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RESEARCH ARTICLE

Bioefficacy of Commercial Vesicular Arbuscular Mycorrhizae on Pomegranate growth

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Abstract

Objective: In the present study, the efficacy of a commercial Vesicular Arbuscular Mycorrhizal (VAM) tablet formulation, Greenical VAM^{TM}, comprising species *Rhizophagus fasciculatus* (NCBI Accession no. OR563927) and *Rhizophagus intraradices* (NCBI Accession no. OR563925), was assessed in relation to pomegranate plant growth. **Methods:** A structured field experiment was conducted with two distinct groups: a test set (inoculated with VAM) and a control set (uninoculated). Several key growth parameters, such as leaf area, shoot dry weight, number of branches per plant, and overall nutrient uptake, were systematically evaluated using GraphPad Prism 10. **Findings:** The results indicated that the green VAM^{TM} inoculated plants exhibited significantly improved pomegranate growth compared to the control group. In 2022, the crop treated with Greenical VAM^{TM} displayed a 2.32-fold increase in yield compared to the control. **Novelty:** This research represents the first study to apply the Greenical VAM^{TM} formulation specifically to pomegranate plants. Furthermore, the findings have significant practical implications, suggesting that farmers can achieve enhanced pomegranate yields by utilizing this particular VAM formulation. Notably, each tablet exhibits a remarkably high sporic concentration (50,000 spores per gram) surpassing existing VAM formulations.

Keywords: Field trial; Greenical VAM TM; Plant Growth; Punica granatum L; Vesicular Arbuscular Mycorrhiza (VAM)

1 Introduction

Pomegranate (*Punica granatum L.*) is a commercially significant fruit plant species associated with the Punicaceae family. It is among the most ancient edible fruits, cultivated in sub-tropical, arid, and semi-arid regions across the globe [(1)]. Pomegranate, often referred to as a ”superfruit” in the global food industry, is rich in proteins, carbohydrates, minerals, antioxidants, and vitamins A, B, and C [(2)]. It has been employed in traditional medicine for treating conditions like fever, hyperacidity, leprosy, diarrhea, tuberculosis, and abdominal pain. It displays remarkable resilience, withstanding harsh climates, heat, drought, and moisture deficits. In Ayurveda, it is known as ’Dadima’ due to its versatile therapeutic applications [(3)]. India is the world’s largest pomegranate producer, with 2.22 lakh hectares of cultivation, yielding an annual production of 26.34

lakh tonnes at a rate of 11.86 tonnes per hectare, surprisingly exports only 1.79% of its total production [4]. This is due to the challenges like fruit sunburn, cracking, and infestations by the pomegranate fruit moth, which greatly affect fruit quality and market appeal [5].

Biofertilizers have gained momentum in recent years as an alternative to chemical fertilizers, primarily due to their lower cost and reduced environmental hazards [6]. This shift aims to boost plant growth and increase crop yields sustainably. When applied to various fruit crops, many forms of biofertilizer have proven to be effective [7]. Biofertilizers such as arbuscular mycorrhizae, composts, animal manure, and humus have demonstrated favorable effects on the vegetative growth and fruit yield of fruit trees [8,9]. However, mycorrhizae application has notably outperformed other biofertilizers in terms of promoting plant growth. Mycorrhizae play crucial roles in benefiting crops by facilitating enhanced nutrient uptake, promoting advantageous root formation, and contributing to disease resistance [10].

Numerous studies have shown that various beneficial soil microorganisms are prevalent in soils and naturally form associations with many fruit plants [11]. Therefore, the present study aimed to investigate the effectiveness of commercial Greenical VAM^{TM} on pomegranate, with the results being compared to a control group (uninoculated).

2 Methodology

2.1 Experimental design

Mycorrhizal inoculum Greenical VAM^{TM} with Rhizophagus fasciculatus and Rhizophagus intraradices (NCBI accession numbers OR563927 and OR563925) was used as AMF. The one treated with Greenical VAM^{TM} served as the test, while the one without any inoculation served as the control. The design was entirely randomized and duplicated three times.

2.2 Field experiment

A field experiment in the years 2021 and 2022 was conducted between June and September. 48 trees (7 years old) were arranged in a randomized complete block design (RCBD), with each of the eight treatments evaluated on six sets (one replication equals one tree). The test set was inoculated with 5 g/acre of Greenical VAM^{TM} in tablet formulation, which is comprised of 50,000 spores/g, whereas the control set was uninoculated. However, the control trees received the same conditions as the test trees. The inoculation of the fungi was achieved through the sub-soil placement method. Subsoil placement was achieved at a depth of 30 centimeters beneath the tree canopy and a radius of 50 centimeters out from the trunk. Trees were watered every four days using four dedicated drippers per tree.

2.3 Soil Sampling and Observation

Plant debris was removed meticulously before soil samples were taken via both center rows within every plot. The soil was sieved through a 2-millimeter mesh and subsequently dried in an oven. The chemical properties of soil, encompassing vital nutrients like total phosphorous (P), nitrogen (N), potassium (K), and available P, were precisely analyzed.

Root samples of ten plants from each treatment were sampled, and dirt was rigorously cleaned before being kept in 50% ethanol. The roots were rinsed in running water, then cut into 1 cm pieces before being cleaned with 10% KOH at 90°C for 1 hour. The percentage of root colonization by arbuscular mycorrhizal fungi (AMF) was assessed by mounting stained root slices onto slides and studying them under a light microscope.

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2.4 Plant Productivity

Plant productivity in terms of number of branches per tree, shoot dry weight, number of fruits per tree, and total yield in kg was evaluated. The overall nutrient uptake by the plant was evaluated in terms of total nitrogen (N), potassium (K), and phosphorous (P) available in the soil.

2.5 Statistical analysis

The data was statistically signified by Turkey's honestly significant difference test and Analysis of Variance (ANOVA) by GraphPad Prism 10.

3 Results and Discussion

3.1 Root Colonization and Spore Density

In a comparative study conducted over two consecutive years (2021 and 2022), it was observed that the spore density in test plots was consistently and significantly higher than that in control plots. In 2021, the control plot registered a spore density of 97 spores per gram of soil, while the test plot showcased a considerably higher count, with 368 spores per gram. The ensuing year, 2022, witnessed a progression in the numbers. The control plot experienced a rise to 112 spores per gram, whereas the test plot, maintaining its trend of heightened spore density, reached 399 spores per gram, as displayed in Figure 1. This is in line with the study, which demonstrated that certain soil treatments, especially those rich in organic matter, can promote higher mycorrhizal spore density[12]. Increased spore density is often correlated with greater root colonization, enhancing the plant's ability to absorb nutrients. Similar observations were established in the study, where there was a strong correlation between spore density and root colonization, suggesting improved nutrient and water uptake in plants with higher colonization[13].

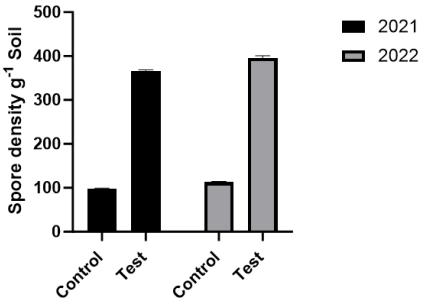

Fig 1. Number of AMF spores in pomegranate plot for two consecutive years

Similarly, marked differences were noted in root colonization between the control and test plots. While the control exhibited an increase in colonization, the test plot consistently achieved full colonization, as shown in Figure 2.

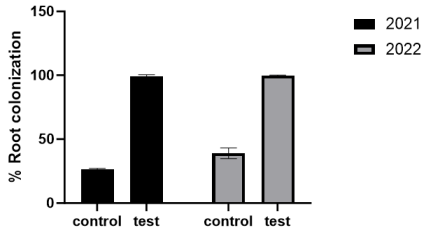

Fig 2. Percentage root colonization by AMF for two consecutive years

3.2 Soil Properties

Table 1 displays the concentrations of nitrogen (N), phosphorus (P), and potassium (K) in control soil and soil treated with mycorrhizae (Greenical VAM^{TM}) inoculum. The treated soil exhibited markedly higher values for N (447 mg Kg^{-1}) and P (293 mg Kg^{-1}) compared to the control.

Table 1. Effect ofGreenical VAM^{TM} on rhizosphere chemical properties of Pomegranate. Values represent mean ± SD (n = 3)

	Total N (mg Kg^{-1})	Total P (mg Kg^{-1})	Total K (mg Kg^{-1})
Control	152±6	67±9	301±15
Test	447±57	293±71	501±18

3.3 Effect of Greenical VAMTM on Plant growth and nutrient uptake

Significant variations were found between the control and test during the course of two-year research conducted in 2021 and 2022. In every measure, including branching, leaf area, shoot dry weight, fruit productivity, and yield, the experimental plants much surpassed the controls. The test plants had 17 and 22 branches in 2021 and 2022, whereas the controls only had 5 and 9. The number of fruits produced by control plants increased to 83, whereas the number produced by test plants increased remarkably from 108 to 136. The production of the control plants grew marginally, from 14 to 22, whereas the yield of the test plants ascended substantially, from 44 to 57. As displayed in Figures 3, 4, 5, 6 and 7. A study on wheat plants observed similar results, attributing enhanced growth to improved nutrient absorption capacities, potentially due to higher root colonization and symbiotic relationships with beneficial soil microbes. The expansive leaf area, as seen in the test group, can also indicate a plant's enhanced photosynthetic capacity, leading to better overall growth [14]. Such productivity boosts can be attributed to the cumulative effects of all the observed growth benefits. In parallel, a study on strawberries correlated increased fruit count and yield with superior nutrient uptake and healthier root systems [15].

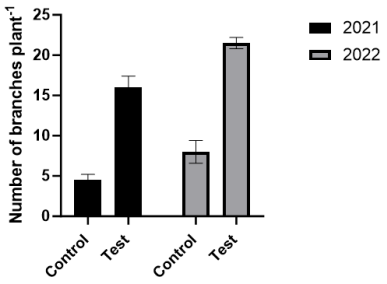

Fig 3. Effect of Greenical VAM^{TM} on the numberof branches

Fig 4. Effect of Greenical VAM^{TM} on leaf area for two consecutive years

Table 2 illustrates the nutrient uptake by pomegranate plants over two years. Notably, in 2022, the nitrogen uptake for the test group was significantly higher at 47 ± 0.16 compared to the control having 21.2 ± 0.48. Similarly, potassium uptake in the test group dramatically increased to 27.9 ± 0.08, while the control only registered 2.12 ± 0.23. These prominent disparities highlight the test group's enhanced ability to absorb essential nutrients compared to the control group. Improved nutrient uptake is often a cascading benefit of enhanced root colonization, as mycorrhizal associations are known to assist plants in nutrient absorption, especially phosphorus. A similar result was documented in a study that emphasized the crucial role mycorrhizae play in bolstering nutrient uptake, especially in soils deficient in essential elements [16].

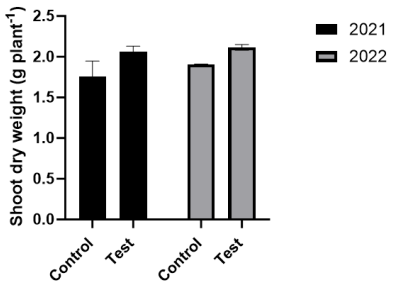

Fig 5. Effect of Greenical VAM^{TM} on Shoot dry weight for two consecutive years

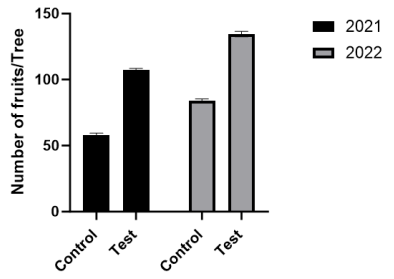

Fig 6. Effect of Greenical VAM^{TM} on Pomegranate fruit productivity for two consecutive years

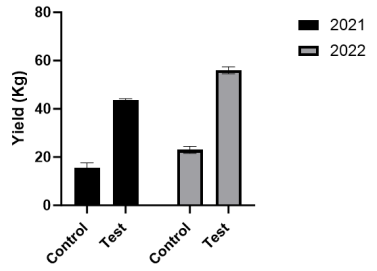

Fig 7. Effect of Greenical VAM^{TM} on Pomegranate yield for two consecutive years

Table 2. Effect ofGreenical VAM^{TM} on plant nutrient uptake

	N		P		K	
	2021	**2022**	**2021**	**2022**	**2021**	**2022**
Control	19 ± 0.42	21.2 ± 0.48	2.9 ± 0.33	3.7 ± 0.09	1.39 ± 0.18	2.12 ± 0.23
Test	31 ± 0.22	47 ± 0.16	7.9 ± 0.46	8.2 ± 0.31	22.9 ± 0.05	27.9 ± 0.08

4 Conclusion

Greenical VAM^{TM} is a unique tablet formulation of Vesicular Arbuscular Mycorrhizal (VAM) that has been shown to greatly improve pomegranate plant growth and yield when applied to the soil. In terms of improving soil health, nitrogen management, and root colonization, its high spore concentration of 50,000 spores per gram is unparalleled. The wide range of benefits shown in pomegranates implies that Greenical VAMTM has great potential among many plant species, providing a versatile and novel approach to enhancing crop yield and overall plant health in a variety of ecological environments.

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