formula $\sum N_n/n$.

All treatments with higher planting density and/or a lesser amount of nitrogen fertilizer showed the least in *R. glutinosa* root growth and development (Table 3).

There were significant interactions between the plant spacings and the nitrogen fertilization in the period of 170 days after planting for the yield fresh roots (Table 2). At the spacings of 30×20 cm (S3), plants fertilized with 180 kg N(N4) and 240 kg N (N5) showed greater production of fresh roots of *R. glutinosa* ($p<0.05$), compared with the treatments with any of lower amount of N and higher density of plants. These combinations of fertilizer and spacing (S3N4 and S3N5) also stimulated the production of roots and their weight and thereafter further increased the average weight of roots $plant^{-1}$ while plants treated with no nitrogen fertilization showed the least values in the development of roots.

Effect of phosphorus fertilizer and plant spacings on fresh roots yield: Planting with different spacings or phosphorus fertilization significantly affected the number of roots, average weight $root^{-1}$, total weight $plant^{-1}$ and total yield ha^{-1}. The highest mean values of these parameters were 3.8 roots, 40.2 g, 167.5 g and 264.3×10^2 kg when fertilized with 180 kg P and 3.84 roots, 47.6 g, 195.6 g and 219.1×10^2 kg due to 30×20 cm spacing, respectively (Table 4). In contrast, plants fertilized with the none-phosphorus application or planted in the highest density of 33 plant m^{-2} showed the least performance in root development and the total yield.

Table 3: Effect of nitrogen fertilization and plant spacing on the development and yield of *R. glutinosa* roots

Plant spacing	Nitrogen fertilizer	No. of root/ plant	Average weight/ root (g)	Average root/ weight plant (g)	Root yield ha^{-1} ($\times$100 kg)
S1	N1	2.8	16.4	45.92	109.11
	N2	3.0	19.6	58.80	139.71
	N3	3.2	22.2	71.04	168.79
	N4	3.4	26.2	89.08	211.65
	N5	3.5	30.2	105.70	251.14
S2	N1	3.2	18.7	59.84	107.71
	N2	3.3	23.6	77.88	140.18
	N3	3.4	28.8	97.92	176.26
	N4	3.6	32.7	117.72	211.90
	N5	3.8	36.5	138.70	249.66
S3	N1	3.4	37.4	127.16	146.49
	N2	3.6	41.7	150.12	172.94
	N3	3.8	47.5	180.50	207.94
	N4	4.1	55.4	227.14	261.67
	N5	4.4	50.2	220.88	254.45
P_N /$LSD_{0.5}$		<0.05/0.09	<0.05/0.91	<0.05/4.28	<0.05/8.99
P_S/$LSD_{0.5}$		<0.05/0.11	<0.05/3.91	<0.05/16.51	<0.05/7.13
$P_{N\&S}$/$LSD_{0.5}$		<0.05/0.16	<0.05/1.57	<0.05/7.41	<0.05/15.57

N fertilization: N1: 0, N2: 60, N3: 120, N4: 180 and N5: 240 kg N ha^{-1}, Plant spacing: S1: 15$\times$20 cm (33 plants m^{-2}), S2: 20$\times$20 cm (25 plants m^{-2}), S3: 30$\times$20 cm (16 plants m^{-2})

Table 4: Effect of plant spacings and phosphorus fertilization on the development and yield of *R. glutinosa* roots

Plant spacing	Phosphorus fertilizer	No. of root/ plant	Mean weight/ root (g)	Total root/ weight plant (g)	Root yield ha^{-1} ($\times$100 kg)
S1	P1	2.8	17.6	49.6	114.6
	P2	3.0	20.8	63.5	146.7
	P3	3.2	23.4	76.7	177.2
	P4	3.4	27.4	96.2	222.2
	P5	3.5	31.4	114.2	263.7
S2	P1	3.3	19.9	64.6	113.1
	P2	3.3	24.8	84.1	147.2
	P3	3.4	30.0	105.8	185.1
	P4	3.6	33.9	127.1	222.5
	P5	3.7	37.7	149.8	262.1
S3	P1	3.4	38.6	137.3	153.8
	P2	3.6	42.9	162.1	181.6
	P3	3.8	48.7	194.9	218.3
	P4	4.1	56.6	245.3	274.8
	P5	4.3	51.4	238.6	267.2
P_L/$LSD_{0.5}$		<0.05/0.13	<0.05/0.85	<0.05/2.84	<0.05/6.93
P_S/$LSD_{0.5}$		<0.05/0.07	<0.05/3.70	<0.05/10.97	<0.05/5.50
$P_{L\&S}$/$LSD_{0.5}$		>0.05/0.22	<0.05/1.47	<0.05/4.93	<0.05/12.01

P fertilization: P1: 0, P2: 40, P3: 80, P4: 120 and P5: 180 kg P ha^{-1}, Plant spacing: S1: 15$\times$20 cm (33 plants m^{-2}), S2: 20$\times$20 cm (25 plants m^{-2}), S3: 30$\times$20 cm (16 plants m^{-2})

Similar to the influence of nitrogen fertilizer, phosphorus fertilization and plant spacing interaction also had a profound effect on the development and yield of *R. glutinosa* roots. Although the number of roots was similar, the average weight of roots, the total weight of roots $plant^{-1}$ and root yield ha^{-1} were significantly different due to this interaction. Plants treated with spacing 30$\times$20 cm and fertilized with 120 kg ha^{-1} produced the highest yield of 274.8$\times 10^2$ kg ha^{-1}, the average weight of root and total root weight $plant^{-1}$ while those planted without phosphorus fertilizer in 15$\times$20 cm rows produced the lowest yield and weight of roots.

Fresh roots quality of *R. glutinosa*: Plant spacings and chemical fertilization in particular and their interaction had a significant impact on the quality of *R. glutinosa* roots. Results presented in Table 5 revealed that plants with a spacing of 30$\times$20 cm fertilized with 180 N or with 120 P produced the best quality of roots which were 21.4 cm height, 3.0 cm diameter and 21.6 cm height, 3.36 cm diameter, respectively. Plants without nitrogen or phosphorus fertilizers gained a relatively small increase in the size of roots which were two times less than the roots produced from the combination. All treatments with

Table 5: Effect of plant spacing and chemical fertilization on the quality of *R. glutinosa* roots

	Nitrogen fertilization			Phosphorus fertilization		
Plant spacing	N	Root length (cm)	Root diameter (cm)	P	Root length (cm)	Root diameter (cm)
S1	N1	9.5	1.45	P1	10.4	1.63
	N2	10.8	1.58	P2	11.9	1.77
	N3	11.9	1.68	P3	13.1	1.88
	N4	13.6	1.84	P4	14.9	2.06
	N5	15.2	2.00	P5	16.8	2.24
S2	N1	10.4	1.54	P1	11.5	1.73
	N2	12.5	1.74	P2	13.7	1.95
	N3	14.7	1.94	P3	16.1	2.18
	N4	16.3	2.10	P4	17.9	2.35
	N5	17.9	2.25	P5	19.7	2.52
S3	N1	15.8	2.29	P1	16.2	2.56
	N2	16.7	2.46	P2	17.4	2.75
	N3	18.4	2.69	P3	18.7	3.01
	N4	21.4	3.00	P4	21.6	3.36
	N5	19.6	2.79	P5	19.4	3.13
P_N/$LSD_{0.5}$		<0.05/0.70	<0.05/0.3E-01	P_P/$LSD_{0.5}$	<0.05/0.76	<0.05/0.56E-01
P_S/$LSD_{0.5}$		<0.05/3.61	<0.05/0.13	P_S/$LSD_{0.5}$	<0.05/2.19	<0.05/0.24
$P_{N\&S}$/$LSD_{0.5}$		<0.05/1.21	<0.05/0.52E-01	$P_{P\&S}$/$LSD_{0.5}$	<0.05/1.32	<0.05/0.96E-01

N fertilization: N1: 0, N2: 60, N3: 120, N4: 180 and N5: 240 kg N ha^{-1}, P fertilization: P1: 0, P2: 40, P3: 80, P4: 120 and P5: 180 kg P ha^{-1}, Plant spacing: S1: 15×20 cm (33 plants m^{-2}), S2: 20×20 cm (25 plants m^{-2}), S3: 30×20 cm (16 plants m^{-2})

phosphorus fertilization appeared to have a better quality of roots compared to the corresponding treatments with nitrogen.

In short, chemical fertilization and their interaction with plant spacings significantly contributed to the differences of fresh root yield and root quality of *R. glutinosa* among experimental plots. Of those, plants treated with a higher amount of fertilizers with larger space for less water and nutrients competition showed better growth and development of roots.

DISCUSSION

Data presented in Table 1 and 2 revealed that neither the interaction of nitrogen nor the phosphorus fertilization with plant spacing was associated with some initial growth parameters such as sprouting rate, survival rate and probably some leaves growth-related parameters. The result also indicated that leaves and plant height were mainly influenced by N rather than P levels.

Nitrogen fertilization and plant spacing considerably increased the number of leaves, plant height and canopy diameter as the result of the increment of nitrogen fertilizers and extending spaces between plants. However, this effect was not clearly applied for phosphorus fertilizer as the increment of P did not significantly stimulate the growth of *R. glutinosa*. The adequate fertilization of nitrogen stimulated the extension of the leaf surface and the increase of photosynthesis capacity as this macro-element is a primary constituent of protein molecules and chlorophyll[20]. Significant increases in general plant growth due to the application nitrogen fertilizers were also observed in many recent studies on other plant species such as cotton[21], lettuce[22], chervil plant[23], while the opposing reported by Chrysargyris *et al.*[15] as nitrogen had no a pronounced effect on plant growth-related parameters of lavender. This lavender species growth was reported mainly affected by P levels. For all or some of these studies also confirmed that vegetative growth other to N above 150 kg ha^{-1}. This result was in line with the current study when *R. glutinosa* showed the highest growth when being fertilized with from 180 kg to 240 kg ha^{-1}. It is worth considering that applying a lower amount of chemical fertilizer for cost-effectiveness and environmental protection as *R. glutinosa* showed no significant difference in vegetative growth between N4 and N5 fertilization in the current study.

High phosphorus levels often accelerate the growth of the plants as it is a key player in a wide range of metabolic and biosynthetic processes[16]. Higher P levels were effective in increasing the leaf expansion of *R. glutinosa* (identified by canopy diameter) but did not increase the number of leaves and plant height as N did in this experiment. It was also found that plant height and leaf length did not benefit from increased P concentration of lavender[15]. However, the leaf biomass of garden sage[24] and *Calendula officinalis*[25] increased with the application of higher P levels. In this study, since none of the growth parameters were affected by P levels and the leaf biomass was not intended for measurement, the difference in canopy diameter was probably associated with other unknown factors.

Plant spacing affected leaf growth parameters within nitrogen fertilizer experiments but did not occur in those with phosphorus ones. It was occasionally reported that taller plants and less vegetative biomass resulted from higher plant density or less plant spacing because of inter-plant competition for nutrient and light[21,26]. Moreover, vegetative growth was often stimulated by nitrogen fertilization, including plant height and vegetative biomass. However, the current study revealed the opposite result as *R. glutinosa* showed better growth (plant height and number of leaves plant^{-1}) in less dense plots. This was probably due to an adequate level of nitrogen fertilization that might have played a significant role in reducing competition for photosynthates and nutrients with other plants. This event showed that well-spaced plants received more solar radiation, which leads to more photosynthetically efficient activity than closely-spaced ones[27]. Furthermore, the opposite results from other researchers on vegetables have shown that total dry matter yield increase linearly when the inter-row plant spacing is reduced, therefore closing spacing appeared to have favored dry vegetative biomass production. The present results indicate a positive response of various growth attributes to higher plant spacing with sufficient nitrogen fertilization and corroborate findings of several researchers[28,29].

The linear increment of root yield and root quality observed in this study might be associated with N and P fertilization which ensures the efficiency of photosynthetically active leaves for longer duration and formation of new vegetative organs and parts. In other words, nutrients exert a significant influence on biomass production including the yield of fresh roots. In agreement with the current finding, various researchers reported similarly as a significant increment in root and tuber yield of various crop plants in response to N application[30-31] but fewer have been reported as the result of P application. It is notably recognized that the highest level of N application of 240 kg ha^{-1} and P application of 180 kg ha^{-1} did not follow the linear increment of the total experiments as the root yield and root yield components stopped increase. This may be due to the condition that vegetative growth of the aerial's parts can be stimulated and therefore prevented the transformation of photosynthetic matters into the storage parts such as roots. From the economic point of view with high yield performance, the amount of N and P fertilizer should be less than 240 and 180 kg ha^{-1}, respectively for more profitable production.

The quality of *R. glutinosa* roots for market purposes is often determined by two factors, which are root length and diameter. The largest proportion of unmarketable (smaller sizes in length and diameter) roots obtained in the nil application of N, P at the closest plant spacing might result from the stiff inter-plant competition for growth factors, which allowed plants to produce higher numbers of undersized roots. This pattern was also reported by other researchers who claimed that closer spacing and fewer nutrients led to a significantly higher yield of small and medium-sized roots[32]. Conversely, chemical fertilizer significantly promotes the growth of large-sized root percentage as the increment of N, P and plant spacing. Getie[31] also reported that chemical fertilizers increased the quality of roots determined by the root length and diameter. The larger roots in wider spacing were probably due to less competition among the plants for space, light, water and nutrients which were facilitated to foster growth and development of roots thereby increasing tuber size in wider spacing as compared to closer spacing[33].

Similarly, the increased number of roots in response to the higher levels N and P could be the result of gibberellins and auxin biosynthesis which play a key role in shoot and root division and expansion. Our findings coincided with some earlier findings of Ezz El-Din *et al.*[34] and Zewide *et al.*[35] who claimed that application N and P significantly increases marketable tuber number in potato. However, Vreugdenhil and Sergeeva[36] revealed the insignificant correlation between chemical application and the number of tubers in potato and explained that this event may be attributed to the increased number of main roots per unit area.

The study has shown that plant spacing significantly influenced the yield and yield components of *R. glutinosa* in which S3 (20×30 cm) exerted the highest production of roots. It is generally accepted that with an excessive supply of environmental factors and nutrients, plants respond positively and probably achieve maximum growth and development of both vegetative and reproductive parts. Although, others reported that planting in closer spacing meant more plants and their biomass will be produced therefore it increases the total yield per unit area[37]. The current results showed the opposite as wider spacing led to higher yield and can be explained by the increase in root numbers and large-sized roots. Furthermore, in combination with N application of 180 kg ha^{-1} and P application of 120 kg ha^{-1}, plants spaced at 20×30 cm produced the highest yield of roots as well as their quality. Wider spacings than 20×30 cm were designed in Mangani *et al.*[33] and Ogbonna *et al.*[37] and were found to be optimal for crops while Mangani *et al.*[33] and Getachew *et al.*[38] revealed that the total yield reached the peak at the standard spacing of 30 cm and observed a fall in total yield when the in-row was further increased to 40 cm. Nevertheless, it is still necessary to determine the influence of plant spacing that is wider than the ones tested in the present experiments.

Application rates of N and P fertilizers combined with plant spacing significantly affect the vegetative growth, root yield and quality of *R. glutinosa* plants. The implications of the optimal doses of chemical fertilizers double with the right planting density in this plant which in turn ultimately might increase the productivity of the commercial medicinal plantation. Although the increasing size of the fresh root of *R. glutinosa* was positively correlated to catalpol content, it is worth to conduct an independent investigation further understanding the effect of fertilization and plant spacing on some key bioactive compounds of this medicinal plant in the future.

CONCLUSION

There was a significant improvement in the vegetative growth, fresh root yield and quality due to the application of chemical fertilizer and proper use of planting density per unit area in this field experiment. The level of such improvement increased with the rate of application. Nitrogen fertilizers revealed a greater effect on both vegetative and reproductive performance while phosphorus appeared to have a profound influence on yield and quality of fresh root of *R. glutinosa*. Also, less populated plots (20×30 cm) were found to promote all aspects of the plant growth and development considered in the study. Plant spacing and chemical fertilization had a significant interaction on promoting root growth and development of *R. glutinosa*. It can be concluded that for the optimum biomass yield of *R. glutinosa* root, plants may be densely cultivated at a spacing of 20×30 cm and fertilized with 180 kg N ha^{-1} and/or 120 kg P ha^{-1}.

SIGNIFICANCE STATEMENT

This study discovered that the application of N, P and plant spacing can be applied to improve the yield of *R. glutinosa* tuber roots and well as their quality. This study will help the researchers to uncover the critical areas of cultivation techniques that many researchers were not able to explore. This also helps the farmers to use fertilizers economically so as to increase profit in the commercial production of *R. glutinosa* for medicinal plant materials.

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