9-4-2023

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Recommended Citation

Aryan, S., Gulab, G., Safi, Z., Durani, A., Raghib, M., Kakar, K., Zahid, T., Baber, B., Ahlawat, Y., Moussa, I., & Elansary, H. (2023). Enhancement of Propagation Using Organic Materials and Growth Hormone: A Study on the Effectiveness of Growth and Rooting of Pomegranate Cuttings. *Horticulturae, 9*(9). http://doi.org/10.3390/horticulturae9090999
Retrieved from: https://digitalcommons.mtu.edu/michigantech-p2/199

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Enhancement of Propagation Using Organic Materials and Growth Hormone: A Study on the Effectiveness of Growth and Rooting of Pomegranate Cuttings

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Abstract: Pomegranate production in Afghanistan is increasing remarkably, but nursery services are lacking, leading to inconsistent growth and low rooting percentages in cuttings. Hence, this research was conducted at the greenhouse of Nangarhar University, Faculty of Agriculture, to investigate the efficacy of indole-3-butyric acid (IBA) and organic materials, namely banana, garlic, aloe vera, and panchagavya, as rooting promoters on cuttings of the ideal seedless pomegranate cultivar (Bedana). The cuttings were derived from one-year-old dormant branches in mid-February of 2023. The findings indicated that panchagavya and aloe vera significantly ($p < 0.05$) accelerated the sprouting process of pomegranate cuttings, resulting in 100% rooting, while IBA showed a delayed initiation of sprouting. In contrast, banana had a detrimental effect on the sprouting of the cuttings. IBA and organic materials, except banana and garlic, significantly influenced all the shoot parameters. Among the treatments, panchagavya (21.1%), IBA (23.4%), and aloe vera (26.8%) demonstrated the highest number of shoots per cutting compared to the control. A consistent soil plant analysis development (SPAD) pattern was observed across treatments, positively correlating with shoot and root parameters (range, r = 0.30–0.97). The application of organic materials significantly ($p < 0.05$) impacted root length, acrobasal roots, root diameter, total root length, and root fresh weight. However, banana and garlic yielded contrasting results in most of these parameters. In conclusion, aloe vera and panchagavya are found to be more suitable for propagating pomegranates through cuttings, making them excellent alternatives to IBA for nurserymen and companies.

Keywords: aloe vera; IBA; panchagavya; pomegranate cuttings; root attributes

Citation: Aryan, S.; Gulab, G.; Safi, Z.; Durani, A.; Raghib, M.G.; Kakar, K.; Zahid, T.; Baber, B.M.; Ahlawat, Y.K.; Moussa, I.M.; et al. Enhancement of Propagation Using Organic Materials and Growth Hormone: A Study on the Effectiveness of Growth and Rooting of Pomegranate Cuttings. *Horticulturae* **2023**, *9*, 999. https://doi.org/10.3390/horticulturae9090999

Academic Editor: Sergio Ruffo Roberto

Received: 10 August 2023
Revised: 31 August 2023
Accepted: 2 September 2023
Published: 4 September 2023

1. Introduction

Pomegranate (*Punica granatum* L.) has been cultivated for thousands of years and is one of the first domesticated fruit crops [1]. Among its native homelands, Afghanistan stands out as a significant origin of this valuable fruit [2]. Recently, pomegranate has achieved significant acclaim due to its delightful taste and potent antioxidant activity [3]. The renowned pomegranate cultivars in Afghanistan are Kandahari and Bedana (seedless). The propagation of these cultivars is traditionally done by collecting cuttings from productive trees. However, there is a lack of a formal and well-documented procedure for this practice [4]. Furthermore, pomegranate cuttings are typically left untreated, which may not

optimize the rooting process or produce healthy saplings. Surprisingly, there have been no prior reports on treating cuttings with organic materials, and only limited studies have explored the use of auxins for this purpose [5].

Rooting compounds play a vital role in facilitating the rooting process of moderate and hard-to-root species. They expedite root initiation, enhance uniformity in rooting, increase the number of roots generated, and effectively minimize shrinkage and rooting time [6]. Indole-3-butyric acid (IBA) stands as the predominant root-promoting compound widely used in the nursery industry [7], a 2 g/L concentration of which results in a higher than 90% rooting success rate [8]. However, a challenge persists for nurserymen and orchardists in developing countries, as they face difficulties in acquiring such hormones from the local market and in determining the optimal concentrations. Hence, there is a need to identify the best alternative to IBA that is readily available and accessible in a local market at any time.

Aloe vera, an organic material, has emerged as a promising natural alternative to synthetic rooting hormones [9]. Aloe vera comprises a multitude of complex ingredients, such as polysaccharides, glycoproteins, phenolic compounds, salicylic acid, lignin, hormones, amino acids, vitamins, and enzymes. These constituents contribute to its numerous beneficial properties, which encompass antibacterial, antifungal, antioxidant, and immune-enhancing effects [10]. This readily available plant substance can be easily accessed and utilized by farmers and orchardists. In addition, garlic is reported to be a good influencer of cucumber root growth as an allelochemical [11]. However, no information is available about the effectiveness of banana on the rooting of pomegranate cuttings.

Panchagavya (a mixture of organic materials) plays a vital role in organic farming practices, serving as the most beneficial organic manure for agricultural fields [12]. By utilizing Panchagavya, farmers can eliminate the need for harmful synthetic fertilizers, pesticides, insecticides, and antibiotics, making it an environmentally friendly and sustainable choice [13]. In addition, it contains various hormones, including gibberellic acid and its derivatives, such as Gibberellin A70, Gibberellin A45, and Gibberellin A61, along with other growth hormones [12,14]. Therefore, this fertilizer was chosen and applied to pomegranate cuttings to assess its effects on growth and rooting performance.

This study represents the initial endeavor to investigate the effects of organic materials, including aloe vera, panchagavya, garlic, and banana, on the rooting and growth of pomegranate cuttings. Additionally, it compares their performance with the synthetic hormone IBA. Our hypothesis suggests that these organic materials, being abundant sources of plant hormones, have the potential to serve as excellent alternatives to hormone application. Moreover, their accessibility and utilization make them particularly suitable for implementation and cost-effective in the context of Afghanistan and developing countries.

2. Materials and Methods

2.1. Plant Materials and Experimental Site

The experiment was conducted in 2023 in a greenhouse (with 50% light transmission capacity) at the Faculty of Agriculture, Nangarhar University, to investigate the effectiveness of various organic materials and IBA on hardwood cutting growth and rooting attributes using the ideal seedless pomegranate cultivar, commonly known as Bedana in Afghanistan.

Cuttings were derived from one-year-old dormant branches in mid-February of 2023 and the experiment started in early March. These hardwood cuttings were initially enveloped in moist paper towels and enclosed within a plastic bag for transport to the experimental site. Upon their arrival, the cuttings were unsealed from their wrapping, subsequently rewrapped using fresh, dampened paper towels, and then stored in a dark refrigerated condition at a temperature of 5–6 °C. This storage condition was maintained until the preparations for the experiment. The cuttings were prepared with a standardized length of 20 cm and a 1.2 ± 0.2 cm diameter. Each cutting was ensured to have a minimum of 2–3 nodes, with one node positioned above ground level at the time of cultivation.

As part of the wounding treatment, incisions were made on all four sides of a cutting's base, approximately 2 cm in length and 2–4 mm in width. Subsequently, these prepared cuttings were inserted about 5–6 cm deep into black plastic bags, measuring 10 × 20 cm, filled with sandy clay loam soil. The experiment was carried out using a completely randomized design (CRD), and each treatment contained ten cuttings (replications). Based on the requirement, the cuttings underwent careful weeding (conducted twice during the experiment) and were regularly hand-watered throughout the duration of the study. Although the irrigation schedule was not consistent due to fluctuating temperatures, measures were taken to prevent the cuttings from experiencing water scarcity.

2.2. Application of Organic Materials and IBA

A total of six treatments, including organic materials (garlic, aloe vera, banana and panchagavya), IBA and control, were employed in the experiment; details are summarized in Table 1.

Table 1. The treatment procedure for cuttings using different organic materials and IBA is shown in this table.

No.	Treatments	Materials and Procedures	Source of Materials
1	Garlic	The basal part of the cutting was treated by rubbing with a sliced garlic clove.	Organic
2	Aloe vera	A piece of aloe vera (3–4 cm or 13–17 g) was vertically sliced, and the basal part of the cutting was covered before being placed into the rooting medium.	Organic
3	Banana	A 3–4 cm (17–20 g) long piece of banana was cut in the same manner as the aloe vera treatment.	Organic
4	Panchagavya	The cuttings were immersed in a 5% solution for 10 min and, during the second and third consecutive weeks, the same 5% solution was applied to the soil. A comprehensive explanation of the preparation technique and constituent elements can be found in Section 2.3.	Organic
5	IBA	A 2% solution of IBA (purity > 98%; Nacalai Tesque, Japan) mixed with talc was prepared by dissolving the hormone powder into 70% ethanol, and the cuttings were quickly dipped in this solution for 10 min.	Chemical
6	Control	Only water treatment.	Water

2.3. Preparation of Panchagavya

The preparation process of panchagavya was similar to that of [15], with certain modifications incorporated. Briefly, in a plastic container, 5 kg of fresh cow dung was combined with 0.5 kg of butter and left for three days. During this period, the mixture was regularly shaken thrice daily for 15–20 min to ensure thorough mixing. After two days, 5 L of cow urine, 2 kg of yogurt, 2 kg of milk, 12 pieces of banana, and one tablespoon of yeast were added to the mixture. Subsequently, the mixture was stirred twice a day for 15 days. In the final step, the mixture was filtered through a clean cloth to extract pure organic panchagavya, free from inert materials. The resulting 1 L of mixture was mixed with 10 L of water. Subsequently, a 5% solution was prepared, using this mixture, to treat the cuttings.

2.4. Measured Attributes

The experiment lasted 3.5 months, after which the rooted cuttings were carefully uprooted to measure their growth and root parameters. After cultivation, as the initial cutting triggered the sprouting process, daily data were collected to measure the cumulative percentage of sprouting. At the end of the experiment, various shoot-related attributes were measured, including the longest shoot length, apical shoot length, number of shoots per cutting (primary shoots), and total shoot length per cutting, as well as fresh and dry weight of shoots per cutting. Additionally, SPAD values were measured using a SPAD-502 chlorophyll meter (Minolta, Japan) to determine the relative chlorophyll content, which

helps to quantify the overall health of the plants. In addition to the parameters above, several root-related parameters were also measured, including rooting percentage, longest root length (cm), number of roots per cutting, number of acrobasal roots per cutting, total root length, fresh weight of roots, and dry biomass of roots. The cuttings' uprooted roots were thoroughly washed to measure root parameters. Subsequently, root diameter and total root length were recorded using a vernier caliper and an EPISON scanner, respectively. The scanned images were then analyzed using ImageJ software to measure the root attributes.

2.5. Statistical Analysis

The data were analyzed using the statistical programming software R. An analysis of variance (ANOVA) was conducted to assess the differences between the means of the treatments. Prior to conducting ANOVA, the percentage data underwent arcsine transformation. Furthermore, a post hoc Tukey's test was performed to determine the significant differences between treatments at a 0.05% significance level. In addition, a correlation matrix analysis was conducted to comprehend the interrelationships among the parameters.

3. Results

3.1. Vegetative Growth

The impact of various organic materials and IBA on the cumulative sprouting percentage and sprouting time exhibited significant variation among the six treatments, as depicted in Figure 1. The use of organic materials (except banana treatment) and IBA influenced the sprouting process. In contrast, banana treatment positively impacted only a limited number of cuttings but generally resulted in a very poor sprouting percentage. Initially, the control treatment did not show any significant effects, and sprouting began after ten days of cultivation. The highest sprouting percentages were observed in the panchagavya (100%) and aloe vera (100%) treatments, while the banana treatment exhibited the lowest sprouting percentage (Figure 1).

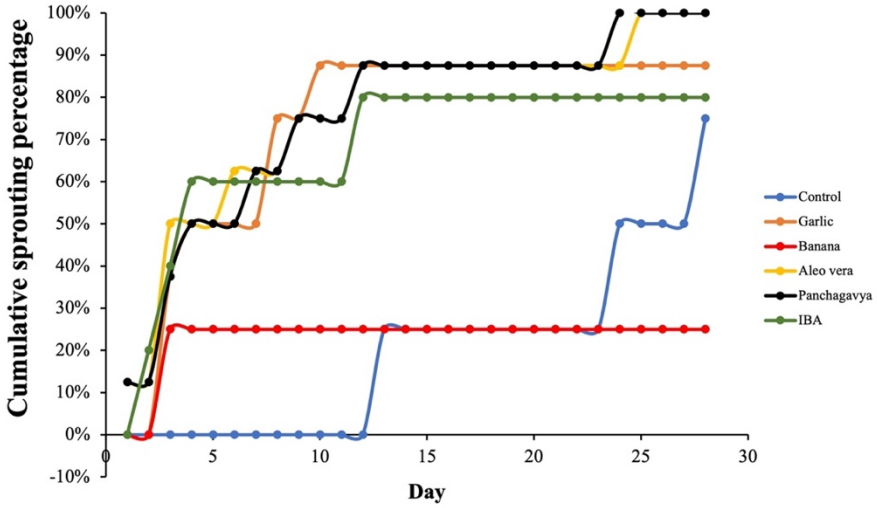

Figure 1. The impact of various organic materials (garlic, banana, aloe vera, and panchagavya) and IBA on the sprouting of Bedana pomegranate cultivar cuttings.

All shoot-related parameters tested here exhibited significant variations ($p < 0.05$) across the different treatments. The sequential application of aloe vera, panchagavya, and IBA notably influenced shoot length, while garlic increased the longest shoot length compared to the control. Conversely, banana had a detrimental effect on shoot length compared to all other treatments. Similar trends were observed for both apical shoot length and total shoot length. Among the treatments, panchagavya (21.1%), IBA (23.4%), and aloe vera (26.8%) showed the highest number of shoots per cutting, representing a

substantial difference compared to the control treatment, while there were no significant differences among control, garlic, banana, and aloe vera. However, garlic (−63.3%) and banana (−50.5%) negatively impacted the number of shoots per cutting compared to the control (Table 2).

Table 2. The influence of different organic materials (garlic, banana, aloe vera, and panchagavya) and IBA on the vegetative growth responses of Bedana pomegranate cultivar hardwood cuttings.

Treatment	Longest Shoot Length (cm)	Apical Shoot Length (cm)	Total Shoot Length per Cutting (cm)	No. of Shoots per Cutting	Fresh Weight of Shoot (g)	Dry Weight of Shoot (g)	SPAD Value (as Reference)
Control	32.66 ± 3.2 b	31.33 ± 3.2 b	104.00 ± 1.3 ab	3.73 ± 1.4 b	5.25 ± 0.04 ab	2.75 ± 1.88 ab	40.43 ± 3.6 bc
Garlic	39.23 ± 1.9 ab	34.03 ± 3.6 ab	83.33 ± 4.5 b	3.33 ± 1.2 b	4.67 ± 0.06 bc	1.95 ± 1.29 ab	45.96 ± 2.7 ab
Banana	16.66 ± 6.1 c	15.66 ± 6.0 c	36.34 ± 5.6 c	2.43 ± 1.5 b	1.52 ± 0.02 c	0.66 ± 0.58 b	33.36 ± 4.1 c
Aloe vera	49.33 ± 2.3 a	46.00 ± 5.2 a	115.66 ± 15.6 a	4.50 ± 1.0 b	7.91 ± 0.20 ab	3.93 ± 0.55 a	53.26 ± 3.4 a
Panchagavya	47.70 ± 4.0 a	43.66 ± 5.5 ab	113.66 ± 4.6 ab	7.33 ± 0.5 a	8.80 ± 0.24 a	4.74 ± 0.89 a	49.23 ± 2.4 ab
IBA	46.00 ± 7.0 a	39.50 ± 3.6 ab	111.25 ± 14.3 ab	5.25 ± 1.1 ab	8.67 ± 0.40 a	3.97 ± 0.89 a	49.86 ± 4.8 ab
p-values	***	***	***	**	***	**	***

The data for each treatment are presented as a mean followed by the corresponding standard deviation. Distinct letters indicate significant differences among the six treatments. The significance level was determined at the 5% level, with ** and *** indicating $p < 0.01$, and $p < 0.001$, respectively.

The application of panchagavya, IBA, and aloe vera in sequence significantly impacted both the fresh and dry weights of the shoot ($p < 0.05$). In Table 2, it can be observed that garlic and banana treatments led to a reduction in the fresh weight of the shoot compared to the control, with percentage decrements of −10.9% and −70.9%, respectively. The dry weight was also affected, with garlic showing a reduction of −29.2% and banana exhibiting the most significant decrease of −75.8% compared to the control.

The chlorophyll content in pomegranate cuttings was significantly influenced ($p < 0.05$) by all organic materials, except banana, and IBA. Among the treatments, the highest chlorophyll content was observed in the aloe vera treatment, followed by IBA and panchagavya. When comparing the sequential differences in SPAD values between aloe vera, IBA, panchagavya, garlic, and banana, it was found that they differed by percentages of 24.0%, 18.9%, 17.8%, 12.0%, and −21.1%, respectively, in comparison to the control treatment.

3.2. Rooting Attributes

Significant variations were observed in the rooting percentage among the different organic materials and IBA treatments. The aloe vera (100%) and panchagavya (100%) treatments exhibited the highest rooting percentages, while the banana treatment had the lowest rooting percentage. Comparing the rooting percentages of garlic, aloe vera, panchagavya, and IBA with the control treatment, differences of 16.6%, 33.3%, 33.3%, and 6.6% were observed, respectively. In contrast, the banana treatment displayed a significantly lower rooting percentage of 66.6% compared to the control treatment (Figure 2).

The root-related attributes of Bedana pomegranate cuttings exhibited significant variations among treatments. IBA treatment displayed the longest root length, followed by aloe vera and panchagavya treatments. Conversely, the garlic and banana treatments showed significantly shorter root lengths compared to the control treatment. Similar results were observed for the number of roots per cutting, with aloe vera, IBA, and panchagavya treatments displaying the longest root length (Table 3). The number of acrobasal roots also varied significantly among treatments, with percentage differences compared to the control as follows: aloe vera (55.5%), IBA (52.3%), panchagavya (50.0%), banana (58.3%), and garlic (91.6%) (Table 3). However, these differences were subdivided into two groups, where no significant differences were observed within each group. The first group comprised aloe vera, panchagavya, and IBA, while the second group consisted of control, garlic, and banana.

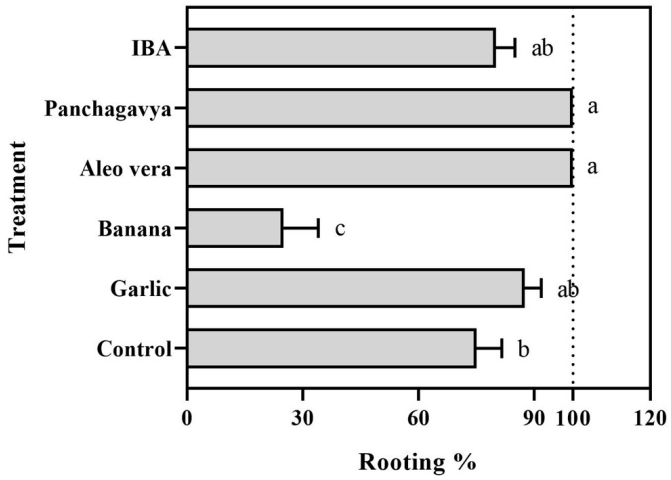

Figure 2. The impact of various organic materials (garlic, banana, aloe vera, and panchagavya) and IBA on the rooting percentage of Bedana pomegranate cultivar cuttings. Distinct letters indicate significant differences among the six treatments.

Table 3. The influence of different organic materials (garlic, banana, aloe vera, and panchagavya) and IBA on the rooting responses of Bedana pomegranate cultivar hardwood cuttings.

Treatment	Longest Root Length (cm)	No. of Roots per Cutting	No. of Acrobasal Roots	Root Diameter (mm)	Total Root Length (cm)	Fresh Weight of Roots (g)	Dry Weight of Roots (mg)
Control	8.00 ± 2.1 bc	10.00 ± 2.0 a	4.22 ± 1.7 b	0.73 ± 0.4 ab	17.66 ± 4.16 ab	0.22 ± 0.04 ab	0.29 ± 0.15 a
Garlic	7.43 ± 0.9 bc	3.66 ± 1.1 b	0.33 ± 0.5 b	0.66 ± 0.2 b	17.35 ± 4.04 ab	0.18 ± 0.06 ab	0.22 ± 0.07 a
Banana	5.33 ± 1.3 c	5.00 ± 1.0 b	1.66 ± 1.5 b	0.43 ± 0.3 b	7.63 ± 2.08 b	0.02 ± 0.02 b	0.23 ± 0.40 a
Aloe vera	13.66 ± 1.5 a	13.66 ± 1.1 a	9.00 ± 2.3 a	1.50 ± 0.2 a	35.33 ± 9.69 ab	0.72 ± 0.20 a	0.43 ± 0.03 a
Panchagavya	11.33 ± 1.7 ab	12.66 ± 1.1 a	8.20 ± 2.0 a	1.50 ± 0.3 a	43.00 ± 10.58 a	0.58 ± 0.24 ab	0.42 ± 0.15 a
IBA	14.66 ± 2.5 a	13.00 ± 2.6 a	8.33 ± 1.5 a	1.10 ± 0.1 ab	39.33 ± 13.86 ab	0.55 ± 0.40 ab	0.59 ± 0.17 a
p-values	***	**	***	**	*	*	ns

The data for each treatment is presented as a mean followed by the corresponding standard deviation. Distinct letters indicate significant differences among the six treatments. The significance level was determined at the 5% level, with *, **, and *** indicating $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. ns: not significant.

Root diameter was significantly influenced by aloe vera, panchagavya, and IBA treatments, while the control treatment had a higher root diameter compared to garlic and banana treatments. Furthermore, there were significant variations among treatments for the total root length (Table 3). The percentage differences in total root length, compared to the control, were 58.9%, 55.0%, 50.0%, −56.6%, and 1.8% for panchagavya, IBA, aloe vera, banana, and garlic, respectively.

Panchagavya, aloe vera, and IBA cuttings displayed significantly ($p < 0.05$) higher fresh weights of roots compared to the control treatment, while garlic and banana treatments exhibited lower values, as presented in Table 3. The percentage variations for panchagavya, aloe vera, and IBA were 68.8%, 61.1%, and 58.7%, respectively. However, the dry weight of roots did not exhibit significant variation among treatments, although IBA, aloe vera, and panchagavya treatments still showed relatively higher values compared to the control treatment.

3.3. Relationship of Vegetative Growth Parameters with Root Attributes

The correlation matrix analysis graph of vegetative parameters with root attributes revealed intriguing findings. The rooting percentage, except for panchagavya and aloe vera treatments, which both displayed 100% rooting, across other treatments ranged between 25% to 87.5%. Consequently, the overall relationship between rooting percentage, vegetative parameters, and root attributes was observed to be negative and weak.

The overall relationship between aboveground and underground parameters exhibited a positive correlation, indicating that well-performing roots were associated with sprouted shoots. Among the shoot parameters, the number of shoots per cutting displayed

a relatively positive relationship with the number of roots per cutting, the number of acrobasal roots, and the total root length. Additionally, root diameter demonstrated a positive correlation with shoot-related parameters (Figure 3). Consequently, this study revealed that, as root diameter increases below ground, the above-ground parameters are significantly influenced.

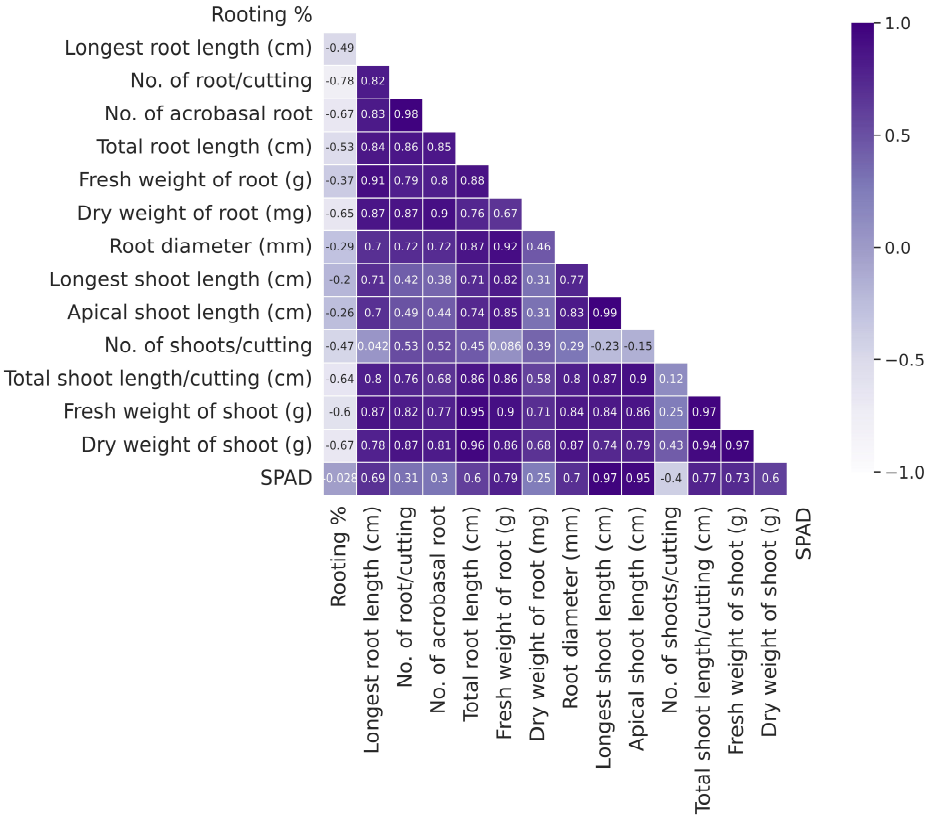

Figure 3. The correlation matrix analysis of vegetative growth parameters with root attributes of Bedana pomegranate cuttings treated with different organic materials and IBA.

The chlorophyll content exhibited a positive correlation with all vegetative parameters and root attributes, except for rooting percentage and the number of shoots per cutting. This indicates that having fewer shoots per cutting and a low rooting percentage in pomegranate cuttings is indicative of a less healthy plant.

4. Discussion

4.1. Sprouting and Vegetative Growth

Significant variations in sprouting percentage and the days to the final sprout of the cuttings were observed among the treatments. All treatments demonstrated a faster sprouting process compared to the control treatment. Notably, the highest sprouting percentages were achieved with panchagavya and aloe vera, followed by garlic and IBA. The banana treatment showed a faster initiation of sprouting than the control, but the sprouting percentage was only 25%, which was lower than the control treatment. IBA exhibited a delayed start to sprouting, but once initiated, the cumulative percentage of sprouting steadily increased, as depicted in Figure 1. Panchagavya has been found to improve growth [16] and increase branching in fruit crops [17], which aligns with our experiment's findings. Additionally, IBA and aloe vera have been shown to influence the sprouting process in pomegranate cuttings and combat fungal rot, facilitating quicker root development and enhancing the success rate of cuttings, respectively [18,19].

In the organic material treatments, panchagavya and aloe vera significantly ($p < 0.05$) promoted the shoot attributes, as depicted in Table 2, with IBA treatment following suit.

However, garlic and banana had a detrimental impact on the growth of cuttings compared to the control. Similar results were observed for SPAD values among the treatments, indicating good plant health. According to [20], panchagavya increased growth, enhanced branching, and the biological efficiency of crops, while aloe vera potentially improved the performance of shoots as well [21]. However, there is no information available on the effects of garlic and banana on the shooting of pomegranates and other crops.

4.2. Rooting Percentage and Attributes

This study marks the first attempt to explore organic materials as alternatives to chemical rooting hormones like IBA. Panchagavya and aloe vera demonstrated remarkable rooting percentages of 100%, even surpassing IBA. However, garlic and banana had negative impacts, achieving only 25% rooting. While IBA was expected to yield higher rooting percentages, panchagavya and aloe vera still outperformed it at 33.3%. Previous research has shown that the application of IBA significantly influences the rooting percentage of pomegranate cuttings [5], while a 2% concentration of IBA is recommended for obtaining a higher rooting percentage [2]. The rooting process of crops can be influenced by aloe vera [19], and panchagavya [22], but no positive effects were found on rooting with the application of garlic [23].

Panchagavya, aloe vera, and IBA treatments significantly increased total root length and longest root length, whereas banana and garlic exhibited lower values than the control treatment. A positive correlation ($r = 0.85$) was observed between total root length and the number of acrobasal roots. Conversely, lower total root length showed a negative correlation ($r = -0.56$) with rooting percentage (Figure 3). Overall, root attributes, including longest root length, number of roots per cutting, number of acrobasal roots, root diameter, total root length, and fresh and dry biomass weight of pomegranate cuttings, were significantly influenced by panchagavya, aloe vera, and IBA treatments, with panchagavya and aloe vera serving as organic alternatives and IBA as a chemical substance (Table 3).

The objective of this study was to identify an optimal alternative method, in lieu of chemical hormones, to enhance rooting and root characteristics in hardwood pomegranate cuttings. Therefore, this study represents the initial endeavor to examine the impact of organic materials on the root attributes of pomegranate cuttings. The outcomes of this experiment demonstrate that organic materials offer a viable alternative, easily accessible to farmers in developing countries. These materials can be effectively employed as root growth promoters for pomegranate cuttings. The future perspective of this study aims to further investigate the impact of organic materials, specifically panchagavya and aloe vera, at varying concentrations and lengths, respectively, on diverse varieties of pomegranate cuttings.

5. Conclusions

The Bedana (seedless) pomegranate cuttings do not easily produce root without treatment with rooting hormones. Organic materials, such as panchagavya and aloe vera, substantially promoted the shoot parameters and root attributes. The highest rooting percentage (100%) was observed with panchagavya and aloe vera, which was higher than that of other organic materials and IBA. This study is the first report to investigate the effectiveness of organic materials on the rooting and vegetative growth of pomegranate cuttings, with a particular focus on the Bedana cultivar.

Author Contributions: Conceptualization, S.A., G.G., Z.S., M.G.R., A.D., T.Z., H.O.E., I.M.M. and K.K.; methodology, S.A., G.G., A.D., K.K., B.M.B. and Y.K.A.; software, S.A., G.G. and K.K.; validation, T.Z., H.O.E., I.M.M., B.M.B. and Z.S.; formal analysis, investigation, S.A., G.G. and T.Z.; resources, Y.K.A., H.O.E., I.M.M., K.K. and M.G.R.; data curation, S.A., G.G., Z.S., K.K. and T.Z.; writing—original draft preparation, S.A., G.G., A.D., K.K. and B.M.B.; writing—review and editing, Y.K.A., K.K., M.G.R., H.O.E., I.M.M. and T.Z.; visualization, supervision, project administration, funding acquisition, S.A., G.G., Y.K.A., H.O.E. and I.M.M. All authors have read and agreed to the published version of the manuscript.

Funding: Researchers Supporting Project Number (RSPD2023R741), King Saud University.

Data Availability Statement: All data are available within this publication.

Acknowledgments: The authors would like to offer their sincere appreciation to Researchers Supporting Project Number (RSPD2023R741), King Saud University, Riyadh, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

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