

## **Effects of AMF Inoculation, Foliar Application of Isabion, and GA<sub>3</sub> on Growth and Leaf Minerals of Pecan Seedlings**

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### **Abstract**

This study examined the response of pecan (*Carya illinoensis* Koch) seedlings to arbuscular mycorrhizal fungi (AMF) inoculation, foliar application of the bio stimulant Isabion® (ISB), and gibberellic acid (GA<sub>3</sub>). The experiment was carried out in the lath house of the Horticulture Department Nursery, College of Agricultural Engineering Sciences, University of Duhok, during 2025. The AMF treatment consisted of two levels: without inoculation (-AMF, 0 g) and with inoculation (+AMF, 50 g). The second factor include foliar application of the commercial bio stimulant Isabion® (ISB) (Syngenta Crop Protection) at three concentrations: 0, 5, and 10 mL·L<sup>-1</sup>, and gibberellic acid (GA<sub>3</sub>) applications at concentrations of 0, 75, and 150 mg·L<sup>-1</sup> as the third factor. A factorial experiment was conducted using a Randomized Complete Block Design (RCBD). The finding results showed that the highest levels of AMF, ISB, and GA<sub>3</sub> significantly enhanced most pecan seedling parameters compared with the control. AMF inoculation at 50 g significantly improved seedling height, leaf area, root colonization, leaf nitrogen, especially phosphorus, and potassium content. Similarly, foliar spraying with 10 mL·L<sup>-1</sup> ISB record the greatest increases in vegetative growth parameters and nutrient concentrations. Additionally, GA<sub>3</sub> at 150 mg·L<sup>-1</sup> significantly stimulated seedling height, stem diameter, leaf area, and improved leaf phosphorus, and potassium accumulation. Several two-way and three-way inter-actions among AMF, ISB, and GA<sub>3</sub> were significant, with the triple combination of producing the highest growth and physiological responses, including the greatest root colonization, nutrient uptake, and biomass accumulation.

Overall, the obtaining results confirm that combining arbuscular mycorrhizal fungi (AMF) inoculation, foliar application of the bio-stimulant Isabion, (ISB) and gibberellic acid (GA<sub>3</sub>) significantly improves growth and nutrient status of pecan seedling.

### **Introduction**

The pecan (*Carya illinoensis* Koch), a deciduous tree in the walnut family (Juglandaceae), is native to temperate North America and continues to be the world's leading producer. Pecan cultivation has spread to a number of nations outside of its natural area, including Australia, Brazil, Canada, Mexico, Israel, and South Africa. The pecan nut, which is well-known for its rich flavor

and buttery texture, has one of the highest fat concentrations of any food originating from plants and is extremely healthy [9].

In addition to its delicious nuts, pecan trees are prized for their fine timber and increasing economic value in a variety of climes. Pecans are long-lived trees that need several years to attain maximum yield, therefore the early phases of seedling growth are very important. However, obstacles including low soil

fertility, nutrient shortages, and environmental stressors can hinder seedling growth, particularly in nurseries. In order to ensure strong establishment, ongoing orchard yield, and improved tolerance to future stress factors, it is imperative to optimize early development conditions [24].

Mycorrhizal fungi (MF) are microorganisms that live in the soil and develop symbiotic relationships with around 90% of agricultural crops and 80–90% of vascular plant species [22]. Mycorrhizae treatment increases pecan trees' effective root surface area, which improves their ability to absorb water and nutrients. [11].

One of the most prevalent symbiotic relationships in nature is mycorrhiza, which is produced between soil-dwelling mycorrhizal fungus and plant roots [17, 25]. In addition to increasing the host plant's resistance to a variety of biotic and abiotic challenges, such as drought, floods, salt, pests, and diseases, mycorrhiza also promotes the absorption of mineral nutrients from the soil. It also affects the architecture of the root system, improves soil structure, boosts photosynthetic efficiency, and improves fruit quality. As a result, mycorrhiza is essential for maintaining environmental stability and sustainable agriculture [7, 15].

Most terrestrial plant species establish arbuscular mycorrhizae, a kind of endomycorrhizal connection, with arbuscular mycorrhizal fungus (AMF), which are among the most common soil fungi [34]. According to [19], mycorrhizal fungi, which include both ectomycorrhizas and endomycorrhizas, improve plant nutrient intake while decreasing the amount of biomass allocated to roots. In particular, under stressful circumstances, arbuscular mycorrhizal (AM) fungi promote phosphorus absorption and improve the physiological and biochemical characteristics of plants. In a similar vein, [8] showed that AMF had a beneficial impact on the physiology of apricot seedlings in northwest China. According to their research, seedlings grown in moderate shade demonstrated better photosynthetic rate,

stomatal conductance, transpiration, and water usage efficiency, as well as increased biomass and AM colonization. The contents of potassium, phosphorus, and nitrogen in roots were further increased under light shade circumstances.

Isabion, a commercial biostimulant that contains amino acids, peptides, and other organic compounds from enzymatic hydrolysis, is frequently used to increase plant metabolism, nutrient uptake, and stress tolerance [5]. Nitrogen assimilation is one of the physiological and biochemical processes that biostimulants affect [2]. Isabion foliar applications have been shown to improve root development, vegetative growth, and chlorophyll levels [10].

[23] demonstrated that gibberellic acid ( $GA_3$ ), stimulates plant elongation by increasing cell division and expansion. According to arecent research [29] on almond seedlings (*Prunus amygdalus*), foliar treatment of  $GA_3$  at  $100 \text{ mg}\cdot\text{L}^{-1}$  resulted in the greatest gains in stem length, leaf area, shoot fresh and dry weight, and root fresh and dry weight. In a similar vein, [18] showed that  $GA_3$  treatments at 100 and  $200 \text{ mg}\cdot\text{L}^{-1}$  greatly improved the root and vegetative development of "Antep" pistachio (*Pistacia vera L.*) seedlings in comparison to untreated controls. Increases in root dry matter, seedling height and diameter, branch count, total chlorophyll content, and leaf potassium concentration were among the impacts noted.

Thus, the current study aimed to investigate the effects of inoculation of arbuscular mycorrhizal fungus (AMF), foliar application of the bio stimulant Isabion, and gibberellic acid ( $GA_3$ ), on the growth and nutritional status of pecan seedlings.

## **Material and Methods**

The lath house of the nursery of the Horticulture Department, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan region, Iraq, which is situated in Sumail city between latitude  $36^\circ$

51° 38.077" N, longitude 42° 52' 02.998" E, and elevation (473 m) above sea level, was the site of this investigation during the growing season (2025). In this investigation, three-year-old pecan (*Carya illinoensis* Koch) seedlings with consistent growth, vigor, and health were employed. The seedlings were cultivated in plastic pots (35-cm-tall and 30-cm-diameter) that were filled with a homogenized substrate made up of a 1:3 mixture of river sand and organic manure.

A factorial experiment was conducted using a Randomized Complete Block Design (RCBD) with three factors, including all possible interactions. The first factor Arbuscular mycorrhizal fungi (AMF) inoculum was obtained from (*Glomus* spp. Commercial formulation), The inoculum consisted of spores, mycelia, hyphae, and colonized root fragments, mixed with a sterile peat moss applied at two levels: without inoculation (-MF) and with inoculation (+MF), using (0 g and 50 g) of AMF. The second factor was foliar application of a commercial bio-stimulant (Isabion®, Syngenta Crop Protection) as (ISB), is a commercial bio-stimulant containing free amino acids, short peptides, and organic nitrogen obtained through enzymatic hydrolysis of natural proteins. About 62% of the product is made up of amino acids and peptides, with 9–10% organic nitrogen supplied at three different concentrations: 0, 5, and 10 mL·L<sup>-1</sup>. Gibberellic acid (GA<sub>3</sub>), which was produced in pure powder form and made into aqueous solutions with a few drops of ethanol for solubility, was the third factor. Using a hand sprayer until runoff, foliar treatments were carried out at three concentrations: 0, 75, and 150 mg·L<sup>-1</sup>. A surfactant (Tween 80) was added to all mixes at a rate of two drops per two liters of solution (about 0.025%) to enhance the adhesion and distribution of the solutions on the leaf surfaces. In order to prevent quick evaporation and guarantee maximum absorption, treatments were administered early in the morning.

Both Isabion and GA<sub>3</sub> treatments were applied four times during the growing season

on the following dates: April 15, May 5, May 25, and June 15. Soil inoculation with Arbuscular mycorrhizal fungi (AMF) was applied once per season at the bud swell stage, in early April.

The experimental layout consisted of 18 treatment combinations (2 AMF × 3 Isabion × 3 GA<sub>3</sub>), with three replications, each containing four transplants per treatment unit, resulting in a total of 216 transplants (2 × 3 × 3 × 3 × 4 = 216).

All the data were analyzed statistically by using SAS programs [30]. Duncan's multiple range test at a 5% level of portability was used

to compare the treatment means according to [4].

At the end of the growth season, in mid-August, the following vegetative growth parameters were measured.

#### 1- Seedling Height -Increment(cm)

At the start of the growth season, a metric tape was used to measure each seedling's height (in centimeters) from the soil's surface to its tallest point. To ascertain the growth in height during the experimental period, the measurement was repeated in mid-August at the conclusion of the season.

#### 2- Seedling diameter- Increment (mm).

The diameter of the seedling was calculated by using Vernier according to [20].

#### 3- Average Single Leaf area (cm<sup>2</sup>). Were taken by [21].

#### 4- Root colonization (%) were taken by [28].

#### 5- Nitrogen percentage (%) were taken by [1].

#### 6- Phosphorus percentage (%) were taken by [26].

7- Potassium percentage (%) were taken by [27].

**Results and Discussion**

**Seedling Height Increment**

The results presented in Table (1) show a clear and significant improvement in pecan seedling height in response to AMF inoculation, foliar application of Isabion (ISB), and GA<sub>3</sub>. The highest levels of each factor—50 g AMF, 10 mL L<sup>-1</sup> ISB, and 150 mg L<sup>-1</sup> GA<sub>3</sub> produced greater height increments compared with their respective controls. The maximum mean values associated with these individual treatments were 22.91 cm, 24.72cm, and 22.54 cm, respectively.

The AMF × ISB interaction had a significant effect on seedling height. The triple interaction (AMF × ISB × GA<sub>3</sub>) demonstrated the strongest synergistic effect. The combined application of 50 g AMF, 10 mL L<sup>-1</sup> ISB, and 150 mg L<sup>-1</sup> GA<sub>3</sub> resulted in the highest recorded seedling height increment (26.73 cm), clearly outperforming all other treatments. In contrast, the lowest height increment

increment, with the combination of 50 g AMF + 10 mL L<sup>-1</sup> ISB one of the highest values (25.91 cm). A similar result was observed for the AMF × GA<sub>3</sub> interaction, where the treatment 50 g AMF + 150 mg L<sup>-1</sup> GA<sub>3</sub> produced the greatest height (24.16 cm).

A significant interaction was also evident between ISB and GA<sub>3</sub>, in which the combination of 10 mL L<sup>-1</sup> ISB + 150 mg L<sup>-1</sup> GA<sub>3</sub> resulted in a substantial increase in seedling height, reaching 24.24 cm.

(10.73 cm) was associated with the untreated seedling (control treatment.

Overall, these results highlight the substantial benefits of integrating AMF inoculation with foliar applications of bio stimulants and GA<sub>3</sub> to enhance early growth and vigor of pecan seedlings.

**Table 1.** Effect of arbuscular mycorrhizal fungi (AMF) inoculation, foliar application of Isabion (ISB), GA<sub>3</sub>, and their interactions on the height increment of pecan seedling.

AMF	ISB	GA3			AMF *ISB	AMF
		0	75	150		
-AMF	0	10.73k	15.40j	16.02ij	14.05e	
	5	18.62gh	20.22fg	22.47de	20.44c	19.34b
	10	23.29cd	22.98cd	24.31bc	23.53b	
+AMF	0	17.27hi	20.13fg	20.64f	19.35d	
	5	21.33ef	24.00b-d	25.09ab	23.47b	22.91a
	10	25.49ab	25.51ab	26.73a	25.91a	
GA3		19.46c	21.37b	22.54a	ISB	

AMF	-AMF	17.55e	19.53d	20.93c		
* GA3	+AMF	21.36c	23.21b	24.16a		
	0	14.00f	17.77e	18.33e	0	16.70c
ISB	5	19.98d	22.11c	23.78b	5	21.96b
*GA3	10	24.39b	24.24b	25.52a	10	24.72a

Means within a row, column and their interaction followed by the same letter are not significantly different according to Duncan’s Multiple Range Test at  $p \leq 0.05$ .

**Seedling Diameter Increment**

Table (2) shows that inoculation with arbuscular mycorrhizal fungi (AMF) did not produce a statistically significant effect on pecan seedling stem diameter. In contrast, foliar application of Isabion (ISB) exerted a clear and significant influence. The highest ISB concentration (10 mL L<sup>-1</sup>) resulted in the greatest increase in stem diameter (1.79 mm), whereas the untreated control exhibited the smallest increment (1.24 mm).

Similarly, gibberellic acid (GA<sub>3</sub>) application significantly enhanced seedling

AMF and 150 mg L<sup>-1</sup> GA<sub>3</sub> achieved the maximum diameter (1.56mm). The ISB × GA<sub>3</sub> interaction was likewise significant, with the 10 mL L<sup>-1</sup> ISB + 150 mg L<sup>-1</sup> GA<sub>3</sub> treatment producing the highest improvement (1.92 mm).

diameter. The highest concentration (150 mg L<sup>-1</sup>) resulted the maximum stem diameter (1.57 mm), exceeding that of the control treatment (1.42mm).

Significant interactions were also observed among the factors. The AMF × ISB interaction indicated that combining 50 g AMF with 10 mL L<sup>-1</sup> ISB produced the greatest diameter increase (1.79 mm). A similar pattern appeared in the AMF × GA<sub>3</sub> interaction, where the combination of 50 g

The strongest overall response occurred under the triple interaction of 50 g AMF, 10 mL L<sup>-1</sup> ISB, and 150 mg L<sup>-1</sup> GA<sub>3</sub>, which produced the highest seedling diameter (1.94 mm). In contrast, the smallest increment (1.08mm) was observed in the untreated seedlings.

**Table 2.** Effect of arbuscular mycorrhizal fungi (AMF) inoculation, foliar application of Isabion (ISB), GA<sub>3</sub>, and their interactions on the diameter increment of pecan seedling.

AMF	ISB	GA3			AMF *ISB	AMF
		0	75	150		
-AMF	0	1.08k	1.22i-k	1.28h-j	1.20d	
	5	1.50e-g	1.51e-g	1.60de	1.54b	1.51a
	10	1.63de	1.82a-c	1.90ab	1.78a	
+AMF	0	1.27h-j	1.43f-h	1.18jk	1.29c	
	5	1.35gh	1.63de	1.55ef	1.51b	1.53a

	10	1.67c-e	1.76b-c	1.94a	1.79a	
GA3		1.42b	1.56a	1.57a		
AMF	-AMF	1.40b	1.52a	1.59a	ISB	
* GA3	+AMF	1.43b	1.61a	1.56a		
	0	1.17f	1.33de	1.23ef	0	1.24c
ISB	5	1.42d	1.57c	1.58c	5	1.52b
*GA3	10	1.65c	1.79b	1.92a	10	1.79a

Means within a row, column and their interaction followed by the same letter are not significantly different according to Duncan’s Multiple Range Test at  $p \leq 0.05$ .

**Average Single Leaf Area**

Table (3) indicates that all three studied factors—AMF soil inoculation, foliar application of Isabion (ISB), and gibberellic acid (GA<sub>3</sub>)—had significant effects on pecan seedling leaf area. For each main factor, the highest leaf area values were recorded under the greatest application levels: 50 g AMF (139.96cm<sup>2</sup>), 10 mL L<sup>-1</sup> ISB (141.38cm<sup>2</sup>), and 150 mL L<sup>-1</sup> GA<sub>3</sub> (135.24cm<sup>2</sup>). These values were significantly greater than their respective controls, demonstrating the positive roles of fungal symbiosis, bio stimulants, and growth regulators in enhancing leaf expansion.

The AMF × ISB interaction showed a significant enhancement in leaf area, where the combined application of 50 g AMF and

10 mL L<sup>-1</sup> ISB produced the highest value (144.22cm<sup>2</sup>). A similar result was seen in the AMF × GA<sub>3</sub> interaction; the treatment integrating 50 g AMF with 150 mL L<sup>-1</sup> GA<sub>3</sub> resulted in the greatest leaf area within this interaction (141.82cm<sup>2</sup>).

Significant effects were also evident in the ISB × GA<sub>3</sub> interaction, with the combination of 10 mL L<sup>-1</sup> ISB and 150 mL L<sup>-1</sup> GA<sub>3</sub> resulting the highest leaf area (143.73cm<sup>2</sup>).

The strongest overall response was observed under the triple interaction of 50 g AMF + 10 mL L<sup>-1</sup> ISB + 150 mL L<sup>-1</sup> GA<sub>3</sub>, which produced the maximum leaf area recorded (145.47cm<sup>2</sup>). In contrast, the smallest leaf area (99.20 cm<sup>2</sup>) occurred in the untreated seedlings (0 g AMF + 0 mL L<sup>-1</sup> ISB + 0 mL L<sup>-1</sup> GA<sub>3</sub>).

**Table 3.** Effects of arbuscular mycorrhizal fungi (AMF) inoculation, foliar application of Isabion (ISB), GA<sub>3</sub>, and their interactions on the average single leaf area of pecan seedlings.

AMF	ISB	GA3			AMF *ISB	AMF
		0	75	150		
	0	99.20f	106.67f	115.47e	107.11e	
-AMF	5	124.27d	125.60d	128.53cd	126.13d	123.93b
	10	134.53bc	139.07ab	142.00ab	138.53b	

	0	129.33cd	134.67bc	137.07ab	133.69c	
+AMF	5	140.67ab	142.27ab	142.93ab	141.96ab	139.96a
	10	144.40a	142.80ab	145.47a	144.22a	
GA3		128.73b	131.84b	135.24a		
AMF	-AMF	119.33c	123.78c	128.67b	ISB	
*GA3	+AMF	138.13a	139.91a	141.82a		
	0	114.27g	120.67f	126.27e	0	120.40c
ISB	5	132.47d	133.93cd	135.73b-d	5	134.04b
*GA3	10	139.47a-c	140.93ab	143.73a	10	141.38a

Means within a row, column and their interaction followed by the same letter are not significantly different according to Duncan’s Multiple Range Test at  $p \leq 0.05$ .

**Root Colonization%**

Table (4) shows that soil inoculation with AMF, foliar application of Isabion (ISB), and GA<sub>3</sub> all exerted significant effects on the percentage of root colonization during the study period. For each factor individually, the highest colonization rates were obtained from the maximum levels of application: 50 g AMF (63.30%), 10 mL L<sup>-1</sup> ISB (47.33%), and 150 mL L<sup>-1</sup> GA<sub>3</sub>

(43.44%). These values were all superior to those recorded in the untreated control.

The interaction between AMF and ISB significantly enhanced root colonization. The combination of 50 g AMF with 10 mL L<sup>-1</sup> ISB produced the highest percentage within this interaction (69.11%). A similar pattern was observed in the AMF × GA<sub>3</sub> interaction; the treatment of 50 g AMF together with 150 mL L<sup>-1</sup> GA<sub>3</sub> resulted in the greatest increase (65.33%).

**Table 4.** Effect of arbuscular mycorrhizal fungi (AMF) inoculation, foliar Isabion (ISB), GA<sub>3</sub>, and their interactions on the percentage of root colonization in pecan seedlings.

AMF	ISB	GA3			AMF	AMF
		0	75	150	*ISB	
-AMF	0	13.00j	15.33ij	16.33h-j	14.89f	20.48b
	5	19.33g-i	22.00f-h	21.67f-h	21.00e	
	10	25.33f	24.67fg	26.67f	25.56d	
+AMF	0	51.67e	57.33d	60.00cd	56.33c	63.30a
	5	64.00bc	65.33bc	64.00bc	64.44b	

	10	66.67ab	68.67ab	72.00a	69.11a	
GA <sub>3</sub>		40.00b	42.22a	43.44a	ISB	
AMF	AMF-	19.22c	20.67c	21.56c		
*GA <sub>3</sub>	AMF+	60.78b	63.78ab	65.33a		
ISB	0	32.33f	36.33e	38.17de	0	35.61c
*GA <sub>3</sub>	5	41.67cd	43.67bc	42.83bc	5	42.72b
	10	46.00ab	46.67ab	49.33a	10	47.33a

Means within a row, column and their interaction followed by the same letter are not significantly different according to Duncan's Multiple Range Test at  $p \leq 0.05$ .

Furthermore, the ISB  $\times$  GA<sub>3</sub> interaction demonstrated significant effects on root colonization. The highest value recorded within this interaction (49.33%) occurred with the combination of 10 mL L<sup>-1</sup> ISB and 150 mL L<sup>-1</sup> GA<sub>3</sub>.

The strongest overall response was achieved under the triple interaction among AMF, ISB, and GA<sub>3</sub>. The treatment consisting of 50 g AMF + 10 mL L<sup>-1</sup> ISB + 150 mL L<sup>-1</sup> GA<sub>3</sub> produced the maximum colonization percentage (72.00%). In contrast, the lowest significant value (13.00%) was observed in the control seedling.

### Nitrogen %

Table (5) shows that soil inoculation with arbuscular mycorrhizal fungi (AMF) and foliar spraying with isabion (ISB) had significant effects on leaf nitrogen percentage. The highest values for each factor were recorded from 50 g AMF and 10 mL L<sup>-1</sup> ISB, achieving nitrogen concentrations of

3.37% and 3.40%, respectively. In contrast, the different concentrations of GA<sub>3</sub> did not significantly differ from one another.

The AMF  $\times$  ISB interaction had a significant effect, and the combination of 50 g AMF + 10 mL L<sup>-1</sup> ISB produced the highest nitrogen content (3.51%). The AMF  $\times$  GA<sub>3</sub> interaction was also significant, with the combination of 50 g AMF + 150 mL L<sup>-1</sup> GA<sub>3</sub> resulting in the greatest value (3.41%). A significant effect was recorded for the ISB  $\times$  GA<sub>3</sub> interaction, in which the treatment of 10 mL L<sup>-1</sup> ISB combined with 150 mL L<sup>-1</sup> GA<sub>3</sub> produced the maximum nitrogen concentration (3.40%).

Additionally, the triple interaction among AMF, ISB, and GA<sub>3</sub> demonstrated significant differences. The highest nitrogen percentage overall (3.62%) was obtained from the treatment of 50 g AMF + 10 mL L<sup>-1</sup> ISB + 150 mL L<sup>-1</sup> GA<sub>3</sub>. The lowest value (2.25%) occurred in the control treatment lacking AMF, ISB, and GA<sub>3</sub>.

**Table 5.** Effects of arbuscular mycorrhizal fungi (AMF) inoculation, foliar application of Isabion (ISB), GA<sub>3</sub>, and their interactions on leaf nitrogen percentage in pecan seedlings.

AMF	ISB	GA <sub>3</sub>			AMF *ISB	AMF
		0	75	150		
-AMF	0	2.25f	2.72e	2.98c-e	2.65d	2.96b
	5	2.96c-e	3.08b-e	2.81de	2.95c	
	10	3.23bc	3.43ab	3.18b-d	3.28b	
+AMF	0	3.32a-c	3.20bc	3.18b-d	3.23b	3.37a
	5	3.23bc	3.40ab	3.43ab	3.35ab	
	10	3.45ab	3.46ab	3.62a	3.51a	
GA <sub>3</sub>		3.07a	3.21a	3.20a	ISB	
AMF *GA <sub>3</sub>	-AMF	2.81c	3.08b	2.99bc		
	+AMF	3.33a	3.35a	3.41a		
ISB *GA <sub>3</sub>	0	2.78d	2.96cd	3.08bc	0	2.94c
	5	3.10bc	3.24ab	3.12bc	5	3.15b
	10	3.34ab	3.45a	3.40a	10	3.40a

Means within a row, column and their interaction followed by the same letter are not significantly different according to Duncan’s Multiple Range Test at  $p \leq 0.05$ .

**Phosphorus (%)**

Table (6) shows that soil inoculation with AMF, foliar spraying with ISB, and the application of GA<sub>3</sub> each exerted significant effects on leaf phosphorus concentration. The highest values for each factor were recorded with the elevated levels of 50 g AMF, 10 mL L<sup>-1</sup> ISB, and 150 mL L<sup>-1</sup> GA<sub>3</sub>, reaching phosphorus contents of 0.231%, 0.250%, and 0.236%, respectively, all statistically superior to the untreated control.

A significant interaction between AMF and ISB was observed, with the combination of 50 g AMF and 10 mL L<sup>-1</sup> ISB producing the maximum phosphorus concentration of

0.264%. Similarly, the interaction between AMF and GA<sub>3</sub> showed a remarkable response, where the treatment of 50 g AMF combined with 150 mL L<sup>-1</sup> GA<sub>3</sub> achieved the highest value within this interaction (0.251%). The ISB × GA<sub>3</sub> interaction also significantly influenced phosphorus content, with the combination of 10 mL L<sup>-1</sup> ISB and 150 mL L<sup>-1</sup> GA<sub>3</sub> resulting in the highest mean value (0.280%).

For the triple interaction among AMF, ISB, and GA<sub>3</sub>, the treatment of 50 g AMF + 10 mL L<sup>-1</sup> ISB + 150 mL L<sup>-1</sup> GA<sub>3</sub> produced the greatest phosphorus concentration

(0.305%). The lowest significant value (0.110%) occurred in the control treatment.

**Table 6.** Effects of arbuscular mycorrhizal fungi (AMF) inoculation, foliar application of Isabion (ISB), GA<sub>3</sub>, and their interactions on leaf phosphorus percentage in pecan seedlings.

AMF	ISB	GA <sub>3</sub>			AMF *ISB	AMF
		0	75	150		
-AMF	0	0.110i	0.154 h	0.191 g	0.152e	0.197b
	5	0.192 g	0.203e-g	0.216 d-f	0.204d	
	10	0.222de	0.229 d	0.256b	0.236b	
+AMF	0	0.197fg	0.199fg	0.216d-f	0.204d	0.231a
	5	0.217d-f	0.224 de	0.232 d	0.224c	
	10	0.235 cd	0.252bc	0.305 a	0.264a	
GA <sub>3</sub>		0.196 c	0.210 b	0.236 a	ISB	
AMF *GA <sub>3</sub>	-AMF	0.175d	0.195c	0.221 b		
	+AMF	0.216 b	0.225b	0.251a		
ISB *GA <sub>3</sub>	0	0.154 g	0.177 f	0.203 e	0	0.178c
	5	0.204 e	0.213 de	0.224 cd	5	0.214b
	10	0.229bc	0.240 b	0.280 a	10	0.250a

Means within a row, column and their interaction followed by the same letter are not significantly different according to Duncan’s Multiple Range Test at  $p \leq 0.05$ .

**Potassium (%)**

Table (7) shows that soil inoculation with AMF, as well as foliar application of ISB and GA<sub>3</sub>, significantly increased leaf potassium concentration in pecan seedlings compared with the untreated control. The highest values for each individual factor were obtained with 50 g AMF (2.29%), 10 mL L<sup>-1</sup> ISB (2.47%), and 150 mL L<sup>-1</sup> GA<sub>3</sub> (2.25%), indicating a substantial improvement in K uptake.

A significant interaction occurred between AMF and ISB. The combination of 50 g AMF and 10 mL L<sup>-1</sup> ISB resulted in the greatest potassium concentration (2.63%). Similarly, the AMF × GA<sub>3</sub> interaction showed a notable effect, with the combined application of 50 g AMF and 150 mL L<sup>-1</sup> GA<sub>3</sub> producing the highest value within this interaction (2.42%). The ISB × GA<sub>3</sub> interaction also exhibited significant enhancement, where the treatment of 10 mL L<sup>-1</sup> ISB plus 150 mL L<sup>-1</sup> GA<sub>3</sub> achieved the highest value (2.67%).

The maximum potassium content overall (2.80%) was recorded for the triple interaction of 50 g AMF + 10 mL L<sup>-1</sup> ISB + 150 mL L<sup>-1</sup> GA<sub>3</sub> . Conversely, the lowest

concentration (1.26%) occurred in the control treatment that received no AMF, ISB, or GA<sub>3</sub> .

**Table 7.** Effects of arbuscular mycorrhizal fungi (AMF) inoculation, foliar application of Isabion (ISB), GA<sub>3</sub> , and their interactions on leaf potassium percentage in pecan seedlings.

AMF	ISB	GA3			AMF *ISB	AMF
		0	75	150		
-AMF	0	1.26m	1.66l	1.77j-l	1.56d	
	5	1.74kl	1.86i-k	1.94h-j	1.85c	1.91b
	10	2.12f-h	2.28d-f	2.55bc	2.32b	
+AMF	0	1.76j-l	1.92i-k	2.01g-i	1.90c	
	5	2.19e-g	2.37c-e	2.45b-d	2.33b	2.29a
	10	2.50bc	2.58b	2.80a	2.63a	
GA3		1.93c	2.11b	2.25a		
AMF *GA3	-AMF	1.71e	1.93d	2.09c	ISB	
	+AMF	2.15c	2.29b	2.42a		
ISB *GA3	0	1.51g	1.79f	1.89ef	0	1.73c
	5	1.97e	2.11d	2.19cd	5	2.09b
	10	2.31c	2.43b	2.67a	10	2.47a

Means within a row, column and their interaction followed by the same letter are not significantly different according to Duncan’s Multiple Range Test at p ≤ 0.05.

**Discussion**

The results of this study demonstrate that arbuscular mycorrhizal fungi inoculation (AMF), foliar spraying with the bio-stimulant Isabion and gibberellic acid (GA<sub>3</sub> ), all significantly and complementarily improve pecan seedlings' early growth performance. When these treatments were applied separately or in combination, improvements were

consistently seen in seedling height, stem diameter, leaf area, root colonization, and leaf nutritional content, including nitrogen, phosphorus, and potassium.

AMF inoculation significantly enhanced nutrient intake and vegetative growth characteristics, which is consistent with the documented function of mycorrhizal symbiosis in boosting plant performance. In nursery conditions, where root limitation

frequently inhibits absorption, AMF are known to colonize root tissues and expand the functional root system through their extraradical hyphae, increasing the soil volume investigated and boosting nutrient and water intake [16]. This study's notable increases in leaf nitrogen, particularly phosphorus, and potassium contents are in line with findings that AMF improves nutrient solubilization and translocation to host plants [33].

Furthermore, as was previously seen for other woody species, greater nutrient availability and enhanced photosynthetic activity are responsible for the increased leaf area and seedling height under AMF treatments. [6], [20]. The effective formation of mycorrhizal relationships is further supported by the high root colonization percentages, which probably helped to improve growth vigor.

Early seedling growth was also greatly improved by the use of Isabion application, by providing easily accessible organic nitrogen molecules that promote protein synthesis, chlorophyll production, and enzyme activity, isabion, as bio-stimulant, stimulates plant metabolic activities [13]. Because serve as precursors for growth-related hormones and osmoregulation pathways, the increased leaf area, stem diameter, and nutrient concentrations seen Overall, this study confirms that AMF inoculation, Isabion as bio-stimulant application, and GA<sub>3</sub> treatments all improved early growth of pecan seedlings

## **Conclusion**

The results of the study clearly demonstrate that AMF inoculation at 50 g significantly enhanced root colonization and increased the absorption of essential elements like potassium and nitrogen, especially phosphorus. These improvements led to increased vegetative development, including

in this study can be linked to better nitrogen absorption and stress resistance. These findings are consistent with earlier research demonstrating that bio-stimulants containing peptides and amino acids enhance root formation, nitrogen absorption, and total vegetative growth in horticultural crops [12].

The significant benefit effect observed when ISB was combined with AMF, as well as suggest that enhanced root colonization may increase the efficiency with which plants utilize exogenous bio-stimulant.

Similarly, foliar application of GA<sub>3</sub> significantly enhanced seedling height, stem diameter, leaf expansion, and nutrient accumulation. GA<sub>3</sub> is a well-known growth regulator that promotes cell elongation, stimulates leaf expansion, and enhances shoot growth by stimulate genes associated with cell wall loosening and division [14], [3]. The improved height and leaf area observed in GA<sub>3</sub> -treated seedlings align with findings reported for almond, where GA<sub>3</sub> enhanced vegetative traits and strengthened physiological performance. [32] The enhanced nutrient content under GA<sub>3</sub> application suggests that increased vegetative growth is supported by improved translocation and assimilation of mineral elements.

through complementary mechanisms related to enhanced nutrient uptake, improved metabolic efficiency, and stimulation of vegetative development.

increased seedling height and leaf area. Foliar treatment of ISB shown strong positive benefits on growth and nutrient accumulation, particularly at 10 mL·L<sup>-1</sup>, indicating that Isabion as bio-stimulants are crucial for improving metabolic activity, nutritional absorption, and stress tolerance. Similarly,

GA<sub>3</sub> at 150 mg·L<sup>-1</sup> enhanced vegetative performance, due to promoting cell elongation, leaf expansion, and nutrient transfer,

significantly, applying 50 g AMF + 10 mL·L<sup>-1</sup> ISB + 150 mg·L<sup>-1</sup> GA<sub>3</sub> combined produced the greatest values for all major growth parameters, nutrient concentrations, and root colonization percentage. In conclusion, a practical and effective way to

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enhance pecan seedling vigor, nutritional status, and overall nursery performance is to combine of arbuscular mycorrhizal fungus inoculation (AMF), with foliar application of the bio-stimulant Isabion, and gibberellic acid (GA<sub>3</sub>), this combined treatment can be recommended as an effective management technique to improve the production of high-quality seedlings given the conditions of the study location.

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