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Growth and anthraquinone content of madder plant as affected by propagation method and different sources of potassium

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Abstract
Two field experiments were conducted during the two successive seasons of 2015/2016 and 2016/2017, at Faculty of Agriculture, Cairo University, Giza, Egypt. The aim of this study is to determine the best propagation method of *Rubia tinctorum* L. plant (seed or division) which leads to the highest productivity of the roots, as well as to investigate the effect of three different sources of potassium on the total content of anthraquinones. The results clarified that there were significant differences between plants grown from seed and division in the root fresh and dry weights. The plants propagated by division produced roots yield more than that produced by seed propagation with a significant difference when harvested at the age of 14 months. Young roots (7 months) had a total content of anthraquinones (mmol / g f. w.) higher than the older ones. The treatment of 24 g ammonium sulfate plus 15 g wood ash per plant had a superior effect on roots yield and the total content of anthraquinones. The root anatomical structure supported the plant growth parameters.

Keywords: Anthraquinones, *Rubia tinctorum*, root anatomy, potassium, wood ash

1. Introduction

The choice of natural colorants to be used by industry instead of synthetic ones is certainly due to health concerns. Phytochemicals including colorants from plant extracts exhibit many advantageous functions such as better biodegradability, higher compatibility with the environment. Cotton has been extensively dyed with colorants derived from plant sources such as *Rubia* plants [1]. *Rubia tinctorum* L. plant, commonly known as wild Madder, is a member of the family Rubiaceae. It is a herbaceous perennial plant, blooms in June and seeds ripen in September. The leaves and stem are prickly; leaves have spines along the midrib on the underside. This feature enables them to be used for polishing metal work. The Plant prefers sandy loamy soil and requires well-drained soil. The plant can be grown in acidic, neutral and alkaline soils. The plant can tolerate maritime exposure [2]. Roots of the plant have been known for their dyeing and medicinal properties since ancient times, due to the presence of anthraquinones. *Rubia tinctorum* have been used for treating inflammatory diseases in Iranian traditional medicine. Also, the aqueous extract of *Rubia tinctorum* root exhibited high anti-inflammatory effect which is attributed to the polar compounds in root extraction. The root is aperient, astringent and diuretic. It is taken internally in the treatment of kidney and bladder stones [3]. *Rubia* was cultivated for the red dye obtained from its roots. However, it is still cultivated in Europe as a medicinal dye plant.

The propagation management may affect both yield and qualitative traits of the plant. Since the root is the economic part of the *Rubia* plant, which is the source of the natural dye, so the goal of this study is to determine the best propagation method (seeds or division) resulting in the highest yield of roots. Riahi *et al.* [4] found that vegetative propagation methods on artichoke significantly affected the yield and the marketable and qualitative characteristics of the product, also affected the short-chain sugars ratio. However, Tatyaba [5] said that propagated marigold by seed was found beneficial in increasing the growth and was produced highest yield of flowers than plant propagated by cutting.

Potassium promotes the formation of roots, so three sources of potassium were investigated in this study. From a plant physiological viewpoint, K is considered close to N in importance. Potassium is essential for plant metabolism, and its cationic species, K+, is very dynamic in the soil solution. K is a key in the synthesis of organic acids in the Krebs cycle during carbohydrate metabolism. The evidence is also emerging from molecular biology studies that K may play a regulatory role in plant stress responses [6, 7]. Potassium promotes root growth and thicker cell walls. It also enhances translocation of sugars and starch. Potassium plays a role in turgor- driven cell and organ movements. It is also involved in the activation of enzymes which affects protein, starch and adenosine triphosphate (ATP) production, cell metabolism, and photosynthesis. Thus, plant growth requires large quantities of K^+ ions that are taken up by roots from the soil solution, and then distributed throughout the plant [8].

Wood ash is a good source of potassium, phosphorus, magnesium, calcium as well as several micronutrients. Wood ash was considered to be a waste product of the forest industry sector. Recycling the ash after burning such residues can potentially compensate for the mineral nutrients lost from the soil. Ash characteristics depend on the nature of the original product. Common median concentrations of nutrients and metals in wood ash (nitrogen more than 1 mg/g, phosphorus ranged between 0.5–15 mg/g, potassium ranged between 2–130 mg/g) [9]. Wood ash is used as an agricultural soil amendment. It is organic residue remaining after the combustion of wood. Using wood ash causes growth promotion due to the substantial content of plant nutrients. The impact of using wood ash depends on ash dose, type of wood ash and receiving environment [10]. Some plant nutrients released from wood ash to the soil such as phosphorus, potassium, magnesium, calcium, and sodium. Wood ash is an acceptable alternative liming agent which also provides modest amounts of P and K to soils [11]. Park *et al.* [12] investigate the application of wood ash on a willow plantation. They found that wood ash application significantly increased soil extractable phosphorus, potassium, calcium, and magnesium concentrations.

2. Material and methods

This study was conducted at the experimental nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the two successive seasons of 2015/2016 and 2016/2017 to study the effect of two different methods of propagation, wood ash and chemical fertilizers on the growth, root anatomy as well as chemical composition of *Rubia tinctorum* plant.

2.1 Plant material: Two propagation methods were used; the first was seedlings with age 3 months old obtained from seeds which sown in January 2015 and 2016 in the first and second seasons, respectively. The second method was dividing of mother plants (12 months old).The seedling or offshoots from two methods were transplanted to the experimental field on 1st April for the two seasons. They were cultivated in (1.5x 1.5 m) plots, in rows 50 cm apart at 50 cm between plants (four plants/ m^2), each treatment consisted of 3 replicates and each replicate contained 9 plants.

The mechanical and chemical analyses of the soil were carried out according to [13, 14]. Data reported as follow; coarse sand 7.9%, fine sand 50.2%, silt 21.7%, clay 20.2% and texture was sandy clay loam. EC dSm^{-1} was 0.84, pH 7, soluble Cations were Na^+ 94.99, K^+ 79.17, Ca^{++} 190 and Mg^{++} 30.96 (ppm) and Anions were SO_4^{--}427.2, Cl^- 157.5 and HCO_3^- 244 (ppm). Available NPK were N 70, P 55 and K 180 ppm.

Ammonium sulfate (20.5 % N) was used at the rate of 12 g/plant/season [N1] and 24 g/plant/season [N2] divided into three equal doses started 45 days after planting then four months interval. Phosphorus as calcium super-phosphate (15.5% P_2O_5) was added as a basal dose for all plants during the preparation of the soil at the rate of 15 g/plant/season. Three different sources of potassium were used; Liquid Agricultural Potassium [AP] (36%) at the rate of 10 ml/L was added (the total dose 400 ml/plant/season) divided into eight equal applications started 45 days after planting then 45 days interval. The traditional source of potassium in Egypt potassium sulfate (48% K_2O) was added at the rate of 12 g/plant/season [PS] divided into three equal doses started 45 days after planting then four months interval. Also, wood ash was used as a source of potassium at the rate of 15 g/plant/season [WA], the chemical analysis of wood ash is in (Table A) according to [14].

Table A: Available amounts (mg/kg) of macronutrients in wood ash

Available N	Available P	Available K
115.5	2.6	4800

2.2 Analysis conducted

Herb fresh and dry weights were recorded 14 months after planting. Root fresh and dry weights and total content of anthraquinone glycosides (mmol/g f. w.) of roots for each treatment was determined two times, the first one was in autumn (October) and the second was in summer (June) (7 and14 months after planting), according to [15]. Chemical analysis of fresh leaves was conducted to determine the contents of total chlorophyll (a + b) and total carotenoids (mg/g fresh weight), using the method described by [16]. Total carbohydrates content (%) was determined (14 months after planting) in the dried roots for each treatment according to [17]. Also, N, P and K percentages in root dry matters were determined using the methods described by [18].

For studying the effect of treatments on the root structure of Madder plant which is considered the main source of anthraquinone dyes, the treatment (N1+AP+WA) [the combination of 12g Ammonium sulfate /plant/season + Liquid Agricultural Potassium at the rate of 400 ml/plant/season+ wood ash at the rate of 15 g/plant/season] was selected as the preferred treatment used in producing roots of both propagation methods (Division & Seed), in addition to the untreated controls. Specimens, (1 cm long) were taken from the basal portion of Madder main root through the second season (2016/2017). The materials used for the anatomical study were chosen at 7 months old plant, and microtechnique procedures are given by [19] were followed. Specimens were killed and fixed for at least 48 hours in F.A.A. solution (10 ml formalin, 5 ml glacial acetic acid, 85 ml ethyl alcohol 70%). After fixation, specimens were washed in 50% ethyl alcohol and dehydrated in a normal butyl alcohol series before being embedded in paraffin wax (52-54°C melting points). Transverse sections which were cut on a rotary microtome to a thickness of 15μ were stained with light green / saffranin before mounting in Canada balsam. Sections were examined and counts and the measurements of different tissues were recorded. Photomicrographs of the selected treatments were taken.

All data were statistically analyzed according to [20] using a two-factorial completely randomized design, the main plot was the method of propagation and the subplot was fertilization. A combined analysis was made for the two growing seasons since the results of the two seasons followed a similar trend. All values are means of three replicates. Significant differences were calculated using the least-significant-difference test (L.S.D.) at P =.05 level.

3. Results and discussion

3.1 Herb fresh and dry weight

The data in (Table 1) illustrated the effect of fertilization treatments on herb fresh and dry weights/plant, data showed that all studied treatments significantly increased herb fresh and dry weights per plant compared to control plants.

Concerning the interaction between propagation method and fertilization treatment, the application of the high rate of ammonium sulfate (24g) plus 15 g wood ash per plant (N2+WA) had superior effect on herb fresh and dry weights per plant, which produce the maximum values from plants propagated by divisions with values1671.0 and 250.5g/plant, respectively. Marzieh *et al.* [21] found that using nitrogen fertilizer at the rate 180 kg/ha caused increasing in leaf fresh and dry weights. Leaf dry weight increased about 32% more than control. Zamani *et al.* [22] reported that the performance of shoots varied ranging from 0.75 to 1.75 Kg/m^2. Maximum performance of shoots was recorded under 150 Kg/ha nitrogen and phosphorus.

3.2 Root fresh and dry weight

Data presented in (Table 1) showed that increasing plant age resulted in more production of root fresh and dry yield per plant. This result was in agreement with Lambers *et al.* [23] who mentioned that the capacity for carbohydrates storage depends on the presence of specific organs, such as tuber, bulb, rhizome or taproot. Thus, the capacity to store carbohydrates in the root increases with increasing plant age. Plants propagated by division produced more root fresh and dry yield per plant than plants propagated by seeds at the age of 14 months in most cases.

Concerning the effect of fertilization treatments on root fresh and dry yield per plant, the results showed that all fertilization treatments increased root fresh and dry yield per plant than control plants, these results in agreement with Li *et al.* [24] who reported that maximum benefits from potassium were observed with the application of 450 kg K_2O /ha when balanced with adequate N and P. Also, Akhter *et al.* [25] found that potassium application produced 13-67 percent yield increases in ginger. Utilization wood ash combined with the high rate of ammonium sulfate (N2+WA) produced more root fresh and dry yield per plant than the treatment of potassium sulfate or liquid agricultural potassium combined with the high rate of ammonium sulfate with significant differences at both plant ages.

Regarding the effect of interaction between propagation method and fertilization treatments at the age of 7 months old, plants propagated by division produced the highest root fresh and dry yield per plant when treated with the combination of low rate of ammonium sulfate +liquid agricultural potassium +wood ash (N1+AP+WA) with mean values 102.7 and 17.90 g root fresh and dry weight, respectively. While at the age of 14 months old the maximum root fresh and dry yield per plant were resulted from plants propagated by division and treated with the high rate of ammonium sulfate (24g/plant) plus15 g wood ash/plant (N2+WA) with mean values289.0 and 75.20g/plant root fresh and dry weight, respectively. Seedlings grown in soils from stands amended with wood ash, fine root biomass of *P. abies* seedlings was significantly increased after 120 days of growth [26]. Zamani *et al.* [22] reported that maximum dry root weight was achieved at 100 Kg/ha.

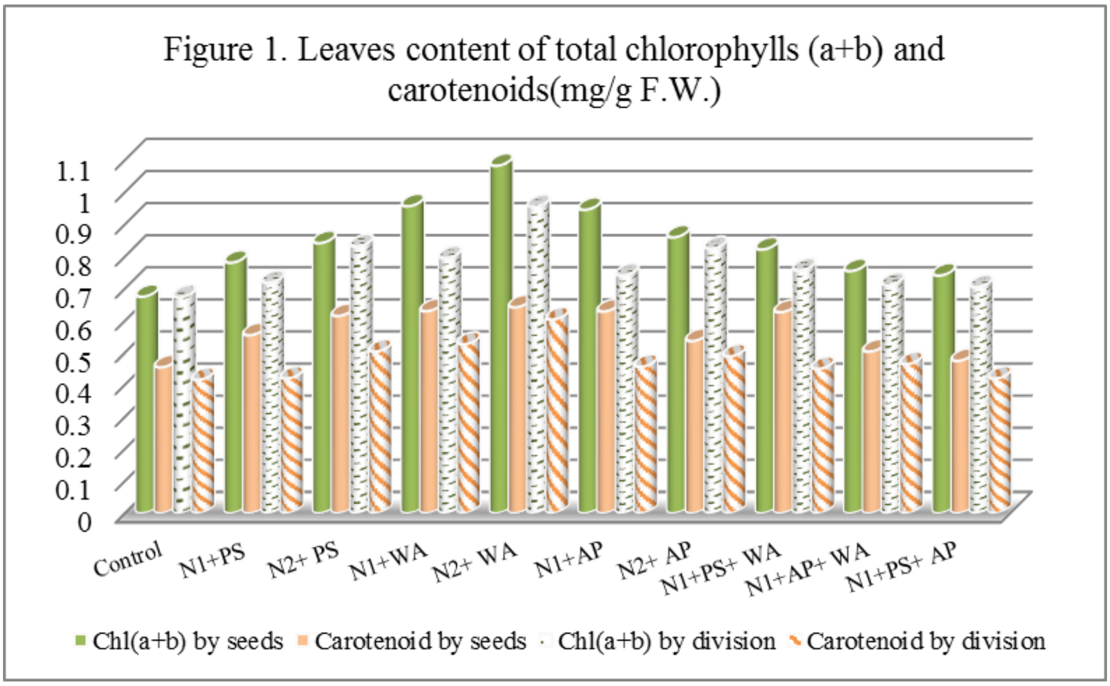
Table 1: Effect of propagation method and different sources of potassium on herb fresh and dry weights g/plant (at 14 months old), root fresh and dry weights g/plant.

	Fresh Herb		Dry Herb		Fresh Root		Dry Root		Fresh Root		Dry Root	
	14 months after planting				7 months after planting				14 months after planting			
Treatments	S	D	S	D	S	D	S	D	S	D	S	D
Control	357.0	167.3	50.4	20.5	14.7	39.3	2.8	6.1	71.3	72.5	21.0	21.3
N1+PS	700.0	248.0	113.0	36.8	24.0	43.0	3.5	6.8	122.0	58.5	37.5	16.5
N2+ PS	625.0	783.5	77.2	116.2	21.0	46.3	3.4	8.3	133.5	144.5	36.3	39.5
N1+WA	1407.0	1105.0	162.1	150.5	29.5	69.0	5.9	12.4	106.5	110.0	29.3	31.9
N2+ WA	1526.0	1671.0	176.5	250.5	43.8	61.0	8.7	11.5	168.7	289.0	45.0	75.2
N1+AP	675.5	470.0	120.7	58.8	27.1	55.0	4.3	10.5	128.5	171.5	38,0	47.5
N2+ AP	825.7	851.0	125.4	140.0	16.5	55.0	3.1	10.5	124.0	135.0	35.0	36.3
N1+PS+ WA	708.0	1017.0	115.2	152.5	26.9	83.0	4.1	14.0	117.0	166.0	34.7	44.0
N1+AP+ WA	677.3	1223.0	124.7	183.4	43.8	102.7	8.5	17.9	144.0	170.5	43.3	48.1
N1+PS+ AP	406.5	540.5	75.0	81.1	17.0	65.0	3.2	12.3	70.0	143.3	21.0	40.2
L.S.D (P= 0.05)	194.1		7.33		7.41		1.49		13.17		4.58	
	153.1		20.59		5.65		0.85		19.67		6.47	
	216.5		29.11		7.98		1.20		27.82		9.16	

S= seed D= division N1=12g/plant Ammonium sulfate N2=24g/plant Ammonium sulfate PS =12g/plant Potassium sulfate WA=15g/plant Wood ash AP = 400ml/ plant Liquid agricultural potassium

3.3. Total chlorophylls (a+ b) and carotenoids

Data presented in (Figure 1.) showed the effect of propagation method, wood ash and chemical fertilizers on leaves content of total chlorophylls and carotenoids. The results revealed that the plants propagated by seeds had more total chlorophyll and carotenoids than those propagated by division. Because of the leaf nitrogen is associated with the photosynthetic apparatus, it could be noticed that the increase in ammonium sulfate from N1 to N2 (from 12 to 24 g/plant) resulted in an increase in total chlorophyll content. In both propagated method, using the high rate of ammonium sulfate in combination with wood ash (N2+WA) resulted in the highest values of total chlorophyll and carotenoids content.



N1=12g/plant Ammonium sulfate N2=24g/plant Ammonium sulfate PS=12g/plant Potassium sulfate WA=15g/plant Wood ash AP=400ml/ plant Liquid agricultural potassium

Fig 1: Effect of propagation method and different sources of potassium on leaves content of total chlorophylls (a+b) (mg/g F.W.) and carotenoids (mg/g F.W.).

3.4. Total content of anthraquinones

It is evident from (Table 2) that, the young roots (7 months) had a higher total content of anthraquinone /g root f. w. than the older(14 months).These results are in agreement with the results obtained by Luciana *et al.* [27] they found that young roots showed higher alizarin content than 15 and 30 month old plants. The young roots (7 months) had taken in autumn (in October) when the temperature is moderate in Egypt, while older roots (14 months) had taken in hot summer (in June) when the plants got flower. So, the previous notice may be explained by a decrease in the total content of anthraquinones in older roots taken in the flowering stage. Also, it is clear that young roots (7 months) produced from plants propagated by seeds had higher total content of anthraquinones /g root f. w. than which produced by divisions with significant increment. Baydar and Karadogan [28] found that the madder plants in Turkey started flowering in the last days of June. The young roots were more dye rich than the older ones. The roots of the seedlings gave the highest dye content. There was a remarkable variability in the dye content of the root depending on growth stages within a year.

Concerning the effect of fertilization on the total content of anthraquinones /g root f. w., results showed that increasing the rate of ammonium sulfate from 12 to 24 g/plant (N1 to N2) caused a significant increase in total anthraquinones content per gram root f. w. in most cases at both plant ages. Marzieh *et al.* [21] found that alizarin content increased about 102% more than control by using nitrogen fertilizer at the rate of 120 kg/ha. Regarding the interaction between propagation method and fertilization treatment, the maximum total anthraquinones content per gram root fresh weight (67.73 mmol) was recorded with young plants (7 months) propagated by seed and treated with the high rate of ammonium sulfate combined with wood ash (N2+WA) followed by the treatment of ammonium sulfate at the low rate plus liquid agricultural potassium plus wood ash (N1+AP+WA) without significant difference between them.

Table 2: Effect of propagation method and different sources of potassium on total content of anthraquinone glycosides m mol/g root fresh weight.

	7 months after planting	**14 months after planting**		
	Seed	**Division**	**Seed**	**Division**
Control	50.13	23.93	26.18	19.23
N1+PS	63.95	26.35	25.95	26.95
N2+ PS	57.56	55.75	29.35	32.57
N1+WA	54.85	36.95	30.00	28.18
N2+ WA	67.73	57.25	40.73	36.03
N1+AP	49.23	36.95	27.08	21.95
N2+ AP	59.08	34.08	34.80	31.35
N1+PS+ WA	64.23	44.85	36.00	21.96
N1+AP+ WA	66.68	57.25	28.63	31.65
N1+PS+ AP	62.73	32.58	25.73	21.50
	5.95		2.23	
L.S.D (*P*= 0.05)	4.10		2.90	
	5.83		4.08	

N1=12g/plant Ammonium sulfate N2=24g/plant Ammonium sulfate PS=12g/plant Potassium sulfate WA=15g/plant Wood ash AP=400ml/ plant Liquid agricultural potassium

5- Content of N, P, K and total Carbohydrates % of root dry weight:

Data presented in (Table 3) indicated that nutrients and total carbohydrates contents in *Rubia tinctorum* root increased with the different fertilizers combinations compared with control plants. Nitrogen content ranged between 0.61-1.02% in the root from plants propagated by seeds and ranged between 0.82-1.14percent in the root from plants propagated by division. Increasing the rate of ammonium sulfate from 12 to 24 g/plant caused an increase in root nitrogen content.

These results were in harmony with the data of Abdul Jalil [29] who reported that the nitrogen percentage of *Rubia tinctorum* root was 1.02%. Also, the data of Marzieh *et al.* [21] found that N content in madder roots increased due to 180 kg/ha nitrogen fertilizer application near 48% compared with control. Also, they found an increase in K content with increasing nitrogen rate. The highest phosphorus content (0.209%) was determined in the roots of plants propagated by division and received the combination of the low rate of ammonium sulfate plus liquid agricultural potassium plus wood ash. The roots of plants propagated by seeds had a higher content of potassium than those propagated by division; these plants were also rich in total chlorophyll and carotenoids contents as shown in ((Figure 1.) Generally, potassium percentage ranged from 0.89 to 1.70% among the two propagation method treatments. These results were in harmony with the data of Abdul Jalil [29] who determined the potassium percentage of *Rubia tinctorum* root by atomic absorption spectrometry and it was found to be 0.833%. Also, Akhter *et al.* [25] found that the amount of K taken up increased in response to raised levels of K application.

Results in (Table 3) represented that the values of total carbohydrates percentage in the roots of plants propagated by seeds had the same trend of those propagated by division. There was an increase in total carbohydrates content in roots with increasing the rate of ammonium sulfate. The highest total carbohydrates content (37.00%) was determined in the roots of plants propagated by seeds and received the high rate of ammonium sulfate plus wood ash which also, caused the highest potassium content (1.70%).

Table 3: Effect of propagation method and different sources of potassium on, N, P, K and total Carbohydrates % of root dry weight.

Treatments	14 months after planting							
	Seed				Division			
	N %	P%	K%	Total Carbohydrates%	N%	P%	K%	Total Carbohydrates%
Control	0.61	0.197	1.10	28.00	0.82	0.199	0.89	29.50
N1+PS	0.81	0.200	1.25	31.50	0.94	0.205	0.95	31.00
N2+ PS	1.02	0.204	1.48	34.00	1.14	0.208	1.15	35.75
N1+WA	0.90	0.201	1.43	33.00	1.01	0.204	1.03	33.50
N2+ WA	0.98	0.203	1.70	37.00	1.07	0.205	1.18	36.00
N1+AP	0.89	0.202	1.20	31.50	1.01	0.203	1.03	31.50
N2+ AP	1.00	0.203	1.60	36.00	1.11	0.205	1.15	35.50
N1+PS+ WA	0.86	0.202	1.28	31.50	0.94	0.206	1.03	31.50
N1+AP+ WA	0.95	0.205	1.48	33.50	1.01	0.209	1.05	35.00
N1+PS+ AP	0.74	0.199	1.23	31.50	0.91	0.199	0.95	31.50

N1=12g/plant Ammonium sulfate N2=24g/plant Ammonium sulfate PS=12g/plant Potassium sulfate WA=15g/plant Wood ash AP=400ml/ plant Liquid agricultural potassium

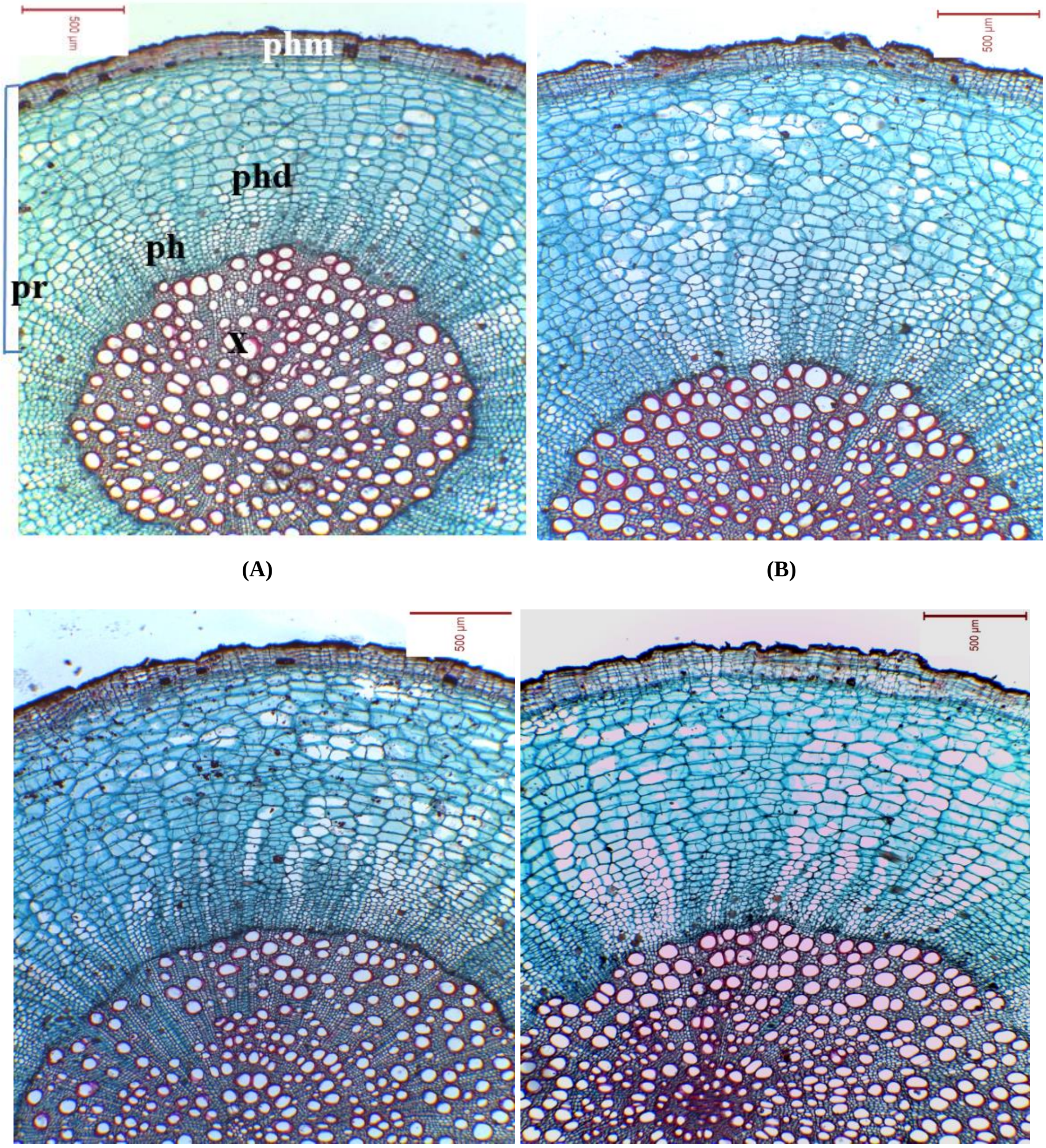
6. Anatomical studies

The results in (Table 4 and Figure 2) show the anatomical differences of the treated Madder plant roots (N1+PA+WA) and cultivated by both methods of propagation (Seeds and Division) in comparison with the control plants. The results appeared that the roots of the treated Madder plants showed an increase in most anatomical parameters compared with the control plant roots. By comparing the roots of the seed-propagated and treated plants with their untreated plant roots, there was an increase in the diameter of the root cross section, where the percentage of increase was 35.2% which attributed to the increase in the thickness of the periderm (23.8%), the phloem tissue (100%) and the diameter of the xylem cylinder (37.5%).

The thickness of the periderm was due to the increase in the thickness of the phelloderm (35.3%) and the number of its layers (12.5%). It was also observed when comparing the roots of division- propagated and treated plants with their untreated plant roots, there was a slight increase in the root cross section (7%) although there was a significant increase in the root vascular tissue thickness, where the rate of increase was 49.7 and 34.5% in both phloem and xylem tissues, respectively. This due to the decrease in the thickness of the periderm being -18.8% which due to the lack of phelloderm thickness (-26.2%) and the number of its layers (-18.2%) despite the increase in the thickness of the phellem (33.6%) and the number of its layers (16.7%).

On the other hand, when comparing the roots of division-propagated and treated plants with the roots of seed-propagated and treated plants, there was also a slight increase in the diameter of the root cross section (8.2%), which mainly attributed to the increase in the diameter of the xylem cylinder (18.2%). Although there was an increase in the thickness of the phellem and the number of its layers amounted to 60.6 and 40%, respectively, there is a shortage in the thickness of the phelloderm amounted to -8.7%, which led to an equal thickness of the periderm in both treatments (1083µ).Similarly, when comparing the control plant roots of division propagation method with their seed propagation counterparts, there was an increase in the diameter of the root cross section being 36.7% as a result to the increase of both the periderm (52.3%), the phloem tissue (33.6%) and the diameter of xylem cylinder (20.9%). Increasing the thickness of the periderm was due to the increase in phellem thickness (20.2%) and the number of its layers (20%) as well as the thickness of phelloderm (67.4%) and the number of its layers (37.5%).

The results were in harmony with the data of Li *et al.* [30] they found that increasing of phosphorus and potassium fertilizers promoted the development of periderm, cortex, and phloem and that potassium fertilizer was better to the development of xylem. Kraus and Warren [31] found the steles of roots of Rudbeckia plants contained more secondary xylem with a mixture of 25ammonium: 75 nitrate than with nitrate alone. They also found that root diameter of Cotoneaster was greater when fertilized with a mix of ammonium and nitrate compared to nitrate alone.



(A) **(B)**

(C) (D)

Fig 2: Transverse sections through the basal portion of the main root of Madder (*Rubia tinctorum* L.) plant as affected by propagation methods, wood ash (WA) and chemical fertilizers (N1+AP). Scale bar: 500 µm. *(A) Control Seed (B) Control Division (C) N1+AP+WA-Seed (D) N1+AP+WA-Division Details: ph, phloem; phd, phelloderm; phm, phellem; pr, periderm; x, xylem.*

Table 4: Counts and measurements in microns of certain anatomical features in transverse sections through the basal portion of the main root of Madder (*Rubia tinctorum* L.) plant as affected by propagation methods, wood ash (WA) and chemical fertilizers (N1+AP). (Means of 10 readings)

Characters	Control Seed (1)	Control Division (2)	± % to Control Seed (1+2)	N1+AP+WA Seed (3)	± % to Control Seed (1+3)	N1+AP+WA Division (4)	± % to Control Division (2+4)	± % to N1+AP+WA Seed (3+4)
Root diameter	4022	5500	36.7	5437	35.2	5883	7.0	8.2
Periderm thickness	875	1333	52.3	1083	23.8	1083	-18.8	0.0
Phellem thickness	104	125	20.2	104	0.0	167	33.6	60.6
No. of Phellem layers	5	6	20.0	5	0.0	7	16.7	40.0
Phelloderm thickness	708	1185	67.4	958	35.3	875	-26.2	-8.7
No. of phelloderm layers	16	22	37.5	18	12.5	18	-18.2	0.0
Phloem thickness	125	167	33.6	250	100.0	250	49.7	0.0
Xylem diameter	2000	2417	20.9	2750	37.5	3250	34.5	18.2

4. Conclusion

The results showed that the younger plants gave a total content of anthraquinones (mmol / g) higher than the older ones. Plants propagated by seeds and supplied with either the treatment of 24g ammonium sulfate plus 15 g wood ash per plant or the combination of 12g Ammonium sulfate + Liquid Agricultural Potassium at the rate of 400 ml + wood ash at the rate of 15 g (for plant/season) resulted in the highest total content of anthraquinones (mmol / g) in fresh roots. It could be recommended to use the division method in the propagation of *Rubia tinctorum* plants and treated with 24g ammonium sulfate plus 15 g wood ash per plant to obtain the maximum fresh and dry weights of the herb as well as the highest root yield when harvesting after 14 months of cultivation. The anatomical root characteristics of the treatment under study were higher in both propagation methods than the control ones and their descending orders were as follows; division treatment, seed treatment, division control and seed control. These results have coincided with vegetative growth behavior of the plant under study.

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