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PHOSPHATE FERTILIZER AND NANO-MAGNESIUM FERTILIZATION EFFECTS ON GENE EXPRESSION, GROWTH, AND YIELD TRAITS OF DATURA (*DATURA STRAMONIUM* L.)

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SUMMARY

A study on the effects of phosphate fertilization and nano-magnesium application on several genes that control alkaloid synthesis, growth, and yield traits in Datura (*Datura stramonium* L.) underwent a field experiment in 2021 at the College of Agriculture, the University of Kerbala, Iraq. Using a randomized complete block design, the experiment had two factors with three replications. The first factor used phosphate (P) fertilizer, i.e., 0, 25, 50, and 75 kg P ha^{-1}, while the second factor included a nano-magnesium application by spraying with concentrations of 0, 60, 120, and 180 mg Mg L^{-1}. The results showed that adding 50 and 75 kg P ha^{-1} caused a decline in the concentration of atropine, hyoscyamine, and scopolamine in Datura leaves (22.77, 81.02, and 68.90 mg g^{-1}) and seeds (40.93, 65.69 and 99.79 mg g^{-1}), respectively. Sequentially, 25 and 50 kg P ha^{-1} generated the most yields of alkaloids in Datura leaves, with an average of 149.10 and 149.12 kg P ha^{-1}. Nano-magnesium application at the concentration of 180 mg Mg L^{-1} caused a significant decrease in the concentration of atropine, hyoscyamine, and scopolamine in seeds and leaves, i.e., with average values in leaves (29.50, 90.25, and 71.25 mg g^{-1}) and seeds (46.25, 82.49 and 121.320 mg g^{-1}), respectively. However, nano-magnesium concentrations of 0 and 120 mg Mg L^{-1} gave the highest yield of alkaloids in the leaves, with average values of 152.30 and 152.81 kg ha^{-1}. The nano-magnesium concentration of 120 mg Mg L^{-1} contributed the largest yield of alkaloids in seeds, with an average of 78.65 kg ha^{-1}**.** The results also showed phosphorus addition significantly decreased the PMT, TR1, and H6H gene expressions, whereas nano-magnesium application only reduced the H6H gene expression. High quantities of fertilizers phosphorus and nano-magnesium boost Datura's vegetative growth and production but lowered the alkaloid yield, thus recommending a balanced proportion.

Keywords: *Datura stramonium* L., genes PMT, TR1, H6H, gene expression, growth and yield traits, atropine, hyoscyamine, scopolamine

Key findings: Spraying nano-magnesium achieved the highest yield of alkaloids from seeds at a low concentration (60 mg Mg L^{-1}), whereas the alkaloids yielded the most from the leaves of Datura (*D. stramonium* L.) when no spraying of nano-magnesium occurred and when sprayed with concentrations of 0 and 120 mg Mg L^{-1}. The best yield of alkaloids from Datura leaves or seeds resulted at low levels of phosphorus (25 kg P ha^{-1})..

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INTRODUCTION

Datura (*Datura stramonium* L.) is a medicinal plant from the Solanaceae family. It contains many pharmaceutical properties because of a large number of chemical compounds content, including alkaloids, flavonoids, amino acids, tannins, saponins, carbohydrates, terpenoids, glycosides, steroids, and phenols. Datura treats many conditions, including digestive and respiratory sickness (Al-Taweel *et al*., 2019). The alkaloids, atropine, hyoscyamine, and scopolamine, derived from the leaves and seeds of the Datura plant, serve as raw materials in producing a wide range of medicinal products worldwide. However, local production of these materials faces several challenges, one of which is the relatively low production of Datura in comparison to its production in other countries, given the availability of environmental conditions necessary for its cultivation and production.

The unsatisfactory care for soils, crop services, and nutrients mainly caused its low productivity even with suitable environmental conditions (Gruhn *et al.*, 2000). Phosphorus is one of the essential nutrients a plant needs for its metabolic and physiological processes. In addition, it helps in cell division and stimulates root growth and higher alkaloid production in plants (Nasir and Khan, 2009).

Plants from the family Solanaceae are characterized by an increased requirement for magnesium (Cristofano *et al*., 2021) because magnesium plays a vital role in the synthesis of chlorophyll and other physiological processes. In addition to its vital role in some biochemical reactions, magnesium also activates a greater number of plant enzymes (Kinay and Erdem, 2020). The foliar feeding method is highly effective and provides the nutrients directly to the leaf tissues in a short time. A foliar nutrient application proved most effective as plants absorb the nutrients more compared with soil application, absorbing through the roots (Kaur and Kaur, 2021). As a result, the foliar nutrient application reduces the antagonism between the elements, easily making absorbing nutrients by the plant**.**

Genes play a significant role in determining how many alkaloids will be produced by Datura plants and how much of them will be accumulated (Kohnen and Kayser, 2019). The activation of genes increases alkaloid production, i.e., scopolamine, atropine, and hyoscyamine. According to Velázquez-Márquez *et al*. (2021), the genes putrescine N-methyl transferase (PMT), Hyoscyamine 6b-hydroxylase (H6H), and Tropinone reductase (TR) are key regulating genes for alkaloid synthesis in Datura plants. The findings of Cinelli and Jones (2021) stated two primary pathways for the biosynthesis of alkaloids linked to genes in the genus *Datura*.

In this process, the first step has the enzyme putrescine N-methyl transferase catalyzing the synthesis of the PMT gene. The enzyme works with methylate putrescine, which then converts to tropinone, and the said gene acts as a catalyst. The alkaloids synthesis is fundamental, and once completing the process through enzyme tropinone reductase, which is responsible for its creation, it takes over the function of the TR gene. This enzyme works to remove a hydrogen atom from tropinone, which results in the formation of tropine. Since this enzyme converts hyoscyamine to scopolamine, Sharma *et al*. (2021) were able to shed light on the function of the enzyme hyoscyamine 6b-hydroxylase (H6H). It is responsible for the manufacture of the H6H gene in Datura species.

After thorough research, 14 genes (ODC, PMT, MPO, PYKS, CYP82M3, TRI ArAT, PPAR, UGT1, LS, LM, HDH, and H6H—THESE are ONLY 12 LISTED) are thought to be responsible for the biosynthesis of alkaloids in Datura plants. Schlesinger *et al*. (2021) reported an increased expression of the TRI gene in the leaves in comparison to decreased expression of PMT and H6H gene, while the expression of the H6H gene increased in roots compared with leaves in *Datura innoxia* L. Moradi *et al*. (2020) noted that the gene H6H expression displayed higher than genes PMT1 and PMT2 in *Datura stramonium* L. They also reported the expression of gene H6H in the vegetative and root system, whereas gene PMT2 expression was limited in the root system, and no expression of the PMT1 gene in either of the two systems.

After further researching the H6H gene in *Datura anoxia* L., Li *et al.* (2020) demonstrated that although this gene's expression is elevated in root cells to a greater extent than in leaves, its accumulation in roots is significantly lower than in the leaves and seeds. According to Kohnen *et al*. (2018), the expression of the genes PMT and TR showed higher in the roots than the H6H gene. However, the H6H gene performed less also in the leaves. The genes PMT, TR1, and H6H are accountable for controlling the synthesis of specific enzymes that are responsible for the accumulation of alkaloids when the various crop plants were exposed to the application of phosphorus and nano-magnesium (Ullrich, 2016), potassium and nano-copper (Al-Yasari,

2022), and nano-fertilizer with zinc and copper (Kareem *et al*. 2022). In light of the previous investigations, the latest study aimed to investigate the phosphorus and nano-magnesium fertilization effects on the relative expression of the three genes PMT, TR1, and H6H, and growth and yield traits in Datura (*Datura stramonium* L).

MATERIALS AND METHODS

The field experiment took place during the crop season 2021 to study the effects of phosphate fertilizer and nano-magnesium fertilization on several genes responsible for controlling the synthesis of the active substance (alkaloids), growth, and yield traits in Datura (*Datura stramonium* L), at the College of Agriculture, the University of Kerbala, Karbala Governorate, Iraq. The experiment underwent a randomized complete block design using two factors with three replications. The first factor consisted of varying doses of phosphate fertilizer (0, 25, 50, and 75 kg P ha^{-1}), while the second factor included the foliar application of nano-magnesium with four different concentrations (zero [control - distilled water only], 60, 120, and 180 mg Mg L^{-1}). The phosphate fertilizer application used the form of triple superphosphate (P_2O_5 - 46%) all at once at planting time. The two stages of different concentrations of the nano-magnesium application proceeded through foliar spray using a 20-liter dorsal sprinkler early in the morning. The first foliar spray of nano-magnesium progressed at the 4-5 leaves stage, while the second spray continued after one month of the first foliar application. The planting of Datura seeds in dishes took place on 20 March 2021 at a rate of four seeds per dish. On 9 April 2021, transfer to the field of the seedlings with 2-3 leaves ensued. The planting took place in furrows, with a spacing of 75 cm between each furrow and a distance of 60 cm among the plants. The field got irrigated regularly, and harvesting occurred on 20 July 2021.

Diagnosis of genes PMT, TR1, and H6H in Datura

Using kits manufactured by Zamo Research USA (Kat. No. R2024), DNA was isolated from the leaves and roots of Datura (*D. stramonium*), and polymerase chain reaction (PCR) primers were constructed to diagnose the genes PMT (F: 5' ACAACCCACGAAGAGCATC'3 and R: 5'GAGCTAGTATGAAGACCG'3), TR1 (F: 5'ATGGACGAATCACAGGTGTCC'3 and R: 5'TTCCTTATGTATCACACCC'3), and H6H (F: 5'ATGGACGAATCACAGGTGTCC'3 and R: 5' TTAGACACATATGGTACGTGCTCC). The PCR test used the kit called MaximeTM PCR PreMix (i-Taq) provided by the Korean business iNtRoN. (KIt.No 25025).

The PCR used a total volume of 25 µl containing the required components. The total volume included the Taq PCR PreMix 10 µl, Forward primer 1 µl, Reverse primer 1 µl, DNA 5 µl, and D.W 8 µl. The reaction mixture utilized a clean tube (one tube for each gene, with a DNA-free tube serving as the negative control), combining its components with a micropipette before placing it in a centrifuge to maintain its integrity. Following the completion of the final volume of the reaction mixture, it was transferred into a PCR machine, employing the procedures detailed in Table 1 to amplify the PMT, TR1, and H6H genes in that order. Electrophoresis took place after dissolving 1 g of agarose in 100 ml of TBE (1X) and heating to boiling point to determine the sizes of PCR fragments and the DNA Ladder marker. After cooling to 40°C–50°C, 2 µl of a red-safe dye was added. The PCR products proceeded to mix with the loading buffer while waiting.

Table 1. PCR conditions for the amplification of genes PMT, TR1, and H6H.

Step	(°C)	Time	Number of cycles
Initial Denaturation	95	3 min	1
Denaturation	95	45 sec	
Annealing	63	45 sec	35
Extension-1	72	2 min	
Extension-2	72	7 min	1

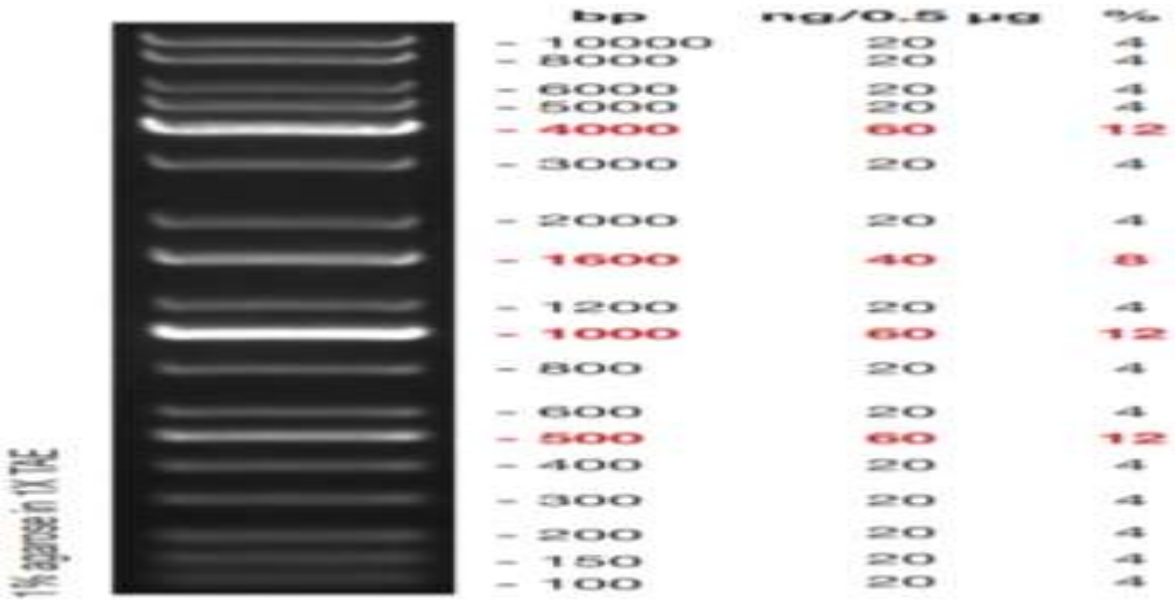

Figure 1. DNA ladders.

After preparing the gel casting tray and positioning the comb to make wells, the dissolved agarose was poured into the tray and allowed to harden at room temperature. After the agarose hardened, the comb underwent removal without deforming or shattering the wells. Upon returning the tray to the electrophoresis equipment, the TBE was poured into the chamber until the agarose layer got submerged in 1 mm. The PCR products were injected into each well of the agarose gel, affixing 5 µl (1Kbp) ladder markers to the wells on the left side of the additional samples (Figure 1). The power source ran at 100 mA for 1 h. Then the agarose layer was placed on a UV transilluminator after electrophoresis.

Relative expression of genes PMT, TR1, and H6H

Studying the relative expression of genes PMT, TR1, and H6H proceeded in the roots and leaves of Datura plants at the 7-8 leaf stage and 50% flowering stage, respectively, by sampling the leaves and roots for each experimental unit. The samples (roots and leaves) underwent RNA extraction, following the specified stages using the kit supplied by the American company Zymo (Kat. No. R2024). The method developed by Livak and Schmittgen (2001) was utilized to estimate the relative expression of genes PMT, TR1, and H6H, with the gene Actin serving as a reference gene through the following equations:

$$\Delta ct = ct_{target\ gene} - ct_{reference\ gene}$$

$$\Delta\Delta ct = \Delta ct_{Test} - \Delta ct_{Control}$$

$$\text{Relative gene expression} = 2^{-\Delta\Delta ct}$$

Since the ct-target gene is the cycle threshold for the target genes (PMT, TR1, and H6H***)***, the ct-reference gene is the cycle threshold for the reference gene (Actin), and the ct-test is the cycle threshold for samples tested for the target genes (PMT, TR1, and H6H***)***, and cT-control is the cycle threshold of the control sample for the target genes (PMT, TR1, and H6H).

The RT-qPCR test ensued for the study parameters according to the required growth stages and plant parts, using the kit (GoTaq® Probe RT-qPCR Master Mix) supplied by Promega (Cat. No A6120), with a total volume of 25 microliters containing the components: GoTaq® RT-qPCR), Master Mix 10 µl, Forward primer of target gene 1 µl, Reverse primer of target gene 1 µl, Forward primer of gene reference 1 µl, Reverse primer of gene reference 1 µl, RNA 5 µl, and D.W 5 µl. The above components were mixed in a rotary mixer device at a speed of 3000 rpm for 10 sec, then put in an RT-PCR device, with the programs employed for each gene (Table 2).

Table 2. RT-qPCR reaction conditions for genes PMT, TR1, and H6H.

Step	(°C)	Time	Number of cycles
cDNA synthesis	50	20 min	Hold
Denaturation Initial	95	10 min	Hold
Denaturation	95	45 sec	
Annealing	62	45 sec	40
Extension	72	1	
Extension	72	5	Hold

Table 3. Retention time for standard samples of compounds diagnosed using HPLC chromatography.

Standard compound	Retention time (min)	Standard compound area
Atropine	3.76	1225
Hyoscyamine	5.40	1011
Scopolamine	5.70	445

Data recorded

Calculating the number of leaves plant^{-1} used an average for five plants in median rows. The leaf samples were taken from all experimental units at 50% of the flowering stage, while the seed samples were taken at the stage of full maturity. The percentage of alkaloids (%) in leaves and seeds estimation followed the methods of Harborne (1973) and Ijarotimi *et al*. (2013). Computing the leave yield kg experimental unit^{-1} followed to convert to kg ha^{-1}**.** The average of fruits plant^{-1} sourced five plants randomly from the median lines**.** Acquiring the seeds fruit^{-1} through the number of fruits taken from the five measured plants transpired. Measuring the total seed yield kg ha^{-1} for each experimental unit took place, then converted to kg ha^{-1}. The concentration of alkaloids atropine, hyoscyamine, and scopolamine in leaves and seeds (mg g^{-1}) went through a High-performance Liquid Chromatography (HPLC) device, depending on the retention (Table 3). Relying on the area of the model to perform the process of calculating, the concentrations were measured according to the following equation:

$$Concentration\ (mg\ g^{-1}) = \frac{Standard\ substance\ concentration}{Standard\ substance\ area} \times \frac{dilution\ factor}{weight}$$

Statistical analysis

The data underwent evaluation according to the analysis of variance to compare the arithmetic means using the Genstat program for all studied traits. The least significant difference ($LSD_{0.05}$) test served for comparison and separation of the means for various traits.

RESULTS

After making all the necessary arrangements for the PCR reaction technique, the PCR products underwent an electrophoresis process using an agarose gel, with the obtained findings shown in Figure 2. The 400 bp band in the sample of roots and leaves represents the gene PMT, responsible for atropine alkaloids biosynthesis. The atropine synthesis pathway states that the tropane ring system originates from either ornithine or arginine via the formation of putrescine, then the enzyme PMT methylating putrescine to form tropinone. Additionally, atropine can be synthesized by reacting with tropine (Dewick, 2009***)***.

The results also showed the presence of a 700 bp band in the root and leaf samples, representing the TR1 gene (Figure 2). The gene TR1 is responsible for converting troponin to tropin by reducing troponin, then reacting tropin and tropic acid to produce littorine, which leads to the compound hyoscyamine in reactions that include several unspecified enzymes (Facchini, 2006***)***. The presence of a 500 bp bundle in the root and leaf samples identifies the gene H6H, which is accountable for the synthesis of scopolamine (Figure 2). The hyoscyamine 6'-hydroxylase (H6H) is responsible for the last significant alteration, which is the production of an epoxide within the molecule. These results further revealed the formation of the scopolamine complex, which is a bifunctional enzyme. Hyoscyamine further transforms into a scopolamine compound (Kramer, 2009).

Subjecting the roots to the influence of phosphate fertilizer and foliar application of nano-magnesium (nano-Mg) to investigate the

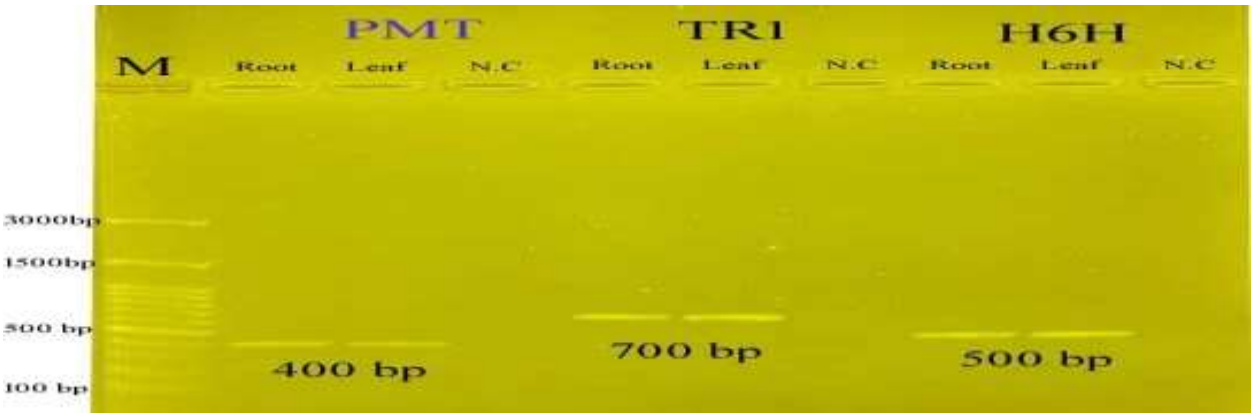

Figure 2. Electrophoresis of PCR product primers of three genes PMT, TR1, and H6H, with a negative comparison (N.C) and DNA ladder attached to the left side of the figure.

relative expression of the genes PMT, TR1, and H6H in Datura roots, the use of RT-qPCR technology amplified the genes**.** An increase in phosphorus content has no significant influence on the expression of the gene H6H. However, an increase in phosphorus led to a significant rise in the CT values of the genes PMT and TR1 while the plant was at the 7-8 leaf stage (Table 4). Two average values of cycles 34.05 and 32.08 resulted from the treatment with 75 kg P ha^{-1}. The relative gene expression calculations also confirmed this, as the relative expression of genes PMT and TR1 in Datura roots at the 7-8 leaf stage displayed 0.51 and 0.50 times higher than the control (33.03 and 31.02 cycles). The relative expression values of these two genes also showed no significant difference between the treatments of 50 and 75 kg P ha^{-1}. In addition, the results of Datura roots at the 7-8 leaf stage also demonstrated that the nano-Mg foliar spray treatments showed no significant effect on the cycle threshold (CT) and the relative expression of the genes PMT, TR1, and H6H (Table 4)**.**

Results revealed that an increase in phosphorus led to a discernible rise in the CT values of the genes PMT, TR1, and H6H in the roots of Datura plants at the flowering stage (Table 5). It provides 35.54, 34.66, and 33.75 cycles, which may cause a significant decrease in gene expression when the treatment of 75 kg P ha^{-1} increased than the no additional treatment. The relative gene expression calculations confirmed this as it decreased by 0.36, 0.38, and 0.35 times more than the non-fertilization treatment. No significant differences showed between the treatment of 50 and 75 kg P ha^{-1} in the relative expression values. Results further exhibited no significant impact of nano-Mg foliar application and the interaction of phosphorus with nano-Mg at the cycle threshold (CT) and the relative expression of genes PMT, TR1, and H6H in Datura roots during the flowering stage.

Results revealed the nonsignificant impact of phosphorus, nano-Mg, and their interaction on the cycle threshold (CT) values and the relative expression of genes PMT, TR1, or H6H in Datura leaves of the plants at the 7-8 leaf stage (Table 6). The phosphorus and the nano-Mg foliar application have no significant effect on the expression of the genes PMT and TR1 in Datura leaves during the flowering stage (Table 7). However, the nano-Mg application caused a significant increase in the cycle threshold for the gene H6H in Datura leaves at the flowering stage. The nano-Mg treatment of 180 mg Mg L^{-1} had the most number of cycles (30.95), which was 0.74 times less than the control. An increase in the number of cycles caused this as the concentration of nano-Mg increased, resulting in a significant decrease in the relative expression of the gene H6H in Datura leaves at the flowering stage.

Results enunciated that adding phosphorus caused a significant decrease in the relative expression of the gene H6H in Datura leaves at the flowering stage, reaching 0.91, 0.92, and 0.93 times more than the control (Table 7). The study observed a significant interaction between the phosphorus and nano-Mg treatments. Nano-magnesium played an essential role in the relative expression of gene H6H in Datura leaves at the flowering stage, and the interaction of treatment 75 kg P ha^{-1} with nano-Mg at the rate of 120 Mg L^{-1} caused a decrease of 0.61 compared with the control.

Table 4. Effect of phosphorus and nano-magnesium foliar application on the relative expression of PMT, TR1, and H6H genes in Datura roots at 7-8 leaf stage.

Phosphorus (kg ha-1)		Genes					
		PMT		TR1		H6H	
		CT	Gene expression	CT	Gene expression	CT	Gene expression
0		33.03	1.00	31.02	1.03	35.54	1.04
25		33.28	0.87	31.30	0.86	35.54	1.02
50		33.94	0.51	31.97	0.50	35.47	1.01
75		34.05	0.51	32.08	0.50	35.55	1.03
$LSD_{0.05}$		0.355	0.133	0.306	0.136	N.S	N.S
Nano magnesium (mg Mg L^{-1})							
0		33.65	0.72	31.67	0.71	35.56	1.05
60		33.70	0.72	31.71	0.73	35.66	1.03
120		33.50	0.72	31.53	0.71	35.48	1.00
180		33.46	0.72	31.47	0.74	35.40	1.02
$LSD_{0.05}$		N.S	N.S	N.S	N.S	N.S	N.S
Phosphorus × Nano-magnesium interaction							
0	0	33.16	1.00	31.16	1.00	35.72	1.00
	60	33.19	0.99	31.12	1.04	35.63	1.07
	120	32.77	1.01	30.79	1.00	35.33	1.01
	180	33.02	0.98	31.01	1.08	35.46	1.07
25	0	33.24	0.88	31.28	0.86	35.40	1.10
	60	32.29	0.89	31.31	0.87	35.60	1.00
	120	33.17	0.85	31.20	0.84	35.49	0.99
	180	33.40	0.87	31.41	0.86	35.67	0.99
50	0	34.15	0.51	32.18	0.49	35.64	1.03
	60	33.95	0.51	31.98	0.50	35.50	1.01
	120	34.07	0.52	32.10	0.51	35.63	0.99
	180	33.60	0.51	31.63	0.51	35.11	1.01
75	0	34.03	0.51	32.06	0.50	35.48	1.07
	60	34.39	0.51	32.42	0.50	35.89	1.01
	120	33.98	0.52	32.01	0.51	35.49	1.02
	180	33.81	0.52	31.84	0.51	35.35	1.01
$LSD_{0.05}$		N.S	N.S	N.S	N.S	N.S	N.S

Table 5. Effect of phosphorus and nano-magnesium foliar application on the relative expression of PMT, TR1, and H6H genes in Datura roots flowering stage.

Phosphorus (kg ha^{-1})		Genes					
		PMT		TR1		H6H	
		CT	Gene expression	CT	Gene expression	CT	Gene expression
0		35.54	1.04	34.66	1.04	33.57	1.03
25		36.28	0.61	35.30	0.66	34.55	0.51
50		36.97	0.35	36.01	0.38	35.01	0.35
75		37.08	0.36	36.11	0.38	35.13	0.35
$LSD_{0.05}$		0.244	0.031	0.254	0.044	0.215	0.022
Nano magnesium (mg Mg L^{-1})							
0		36.56	0.58	35.61	0.60	34.66	0.55
60		36.59	0.60	35.66	0.61	34.68	0.57
120		36.41	0.58	35.44	0.62	34.51	0.55
180		36.31	0.60	35.37	0.62	34.41	0.57
$LSD_{0.05}$		N.S	N.S	N.S	N.S	N.S	N.S
Phosphorus × Nano-magnesium interaction							
0	0	35.72	1.00	34.85	1.00	33.74	1.00
	60	35.63	1.07	34.83	1.03	33.67	1.06
	120	35.33	1.01	34.37	1.08	33.36	1.00
	180	35.46	1.07	34.59	1.07	33.49	1.06
25	0	36.27	0.60	35.29	0.65	34.50	0.52
	60	36.32	0.62	35.35	0.67	34.60	0.51
	120	36.19	0.61	35.21	0.66	34.48	0.51
	180	36.32	0.62	35.36	0.66	34.60	0.52
50	0	37.18	0.35	36.22	0.38	35.24	0.34
	60	36.99	0.35	36.03	0.37	34.99	0.35
	120	37.09	0.36	36.12	0.38	35.14	0.35
	180	36.62	0.35	35.66	0.38	34.67	0.35
75	0	37.06	0.36	36.08	0.39	35.14	0.35
	60	37.41	0.36	36.45	0.38	35.45	0.35
	120	37.01	0.36	36.06	0.38	35.05	0.35
	180	36.83	0.36	35.86	0.39	34.89	0.35
$LSD_{0.05}$		N.S	N.S	N.S	N.S	N.S	N.S

Table 6. Effect of phosphorus and nano-magnesium foliar application on the relative expression of PMT, TR1, and H6H genes in Datura leaves at 7-8 leaf stage.

Phosphorus (kg ha^{-1})		Genes					
		PMT		TR1		H6H	
		CT	Gene expression	CT	Gene expression	CT	Gene expression
0		36.57	1.04	33.57	1.04	32.57	1.03
25		36.58	1.02	33.58	1.02	32.57	1.02
50		36.49	1.01	33.50	1.02	32.50	1.00
75		36.60	1.02	33.58	1.04	32.58	1.02
$LSD_{0.05}$		N.S	N.S	N.S	N.S	N.S	N.S
Nano magnesium (mg Mg L^{-1})							
0		36.62	1.03	33.61	1.05	32.62	1.02
60		36.68	1.03	33.69	1.03	32.69	1.01
120		36.52	1.00	33.51	1.01	32.48	1.02
180		36.43	1.02	33.43	1.03	32.42	1.01
$LSD_{0.05}$		N.S	N.S	N.S	N.S	N.S	N.S
Phosphorus × Nano-magnesium interaction							
0	0	36.75	1.00	33.76	1.00	32.74	1.00
	60	36.67	1.07	33.67	1.08	32.68	1.05
	120	36.38	1.00	33.37	1.01	32.36	1.00
	180	36.49	1.07	33.49	1.08	32.49	1.06
25	0	36.44	1.10	33.48	1.07	32.54	1.02
	60	36.63	1.01	33.63	1.02	32.63	0.99
	120	36.52	0.99	33.51	1.00	32.41	1.06
	180	36.71	0.99	33.71	1.00	32.70	0.99
50	0	36.66	1.03	33.68	1.03	32.68	1.01
	60	36.53	1.02	33.53	1.02	32.53	1.00
	120	36.65	1.00	33.64	1.01	32.66	0.98
	180	36.14	1.01	33.14	1.01	32.14	1.00
75	0	36.62	1.00	33.51	1.08	32.53	1.05
	60	36.91	1.02	33.92	1.02	32.92	1.01
	120	36.51	1.03	33.53	1.02	32.51	1.02
	180	36.37	1.02	33.73	1.02	32.36	1.01
$LSD_{0.05}$		N.S	N.S	N.S	N.S	N.S	N.S

Table 7. Effect of phosphorus and nano-magnesium foliar application on the relative expression of PMT, TR1, and H6H genes in Datura leaves at the flowering stage.

Phosphorus (kg ha^{-1})		Genes					
		PMT		TR1		H6H	
		CT	Gene expression	CT	Gene expression	CT	Gene expression
0		37.60	1.03	33.80	1.05	30.59	1.03
25		37.60	1.02	33.81	1.03	30.78	0.91
50		37.52	1.02	33.73	1.02	30.68	0.92
75		37.63	1.01	33.82	1.03	30.81	0.93
$LSD_{0.05}$		N.S	N.S	N.S	N.S	N.S	0.098
Nano magnesium (mg Mg L^{-1})							
0		37.65	1.03	33.84	1.05	30.66	1.02
60		37.71	1.03	33.92	1.03	30.72	1.02
120		37.55	1.00	33.75	1.01	30.53	1.01
180		37.46	1.02	33.66	1.03	30.95	0.74
$LSD_{0.05}$		N.S	N.S	N.S	N.S	0.238	0.098
Phosphorus × Nano-magnesium interaction							
0	0	37.78	1.00	34.00	1.00	30.77	1.00
	60	37.69	1.07	33.90	1.08	30.69	1.07
	120	37.41	0.99	33.59	1.02	30.38	1.01
	180	37.5	1.07	33.72	1.08	30.53	1.05
25	0	37.48	1.09	33.71	1.08	30.58	1.01
	60	37.65	1.01	33.86	1.02	30.67	0.99
	120	37.55	0.99	33.74	1.01	30.54	0.99
	180	37.74	0.99	33.49	1.00	31.33	0.63
50	0	37.68	1.04	33.90	1.03	30.72	1.01
	60	37.55	1.02	33.77	1.02	30.55	1.01
	120	37.68	1.00	33.87	1.01	30.68	0.99
	180	37.17	1.00	33.37	1.02	30.75	0.67
75	0	37.65	0.99	33.75	1.07	30.56	1.05
	60	37.95	1.01	34.16	1.02	30.95	1.01
	120	37.54	1.03	33.78	1.01	30.52	1.03
	180	37.39	1.02	33.59	1.03	31.20	0.61
$LSD_{0.05}$		N.S	N.S	N.S	N.S	0.476	0.195

Phosphorus caused a significant increase in the number of leaves and leaf yield per plant, and the treatment of 75 kg P ha^{-1} provided the highest average of 168.18 leaves $plant^{-1}$ and 974.50 kg ha^{-1}, excelling the control treatment (83.30 leaves $plant^{-1}$ and 671.59 kg ha^{-1}), respectively (Table 8). The nano-Mg application also caused a significant increase in the number and yield of Datura leaves, and the treatment of 180 mg Mg L^{-1} provided the highest mean values of 145.19 leaves $plant^{-1}$ and 908.09 kg ha^{-1} compared with the control (104.35 leaves $plant^{-1}$, 815.21 kg ha^{-1}). Results further showed a significant interaction between the treatments of phosphorus and nano-Mg in the number and yield of Datura leaves, and the combination of the nano-Mg application (180 mg Mg L^{-1}) with 75 kg P ha^{-1} gave the highest average values of 239.59 leaves $plant^{-1}$ and 1,088.66 kg ha^{-1}, respectively.

For fruits, the addition of phosphorus caused a significant increase in the number of fruits and seeds in Datura plants. The two treatments, 50 and 75 kg P ha^{-1} revealed the highest average, reaching 30.02 and 29.98 fruits $plant^{-1}$ and 228.14 and 217.36 seeds $plant^{-1}$, respectively, compared with the control (20.83 fruits $plant^{-1}$ and 163.22 seeds $plant^{-1}$) (Table 8). However, no significant impact of nano-Mg and its interaction with phosphorus on the number of Datura fruits occurred. The nano-Mg application caused a significant increase in the number of seeds, and the treatment of 180 mg Mg L^{-1} gave the highest average number of seeds reaching 236.14 seeds $plant^{-1}$, which excelled the control (174.36 seeds $plant^{-1}$). The two treatments of nano-Mg application, i.e., 60 and 120 mg Mg L^{-1} revealed the averages of 180.09 and 197.78 seeds $plant^{-1}$.

In Datura seed yield, the addition of phosphorus caused a significant increase in the total seed yield (Table 8). Treatments 50 and 75 kg P ha^{-1} gave the highest average seed yield, reaching 501.36 and 503.49 kg ha^{-1} compared with the control (320.29 kg ha^{-1}). The nano-Mg foliar application caused a significant increase in the total seed yield, and the treatment of 180 mg Mg L^{-1} provided the highest average seed yield of 486.17 kg ha^{-1} than the control (395.49 kg ha^{-1}). A significant interaction existed between phosphorus and nano-Mg for the total seed yield**.** The nano-Mg application at the rate of 180 mg Mg L^{-1}, in combination with treatments of 50 and 75 kg P ha^{-1}, gave the highest total seed yield in the Datura plant, reaching 599.81 and 600 kg ha^{-1}, respectively.

The addition of phosphorus caused a significant decrease in the concentration of atropine, hyoscyamine, and scopolamine in Datura leaves, and the phosphorus at 75 kg P ha^{-1} gave the lowest average of 22.77, 81.02, and 68.90 mg g^{-1}, and seeds (40.93, 65.69 and 99.79 mg g^{-1}) compared with the control (60.12 and 124.12 and 164.35 mg g^{-1}), respectively (Table 9). The nano-Mg application also caused a significant decrease in the concentration of atropine, hyoscyamine, and scopolamine in Datura leaves, and the treatment of 180 mg Mg L^{-1} gave the lowest concentration of 29.50, 90.25, and 71.25 mg g^{-1}, and the same treatment caused a significant decrease in the concentration of atropine, hyoscyamine, and scopolamine in Datura seeds with averages of 46.25, 82.49, and 121.60 mg g^{-1}.

The addition of phosphorus caused a significant decrease in the percentage of alkaloids in Datura leaves (Table 10), and the treatment of 75 kg P ha^{-1} gave the lowest percentage of alkaloids in the leaves (11.06%) compared with the control (20.89%), whereas the two other treatments, 25 and 50 kg P ha^{-1},provided 18.39% and 15.61%, respectively. In the case of Datura seeds, the addition of phosphorus also caused a significant decrease in the alkaloids, and the treatment of 75 kg P ha^{-1} gave the lowest percentage of alkaloids in the seeds (11.84%) compared with the control (20.07%). Meanwhile, the two other treatments 25 and 50 kg P ha^{-1} amounted to 19.24% and 14.71%, respectively, which also revealed lower values than the control.

The nano-Mg foliar application also caused a notable decrease in the percentage of alkaloids in Datura leaves. The treatment of 180 mg Mg L^{-1} gave the lowest percentage of alkaloids (12.95%) compared with the control (19.10%), whereas the two other applications, 60 and 120 mg Mg L^{-1}, amounted to 16.63% and 17.27%, respectively (Table 10). In Datura seeds, the nano-Mg also caused a substantial decrease in the percentage of alkaloids in seeds, and the treatment of 180 mg Mg L^{-1} showed the smallest percentage of alkaloids in seeds (13.07%), compared with the control (17.90%). However, two other treatments of nano-Mg, i.e., 60 and 120 mg Mg L^{-1} gave the alkaloid percentages of 17.95% and 16.94%, respectively.

Results further revealed that the addition of phosphorus, with the doses of 25 and 50 kg P ha^{-1}, caused a significant increase in the yield of leaf alkaloids (149.10 and 149.12 kg ha^{-1}) compared with the control treatment (142.63 kg ha^{-1}) and the treatment

Table 8. Effect of phosphorus and nano-magnesium foliar application on some characteristics of vegetative growth and production of Datura plants.

Phosphorus (kg ha^{-1})		Leaves plant^{-1}	Leaf yield (kg ha^{-1})	Fruits plant^{-1}	Seeds fruits^{-1}	Seed yield (kg ha^{-1})
0		83.30	671.59	20.83	163.22	320.29
25		98.72	797.80	24.35	179.64	384.12
50		122.99	940.76	30.02	228.14	501.36
75		168.18	974.50	29.98	217.36	503.49
$LSD_{0.05}$		17.626	31.850	3.957	22.378	
Nano magnesium (mg Mg L^{-1})						
0		104.35	815.21	25.21	174.36	395.49
60		107.09	822.01	26.93	180.09	405.54
120		116.55	849.33	26.49	197.78	422.07
180		145.19	908.09	26.56	236.14	486.17
$LSD_{0.05}$		35.251	31.80	N.S	22.378	25.905
Phosphorus × Nano-magnesium interaction						
0	0	81.61	650.66	22.56	155.22	310.39
	60	81.52	659.48	22.66	159.46	311.69
	120	83.50	682.58	20.94	165.61	324.29
	180	86.58	693.63	17.15	172.60	334.81
25	0	92.72	789.12	24.65	176.32	365.92
	60	94.54	789.64	250.56	177.48	373.63
	120	100.27	800.58	24.94	182.05	387.77
	180	107.45	811.85	22.23	182.70	409.15
50	0	110.70	910.57	26.86	198.41	451.19
	60	109.49	919.63	29.75	206.75	467.06
	120	124.61	934.61	30.05	218.75	487.37
	180	147.14	1015.24	33.44	288.63	599.81
75	0	132.38	910.48	26.76	167.47	454.44
	60	142.91	919.29	29.73	176.66	469.78
	120	157.83	979.55	30.01	224.70	488.85
	180	239.59	1088.66	33.43	300.61	600.88
$LSD_{0.05}$		17.626	63.700	N.S	44.756	51.905

Table 9. Effect of phosphorus and nano-magnesium foliar application on the concentration of atropine, hyoscyamine and scopolamine (mg g^{-1}) in Datura leaves and seeds.

Phosphorus (kg ha^{-1})		Leaves			Seeds		
		Atropine	Hyoscyamine	Scopolamine	Atropine	Hyoscyamine	Scopolamine
0		48.86	129.86	90.18	60.12	124.12	164.35
25		43.48	112.23	85.92	57.28	98.49	144.22
50		29.57	96.82	80.76	53.84	75.53	128.09
75		22.77	81.02	68.90	40.93	65.69	99.79
$LSD_{0.05}$		4.225	9.046	6.247	4.165	8.751	12.320
Nano magnesium (mg Mg L^{-1})							
0		41.43	113.68	97.67	64.61	102.33	161.62
60		39.90	111.40	82.77	53.93	97.27	130.50
120		33.86	104.61	74.08	47.38	81.75	122.74
180		29.50	90.25	71.25	46.25	82.49	121.60
$LSD_{0.05}$		4.225	9.046	6.247	4.165	8.751	12.320
Phosphorus × Nano-magnesium interaction							
0	0	49.84	130.84	114.25	76.17	148.62	208.61
	60	49.92	130.92	90.58	60.39	140.70	154.59
	120	48.97	129.97	80.46	53.64	103.62	148.56
	180	46.72	127.72	75.44	50.29	103.55	145.65
25	0	45.14	115.14	112.45	74.97	107.69	188.68
	60	45.25	115.25	85.49	56.99	102.52	134.44
	120	43.00	113.00	75.39	50.26	90.33	128.28
	180	40.52	105.52	70.35	46.90	93.43	125.49
50	0	37.62	107.62	89.52	59.68	80.54	137.51
	60	34.91	104.91	83.66	55.77	78.50	130.48
	120	25.88	95.88	76.52	51.01	70.59	122.58
	180	19.87	78.87	73.34	48.89	72.49	121.80
75	0	33.11	101.11	74.46	47.64	72.45	111.69
	60	29.53	94.53	71.34	42.56	67.36	102.49
	120	17.57	79.57	63.93	34.62	62.46	91.55
	180	10.89	48.89	65.86	38.90	60.49	93.45
$LSD_{0.05}$		8.450	18.098	12.494	8.329	17.502	24.641

Table 10. Effect of phosphorus and nano-magnesium foliar application on the percentage (%) and yield (kg ha^{-1}) of alkaloids in Datura leaves and seeds.

Phosphorus (kg ha^{-1})		Alkaloids (%)		Alkaloid yield (kg ha^{-1})	
		Leaves	Seeds	Leaves	Seeds
0		20.89	20.07	142.63	66.72
25		18.39	19.24	149.10	76.45
50		15.61	14.71	149.12	73.55
75		11.06	11.84	106.68	58.86
$LSD_{0.05}$		1.979	1.229	4.367	8.216
Nano magnesium (mg Mg L^{-1})					
0		19.10	17.90	152.30	67.94
60		16.63	17.95	134.14	71.08
120		17.27	16.94	152.81	78.65
180		12.95	13.07	108.27	57.92
$LSD_{0.05}$		1.979	1.229	4.367	8.216
Phosphorus × Nano-magnesium interaction					
0	0	22.66	20.87	145.77	63.73
	60	20.47	20.75	135.72	65.39
	120	20.24	20.55	148.19	78.06
	180	20.21	18.12	140.84	59.70
25	0	20.70	19.79	163.16	71.40
	60	18.76	20.03	149.01	74.80
	120	17.77	19.88	151.45	88.79
	180	16.32	17.28	132.77	70.84
50	0	17.42	16.55	158.33	72.60
	60	15.22	16.48	140.46	75.91
	120	18.66	15.14	183.93	81.86
	180	11.13	10.66	113.78	63.81
75	0	15.63	14.41	141.96	64.03
	60	12.07	14.52	111.39	68.20
	120	12.40	12.22	127.67	65.88
	180	4.15	6.21	45.70	37.31
$LSD_{0.05}$		3.957	2.458	8.734	N.S

of 75 kg P ha^{-1} significantly decreased the yield of leaf alkaloids (106.68 kg ha^{-1}) (Table 10). The nano-Mg application caused a significant increase in the yield of leaf alkaloids, and the treatments 0 and 120 mg Mg L^{-1} gave values of 152.30 and 152.81 kg ha^{-1}, respectively. However, the two treatments of nano-Mg, i.e., 60 and 180 mg Mg L^{-1}, caused a decrease of leaf alkaloids with average values of 134.14 and 108.27 kg ha^{-1}**.** The addition of phosphorus with 25 kg P ha^{-1} gave the highest yield of alkaloids in Datura seeds (76.45 kg ha^{-1}) than the control (66.72 kg ha^{-1}), while treatments of 50 and 75 kg P ha^{-1} gave 73.55 and 58.86 kg ha^{-1}, respectively**.** The nano-Mg at the concentration of 60 and 120 mg Mg L^{-1} caused a significant increase in the yield of seed alkaloids (71.08 and 78.65 kg ha^{-1}) compared with the control (67.94 kg ha^{-1}), while nano-Mg treatment of 180 mg Mg L^{-1} gave the lowest mean yield of the alkaloid (57.92 kg ha^{-1}).

DISCUSSION

The effect of high levels of fertilization with transcription factors causes a decrease in the relative expression of PMT and TR1 genes when increasing phosphate levels. Transcription factors play a critical role in either increasing or inhibiting gene expression, therefore, it is important to understand the whole mechanism of how these proceed and work (Wang *et al*., 2016). Also, according to Pyne *et al.* (2019), alkaloids result from secondary metabolism, produced under environmental stresses to help the plant adapt to its external environment. Additionally, secondary metabolism processes can start due to the lack of nutrients, as cells sense these indicators by molecules. Delicate on their surfaces to give signals to change the metabolic processes within cells by changing the expression of some genes, an increase in phosphorus levels may reduce the signals necessary for the functioning of the PMT and TR1 genes, causing the plant to continue to build cellular material at the expense of by-products (Vafaie *et al*., 2022).

Also, the decrease in the relative expression of the H6H gene in Datura leaves by spraying with nano-Mg can be attributed to the effect of nutrients (magnesium) indirectly in the process of gene expression through changes in hormonal signals and stimulating to

change the vital activity. These changes occur because increased levels of nutrients work to continue metabolic pathways, whereas decreased levels of nutrients work to modify secondary metabolic pathways, increasing their products and some selective metabolic pathways (Li *et al*., 2020).

Phosphorus plays an essential role in the production of enzymes required for the energy reactions that occur during the photosynthesis process. Additionally, phosphorus participates in the representation of nuclear proteins, which increases the number of leaf origins. As a result, the number of leaves on a plant can grow as a direct result of the presence of phosphorus (Vashvaei *et al*., 2019). The foliar application of nano-magnesium also has a major effect as it increases the total number of leaves. As a result, magnesium plays a critical role in increasing the availability of magnesium pectate, involved alongside calcium pectate in the process of sticking cellulose fibers together during the construction of cell walls, thus, increasing the process of cell division, which ultimately increases the number of leaves in the plants (Vashvaei *et al*.,2019).

Moreover, an increase in the number of the Datura leaves led to an increase in the leaf yield, as well as, the role of indirect phosphorus in the absorption of nitrogen and potassium developing the root system, further working to increase vegetative growth, given the presence of phosphorus (Bozhinova, 2016). Phosphate fertilizer and nano-magnesium application induced an increase in the components of Datura yield, which led to a considerable increase in seed yield. It resulted in substantial fruit and seed production**.**

With the phosphorus and nano magnesium influences, the concentration of atropine, hyoscyamine, and scopolamine in the leaves and seeds of Datura. It resulted from a decreased expression of the PMT gene, responsible for encoding the enzyme putrescine N-methyl transferase that assists in the production of atropine alkaloids. There was also a reduction in the relative expression of the TR1 gene. In addition, as the quantity of atropine, hyoscyamine, and scopolamine in the leaves and seeds of Datura decreased, a reduction in the percentage of alkaloids found in the leaves and seeds happen.

An increase in phosphorus level of 75 kg P ha^{-1} caused a significant decrease in the concentration of leaf alkaloids. The increase in yield of leaf alkaloids, at levels 25 and 50 kg P ha^{-1}, may be due to these two levels giving the best amount of yield in leaves and the concentration of leaf alkaloids. Alkaloids found in the leaves had a detrimental impact on the total amount of alkaloids produced. As for the alkaloid yield in the Datura seeds, the level of 25 kg P ha^{-1} treatment achieved the best yield, which resulted in the most appropriate treatment on seed yield and an appropriate proportion of alkaloids in the seeds, thus, obtaining the highest alkaloid yield in the seeds. However, the addition of 50 and 75 kg P ha^{-1} decreased the yield of alkaloids, as shown by the low percentage of alkaloids in the seeds, respectively**.** The increase in the yield of alkaloids in the seeds, due to nano-Mg at the concentration of 60 and 120 mg Mg L^{-1}, might be due to optimum seed yield and eventually, an optimum alkaloid ratio in the seeds. However, increasing the magnesium level would result in reducing the yield of alkaloids in the seeds, as shown by the low percentage of seed alkaloid.

CONCLUSIONS

The study results conclude that the abundance of phosphorus addition and spraying with nano-magnesium led to an increase in the yield of leaves, seeds, and alkaloids of Datura. In light of this, the recommendation to raise the overall production of atropine, hyoscyamine, and scopolamine alkaloids in the leaves and seeds needs immediate application of the phosphate fertilizer at a rate of 25 kg P ha^{-1} and spraying of nano-magnesium on the leaves at a concentration of 120 mg Mg L^{-1}.

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