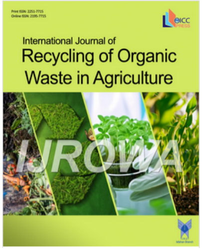

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Exploring the synergistic effect of rose distillation waste and biostimulant beside other organic amendments on *Rosa damascena* seedlings' growth

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Original Research

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Abstract:

Purpose: Floral waste, as a frequently overlooked resource for sustainable agriculture, can be harnessed to produce nutrient-rich soil amendments resulting in an enhancement of soil fertility and augment crop yields. This study was aimed to explore the potential of rose distillation waste and other organic amendments as sustain-able alternatives to traditional synthetic chemicals, particularly in the context of rose production.
Method: The data was collected experimentally in a controlled greenhouse condition. A complete randomized block design with three replicates was applied to conduct the experimental units. The study investigated the combined effects of three organic amendments: goat manure rose distillation waste, and compost, as well as a bio-stimulant containing both Mycorrhizae and Plant Growth Promoting Rhizobacteria.
Results: Rose distillation waste proved to be a promising organic amendment with a significant positive effect on the growth and development of seedlings. Rose distillation waste substrate showed a remarkable increase in terms of leaf area and a maximum apical internode growth of 1.5 mm. The incorporation of bio-stimulant in the rose distillation waste substrate resulted in significant stem height growth of 11.3 cm and a high bud break rate of 73.3%.
Conclusion: The findings of the study offer valuable insights into the sustainable management of rose production. The use of rose distillation waste as an organic amendment not only enhances plant growth but also positively impacts nutrient content in leaves. This highlights the potential of rose distillation waste as a sustainable and easily available resource for use in organic agriculture.

Keywords: Biostimulant; Organic amendment; *Rosa damascena* Mill.; Rose distillation waste; Sustainable agriculture

1. Introduction

The damask rose (*Rosa damascena* Mill.) is an aromatic plant with a major horticultural and possess a significant economic importance (Changizi and Madani, 2020; Hussen and Wulchafo, 2023). It is a hybrid plant derived from crossing *Rosa gallica* and *Rosa phoenicia*. The derived products from this plant are applied in many disciplines, including perfumery, which is highly prized by the fragrance industry for its aromas (Sharmeen et al., 2021). Not only in this case, it is also used in the pharmaceutical, food, agricultural, ornamental and even animal feed industries (Thakur and Kumar, 2021). *Rosa damascena* is produced mainly in Bulgaria, Turkey, Morocco, Iran, and India (Thakur and Kumar, 2021). In Morocco, perfume rose cultivation is mainly localized in Dades Valley (950 ha in 2019). The yield of rose reached 3,900 tons per hectare, and exports reached 9.2 million Dirhams (MDH) (Malahi et al., 2023).

Rosa damascena is defined as one of the most recalcitrant plants. Climatic and edaphic factors influence its production (Hamedi et al., 2022a, 2022b). Otherwise, many abiotic factors, such as light, temperature, drought, and salinity, influence soil properties and, consequently, affect the physiological and morphological behavior, as well as the growth of plants (**vt9**; Araus et al., 2008). Therefore, pre-harvest factors such as soil or substrate fertility, nutrient availability, cultural practices, and harvesting techniques should be taken into account to develop new promising technologies for roses production, especially during the acclimation process in the greenhouse (Danyaie et al., 2011; Kumar et al., 2013). Hence, substrate fertility is an important edaphic factor, influencing plant growth and root development.

On the other hand, globalized agriculture aims not only to eradicate or reduce the use of chemical fertilizers and replace them with organic fertilizers but also to apply new eco-friendly fertilizer products as well, which is widely considered to target a fully organic production (Zim et al., 2022; Thakur and Kumar, 2021).

Although the availability of a multitude of agricultural resources applied to enhance substrate nutrient uptake and promote plant growth, arbuscular mycorrhizal fungi (AMF) remain one of the potentially and widely used biofertilizers. Mycorrhizae are considered potential amendments to substrates to increase plant yields and enhance crop resilience to adverse environmental conditions in agricultural and forestry programs (Johansson et al., 2004). Studies on plant-AMF associations have shown that over 70% of vascular plant species can establish a mutually beneficial symbiotic association for both the host plant and the fungus (Cosme et al., 2018).

The efficiency of AMF has been proved when they are used as an additive or in association with organic amendments, such as compost, manure, or other residues (Ou-Zine et al., 2022). These amendments provide the necessary substantial amount of nitrogen (N) and carbon (C) which are essential nutrients for AMF growth and activity (Arif et al., 2016). By adding organic amendments, AMF diversity and abundance in soil are increased, which in turn promotes their colonization on plant roots and improves plant performance (Maji et al., 2017). Additionally, specific organic amendments like biochar or vermicompost provide stable nutrient sources, increasing soil porosity, and water holding capacity, further promoting AMF activity (Ullah et al., 2021). Studies have shown that using AMF with organic amendments could enhance plant biomass, nutrient uptake, and yield while reducing synthetic fertilizer use (Liu et al., 2019). For instance, a study on soybean production revealed that the combination of AMF and compost increased plant biomass and N uptake while decreasing synthetic N fertilizer use by up to 50% (Kumar et al., 2022). In brief, using AMF with organic amendments promotes sustainable agriculture practices by improving plant growth and yield while reducing the use of synthetic fertilizers.

Currently, rose distillation waste from distillation factories is often discarded—either left in fields or stored near the factories—resulting in environmental burdens and missed resource utilization opportunities. However, our research introduces an innovative solution: utilizing this waste in Damask rose organic production. This approach aims to transform the waste into a valuable resource for farmers, promoting sustainable practices. This eco-friendly application not only reduces environmental pollution but also provides an economically viable option for small rose producers with limited access to manure and expensive organic fertilizers. Moreover, incorporating floral waste-amended substrate along with bio-stimulant practices offers an attractive alternative for farmers transitioning to organic production systems. These efforts contribute significantly to sustainable agriculture and foster a more circular economy within the rose industry.

Floral waste may be an untapped yet significant source of organic matter for substrate amendment, with the potential to offer sustainable nutrients for plant growth (Yadav et al., 2018; Boutasknit et al., 2020). Despite the positive contribution of previous studies on the use of floral waste for plant development, research is still scarce in this area. By exploring the potential of amendments based on flower waste for rose seedlings' growth, our study aims to shed light on the combining effect of substrates and biological activity on the absorption of nutrients and growth of small seedlings of *Rosa damascena* in the nursery. This research is crucial in designing biologically integrated agroecosystems and maintaining an economic production system, thereby contributing to the valorization of natural waste.

2. Material and methods

Experimental site

The study was conducted in an experimental greenhouse located at Hassan II Agronomic and Veterinary Institute (IAV), Agadir Horticultural Complex, Morocco (30°35′31″N, -9°47′71″E). The dimensions of the greenhouse are 10 meters (m) in length and 8 m in width. It is equipped with wire greenhouse benches, which provide efficient drainage. Climatic parameters inside the greenhouse, including temperature, relative humidity, and global radiation during the study period are (Max T = 40° C, Min T = 19° C, RH ∼ 70%, and Sunlight = 209.103 Lux).

Experimental design

In order to investigate the effect of different amendments of substrate on *Rosa damascena* growth and development, 10-month-old seedlings of *Rosa damascena*, from a nursery located in Kelâat M'Gouna, (31°14′10.2″N 6°07′57.5″W) were the subject of this study. The seedlings were raised from cuttings of four-year-old plants of *Rosa damascena*. The irrigation of seedlings was carried out by sprinkling substrates at a regular rate of 2 hours/day.

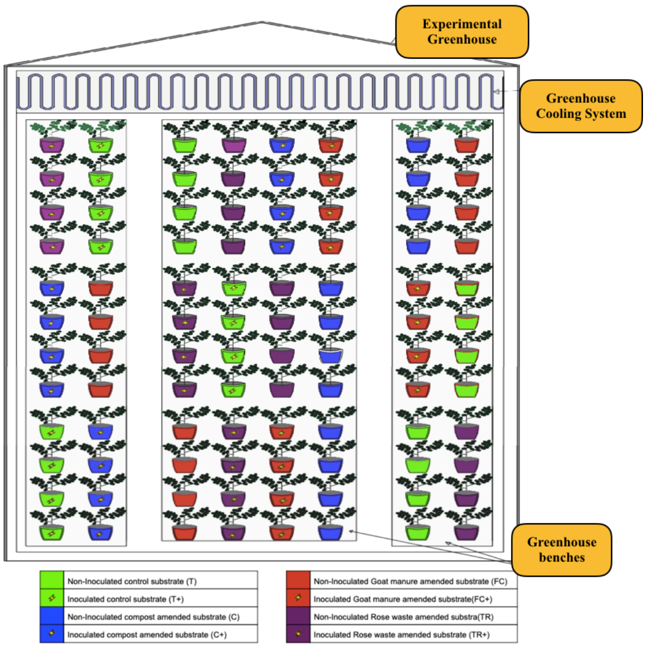

Figure 1. Experimental design used in the study.

Afterward, seedlings were transplanted into polyethylene pots of 3 liters of volume. The culture substrate was a mixture of soil from Kelâat M'Gouna, sand, and perlite at a volume of (2: 2: 1). The substrate was sandy silty (63.8% sand, 23.2% silt, and 13.1% clay) in texture with basic pH (8), low in organic matter (0.9%) and in available nitrogen (N) (198.7 ppm); while the available superphosphates P_2O_5 and available potassium oxide (K_2O) were respectively 118.2 ppm and 240 ppm. The experimental units were grouped in a complete randomized block design (RBD) with three replicates following the gradient of heterogeneity under the greenhouse cooling system conditions. The experiment consisted of comparing the combining effect of three organic amendments, namely: goat manure (FC), rose distillation waste (TR), and compost (C), and a bio-stimulant based on both Mycorrhizae and Plant Growth Promoting Rhizobacteria (PGPR) provided by a commercial product (Actyv plus®). Green Solution inc.). The commercial product was applied by dissolving a quantity of it in a volume of water, respecting the 0.5% dose. The organic amendment was added to the substrate before transplanting seedlings at a volume of (1:5). The inoculum was applied at 100 cm^3/plant after 5 days from the day of transplantation. Each amendment consisted of 12 pots with one plant in each pot. (Fig. 1) below graphically illustrates the experimental protocol used in this study. Each data point in the experimental protocol graph represents 8 individual seedlings, with each one grown in a separate pot.

Substrate analysis and its nutrient content

The mixtures were prepared using four substrates: soil of Kelâat M'Gouna, sand, perlite, and the main organic biological amendment at a volume of (2:2:1:1). Organic matter content was examined by the Walkley and Black method, available (N) of samples was analyzed according to the method of Kjeldhal. Available P_2O_5 was examined by the method of Olsen and available K_2O was analyzed by the method of Mehlich. The measurement of magnesium and

Table 1. Chemical properties of different analyzed substrates.

Parameters	T	FC	TR	C
pH	$8.0^a \pm 0.03$	$7.9^a \pm 0.06$	$7.5^b \pm 0.03$	$7.9^a \pm 0.04$
MO (%)	$0.9^b \pm 0.40$	$2.0^a \pm 0.07$	$1.2^{ab} \pm 0.03$	$2.1^a \pm 0.67$
N (ppm)	$198.7^d \pm 7.8$	$489.7^b \pm 22.09$	$228.3^c \pm 16.80$	$289.7^{bc} \pm 11.60$
P_2O_5 (ppm)	$118.2^c \pm 13.93$	$302.8^a \pm 16.08$	$144.5^b \pm 39.40$	$71.6^d \pm 4.00$
K_2O (ppm)	$240.0^b \pm 16.59$	$2019.4^a \pm 78.00$	$358.5^b \pm 57.50$	$357.5^b \pm 6.27$
EC (mS/cm)	$3.6^b \pm 0.34$	$7.7^a \pm 0.22$	$3.5^b \pm 0.92$	$3.3^b \pm 0.19$
Ca (méq/100 g)	$43.8^a \pm 1.60$	$45.7^a \pm 6.37$	$45.2^a \pm 3.75$	$47.8^a \pm 0.76$
Mg (méq/100 g)	$41.3^b \pm 2.57$	$37.5^c \pm 0.5$	$38.5^{bc} \pm 0.50$	$42.7^a \pm 0.29$
Na (méq/100 g)	$0.91^b \pm 0.07$	$0.89^b \pm 0.26$	$0.92^b \pm 0.17$	$2.4^a \pm 0.16$
CEC (méq/100 g)	$8.70^a \pm 0.65$	$9.35^a \pm 0.22$	$8.63^a \pm 0.56$	$8.4^a \pm 0.74$

(T: control, Fc: goat manure, TR: rose distillation waste, and C: Compost). Means followed by the same letter are not statistically different at $p < 5\%$ according to the Tukey test.

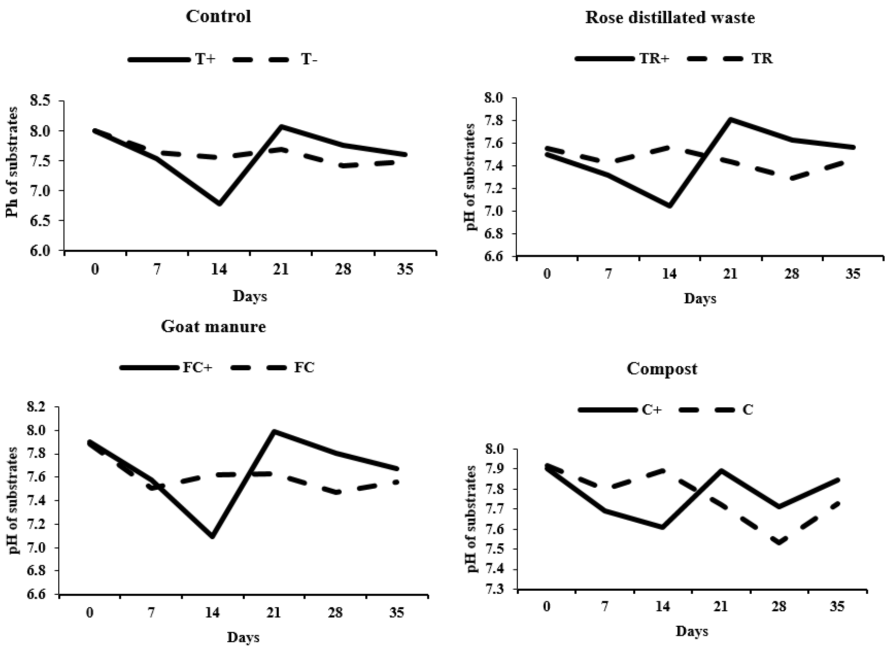

Figure 2. Evolution of the pH of the substrates during the experimentation period.
TR: Non-inoculated rose distillation waste amended substrate; TR+: Inoculated rose distillation waste amended substrate, FC: Non-inoculated goat manure amended substrate; FC+: Inoculated goat manure amended substrate, T: Non-inoculated control substrate; T+: Inoculated control substrate, C: Non-inoculated compost amended substrate; C+: Inoculated compost amended substrate.

calcium was carried out by atomic absorption spectrometry. The cation exchange capacity was calculated using the exchangeable basis method. Soil texture and pH of the soil were estimated by the method of hydrometer and pH meter of the glass electrode. Electrical conductivity was measured by the saturated paste method.

Irrigation monitoring and climatic conditions

The monitoring of temperature and relative humidity, inside the greenhouse, was performed daily using a data logger (TZone-TempU03®) while the solar radiation is done by a luxmeter (Lutron LX-105®).

Measurement of electrical conductivity and pH

The salinity and pH of draining were measured using the EC-meter (HANNA® Instruments) and pH-meter (Jenway TM). Regarding the electrical conductivity and the pH of substrates, measurements were carried out every seven days. A quantity of 10 g of substrate was sufficient to measure the electrical conductivity by the 1/5 method (Slavich and Petterson, 1993).

Water holding capacity of substrates

The water retention capacity was assessed by measuring the draining volume. The presentation of the drain-age of each treatment was carried out by choosing two individuals at random, which is worth 20 volumes to be measured in total. The draining solution was collected by placing small basins under each treatment. Then, the measurement of the draining volume was done using a test tube in the laboratory. This volume directly indicated the water retention capacity of each substrate.

Assessment of growth attributes

Plant growth was monitored by measuring stem height, bud-break rate, growth of apical internodes and leaf area. The stem height and apical internodes were measured with a tape meter. The bud-break rate was monitored based on the calculation of the percentage of bud breaks on the totality of existing ones. The monitoring of this vegetative progress is done periodically with an interval of 7 days. The measurement of the value of the leaves surface was carried out using the 'Mesurim' software.

Foliar analysis

To confirm the seedlings' absorption of available elements supplied by various amendments, foliar analysis was performed. Mineral elements of leaves were analyzed, after calcination at 600° C and were extracted with hydrogen chloride. The abundance of nutrient elements in different substrates was subject to developing a graded heat map for nutrient abundance classification.

Statistical analysis

All collected data on growth parameters of *Rosa damascena* were subjected to standard statistical analysis of

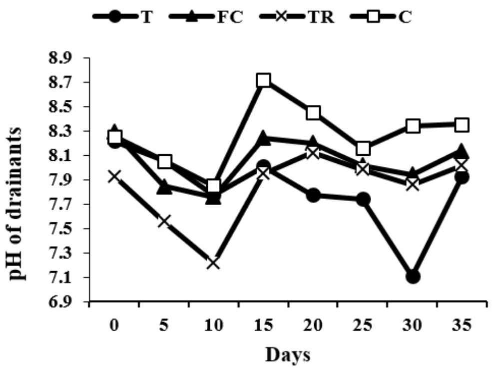

Figure 3. Evolution of the pH of the drains during the study period.
TR: Rose distillation waste, FC: Goat manure, T: Control, C: compost.

variance (ANOVA). ANOVA was performed using Minitab statistics to explore the relationship between growth parameters and different treatments studied to validate the correlation between substrate amendment inoculated with Mycorrhizae and plant growth and development. The confidence intervals were set at 95% and p values were used as statistical significance where $p \leq 0.05$ means significant difference and $p \leq 0.01$ means highly significant difference between two different treatments. The tests that were used to compare the means between the different treatments studied at a significance level of $p \leq 0.05$ are the Tukey test and the Gupta test (parametric tests).

3. Results and discussion

Effect of organic amendments on physicochemicals properties of substrates

The statistical analysis showed that organic amendments have a highly significant effect ($p < 0.001$) on the physicochemical parameters of the substrate as mentioned in (Table 1). The pH values of the substrates were observed to vary between 7.5 and 8.0, indicating a range from slightly alkaline to nearly neutral. The substrate amended with compost (C) and goat manure (FC), as well as the control substrate (T), showed moderately alkaline pH values, while the substrate amended by rose distillation waste (TR) had a slightly alkaline pH. High levels of mineral elements were detected in all substrates analyzed, and salinity significantly differed among them ($p < 0.001$). The FC substrate had the highest mineral salt content. The assimilable phosphorus content showed a significant difference ($p < 0.001$) among substrates, with FC exhibiting the highest concentration, and the remaining three substrates displaying statistically homogeneous concentrations. Similarly, the content of potassium varied highly significantly ($p < 0.001$), with FC having higher potassium content compared to other substrates. Calcium content was found to be homogeneous among all substrates analyzed ($p > 0.05$), while magnesium content varied significantly ($p < 0.01$), with substrates being rich in this element. The organic matter content showed a significant difference ($p < 0.01$) among the

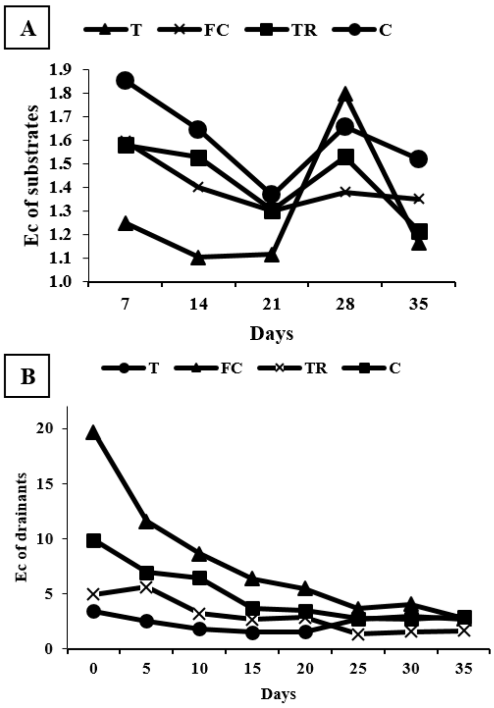

Figure 4. Electrical conductivity evolution of substrates and drained solutions during the study period.
(A) Electrical conductivity evolution of different substrates; (B) Electrical conductivity evolution of drained solutions.
TR: Rose distillation waste, FC: Goat manure, T: Control, C: compost.

different treated substrates, with C having the highest percentage of organic carbon. Sodium content also differed highly significantly ($p < 0.001$) among substrate mixes, with the highest concentrations found in C, while TR, T, and FC had relatively low values. Notably, the cation exchange capacity (CEC) was found to be homogeneous ($p > 0.05$) among the substrates studied. These findings suggest that the substrates analyzed offer a diverse range of mineral and nutrient compositions, which could be further studied and applied in various fields.

The results indicated that the pH values of the substrates were within the range of 7.5 to 8.0, which was slightly alkaline, a condition preferred by *Rosa damascena* (Baser and Arslan, 2014). Specifically, the FC substrate exhibited the highest concentration of assimilable phosphorus, an essential nutrient for plant growth and development (Paz-Ares et al., 2021). The potassium content in FC was also higher compared to other substrates, which can potentially benefit the growth and development of *Rosa damascena* (Karlik et al., 2003). Furthermore, the higher magnesium content in some substrates can potentially benefit the growth and development of *Rosa damascena*, as magnesium was an essential nutrient involved in various physiological processes. The organic matter content in the C substrate was found to be the highest, which could improve soil health and benefit plant growth by enhancing soil structure, water-holding capacity, and nutrient availability. However, high sodium levels, which observed in the C substrate, could negatively impact plant

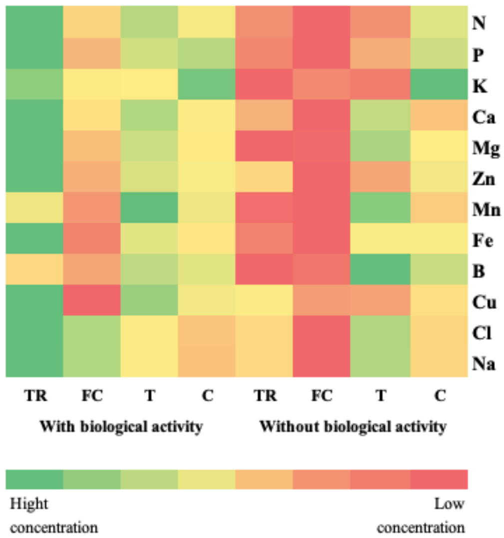

Figure 5. Heat map representing dynamics of the nutrients as influenced by different treatments tested.
The different colors from the left to the right end of the heat map legend showed, a decrease of mineral compounds abundance. TR: Rose distillation waste, FC: Goat manure, T: Control, C: Compost.

growth (Ait-El-Mokhtar et al., 2021). The lower sodium content in some substrates, such as TR, T, and FC, might potentially be beneficial for *Rosa damascena* growth. Our results demonstrated that the cation exchange capacity (CEC) was homogeneous among the substrates studied, which indicated good soil fertility and nutrient availability. Although the goat manure-amended substrate exhibits significantly higher mineral element levels, the positive impact of the rose distillation waste-amended substrate should not be underestimated. While the high mineral content in the goat manure-amended substrate might lead to excessive salinity, making it less suitable for *Rosa damascena*, both substrates provide essential nutrients crucial for plant growth. In this regard, the rose distillation waste-amended substrate emerges as a promising choice, offering a well-balanced supply of vital elements without the risk of excessive salinity, thus promoting the successful cultivation of *Rosa damascena*.
Investigated on pH level in substrates revealed that during the first week of the study, pH slightly decreased in substrates amended with goat manure and rose distillation waste with no mycorrhizae and rhizobacteria incorporated as well as for non-inoculated control. Whereas, pH analysis of substrates inoculated with mycorrhizae demonstrated a clear decrease of pH after one week of application, then showed an abrupt increase during the 3rd week of the study. Of note, the curves illustrated in (Fig. 2) resulted in the measurement of the pH of mycorrhizae substrates, in general. a sinusoidal evolution during the experiment. The compost with mycorrhizae inoculum had a slightly different pH evolution compared to other substrates (Fig. 2).
Fig. 3 represents the graphic of the pH evolution of water drainage during the study period.
The pH of drainage water evolved in the same way as the pH of the substrate. However, the pH level of drainage did not differ neither in the inoculated nor in non-inoculated substrates.
Fig. 4 represents graphically the evolution of EC of water drainage during the study period. Concerning the EC, similar conductivity values evolution has been found in all EC analyses of different substrates inoculated during the whole period of the experiment (Fig. 4A). No effect of substrate inoculated with mycorrhizae was detected on the EC. Our results in Fig. 4A indicate that EC decreases during the first 21 days, goes up the fourth week then subsequently decreased again at the end of the study. Concerning the EC of water drainage decreased with time. All drainage of different substrates decreased to drop almost the same value towards the end of the experiment. Fig. 4B represents graphically the evolution of EC of water drainage during the study period.

Table 2. Volume of water drained per day for each substrate.

	TR	FC	T	C
The volume of drained water in (mL/d)	$36.75^c \pm 7.38$	$47.92^b \pm 6.92$	$69.03^a \pm 5.86$	$52.89^b \pm 5.91$

TR: rose distillation waste, FC: Goat manure, T: Control, C: compost, Means followed by the same letter are not statistically different at $p < 5\%$ according to the Tukey test.

Table 3. Effect of substrates amended with different organic amendments inoculated and no-inoculated with biostimulant-based mycorrhizae and PGPR on growth traits of *Rosa damascena* seedlings.

	Inoculated				Non-inoculated			
	T	FC	TR	C	T	FC	TR	C
Stem growth in (cm)	$9.48^{a} \pm 1.54$	$6.94^{b} \pm 2.50$	$11.3^{a} \pm 2.71$	$6.38^{b} \pm 1.36$	$2.26^{c} \pm 0.85$	$1.00^{cd} \pm 0.62$	$2.29^{c} \pm 1.40$	$6.99^{b} \pm 1.56$
Bud break rate in (%)	$63.3^{a} \pm 23.9$	$44.2^{bc} \pm 17.3$	$73.3^{a} \pm 18.7$	$70.0^{a} \pm 13.5$	$43.3^{c} \pm 15.0$	$30.8^{c} \pm 17.3$	$27.5^{c} \pm 11.4$	$62.5^{ab} \pm 8.7$
Growth of apical internodes (cm)	$0.60^{c} \pm 0.32$	$1.33^{a} \pm 0.69$	$1.50^{a} \pm 0.30$	$0.80^{bc} \pm 0.40$	$0.55^{c} \pm 0.21$	$0.69^{bc} \pm 0.21$	$1.06^{ab} \pm 0.54$	$1.10^{ab} \pm 0.40$
Leaf area (cm^2)	$131.74^{a} \pm 41.75$	$67.32^{bc} \pm 14.52$	$135.42^{a} \pm 44.14$	$93.14^{b} \pm 12.72$	$74.05^{bc} \pm 32.77$	$41.91^{c} \pm 19.61$	$45.46^{c} \pm 11.42$	$86.79^{b} \pm 33{,}47$

(T: Control, FC: Substrate amended with goat manure, TR: substrate amended with rose distillation waste and C: substrate amended with compost). Means followed by the same letter are not statistically different at $p < 5\%$ according to the Tukey test.

The application and role of nutrients in organic fertilization is an issue that concerns several agronomists and researchers working on different crops. In case of *Rosa damascena* Mill. studies are limited. In this first-ever experiment of both amendments with different substrates including rose distillation waste and inoculated with mycorrhizae and (PGPR) in potting *Rosa damascena*, Organic substrate amendment inoculated with mycorrhizae and PGPR impact on *Rosa damascena* growth and development were assessed in the current experiment. Thus, substrate colonization by mycorrhizae not only enhanced the shoot and leave the surface of the plant, but also showed a buffering indicator of the substrates, and therefore, the rhizosphere itself (pH and conductivity regulation) (Table 1). During the trial period, the measured pH of substrates ranged from 7 to 8. It appears that the rose distillation waste substrate was essentially characterized by a slightly alkaline pH (pH $\sim$ 7.6) (Fig. 2). The optimal range in terms of pH for Damask rose cultivation was about 6 to 8, as reported by Baser and Arslan (2014). It was also mentioned by Karlik et al. (2003) that a pH close to neutrality was suitable for damask rose plants. However, highly acid soil causes a nutritional imbalance in plants of the same plant (Ghavam, 2021). On the other hand, calcareous soil was unfavorable for the cultivation of the fragrant rose, especially if the irrigation water was alkaline (Chevalier and Sourzat, 2013). The substrate amended with rose distillation waste possessed a range of 1,43 mS/cm in terms of electrical conductivity throughout the experiment (Fig. 4). The recorded mineral salt content was below 2 mS/cm, corresponding perfectly to the threshold required by *Rosa damascena* crop, which was suggested as ideal for the growth of this plants (Karlik et al., 2003).

Water retention capacity in substrates was measured to explore the effect of different substrate amendments on water holding capacity, and therefore, *Rosa damascena* growth. Table 2 summarizes volumes of irrigation water drained by different substrates per day. A difference in water retention capacity from one substrate to another was observed. The substrate amended with rose distillation waste was the least substrate to drain (36.75 mL/d). As for control, which does not contain organic amendments, irrigation water leaches out more (69.03 mL/d).

Substrate amendments might impact the particle constitution of substrates and increase the instantaneous water-holding capacity. It was already proved that amended soil or substrate contained high moisture content and boosted plant dynamic growth. Also, it was shown that substrate amendment could reduce the risk of plant death in the event of water shortage. In its study Fields et al. (2017) pointed out that improved nutrient uptakes and water use influenced the formation of vegetative growth in shrubs. Substrate amendment might serve as an enhancing nutrient reservoir and a water support container for plant growth, especially in potted plant production (Yangyuoru et al., 2006; Daniels et al., 2012). Indeed, for roses, it was reported that a well-aerated soil was required with a large amount of humus (Ghavam, 2021).

Effect of substrate on growth traits

Among four treatments inoculated with mycorrhizae and rhizobacteria, rose distillation waste and control satisfied the substrate nutrient requirement for stem enhancement in *Rosa damascena*. Significant ($p < 0.001$) improvement in stem height growth was observed when rose distillation waste was enriched with mycorrhizae and rhizobacteria indicating the highest value 11.30 cm. Furthermore, the inoculated control showed also a significant stem height growth enhancement compared to the other treatments with a stem height of 9.48 cm, while goat manure and compost substrates resulted in producing the lowest stem height with 6.94 cm and 6.38 cm respectively (see Table 3). The rose distillation waste inoculated substrate was able to enhance approximately five-time stem heights in *Rosa damascena* compared to the non-inoculated control. Seedlings amended with goat manure and without mycorrhizae and rhizobacteria incorporation showed a very low stem height growth (1 cm) compared to other treatments. The incorporation of mycorrhizae and rhizobacteria to different substrates showed significant enhancement of the stem height of the plant except for the substrate amended with compost (Table 3). The observed bud break rate ranged between 27.5 ± 11.4 cm and 73.3 ± 18.7 cm. The incorporation of the biostimulant into the substrate induced a highly significant ($p < 0.001$) increase in the bud break rate of *Rosa damascena* seedlings grown on the control substrate

Table 4. Effect of substrates amended with different organic amendments inoculated and no-inoculated with biostimulant-based mycorrhizae and PGPR on Nutritional status of leaves.

Mineral elements	Inoculated				Non-inoculated			
	T	FC	TR	C	T	FC	TR	C
Nitrogen (N) (mg)	$446.62^{a} \pm 94.52$	$184.69^{cd} \pm 4.38$	$316.92^{b} \pm 38.37$	$219.49^{bc} \pm 34.41$	$130.92^{d} \pm 7.12$	$101.12^{d} \pm 3.78$	$131.35^{d} \pm 15.58$	$255.17^{bcd} \pm 47.79$
Phosphorus (P) (mg)	$37.42^{a} \pm 5.82$	$14.12^{c} \pm 1.46$	$24.59^{b} \pm 4.02$	$27.86^{b} \pm 3.98$	$9.39^{c} \pm 0.50$	$6.31^{c} \pm 0.47$	$13.16^{c} \pm 1.22$	$24.99^{b} \pm 4.31$
Potassium (K) (mg)	$242.37^{a} \pm 49.28$	$187.77^{ab} \pm 5.90$	$188.61^{ab} \pm 24.08$	$253.16^{a} \pm 41.32$	$82.44^{c} \pm 4.59$	$108.57^{bc} \pm 3.63$	$99.44^{c} \pm 9.48$	$262.50^{a} \pm 48.62$
Calcium (Ca) (mg)	$232.26^{a} \pm 48.66$	$93.63^{cd} \pm 2.76$	$164.98^{b} \pm 20.42$	$100.70^{cd} \pm 18.39$	$78.31^{d} \pm 4.16$	$54.24^{d} \pm 2.78$	$149.87^{bc} \pm 16.81$	$84.02^{d} \pm 15.34$
Magnesium (Mg) (mg)	$62.77^{a} \pm 12.28$	$36.69^{bc} \pm 2.22$	$50.99^{ab} \pm 6.27$	$44.77^{ab} \pm 8.20$	$20.28^{c} \pm 1.06$	$21.08^{c} \pm 0.52$	$54.52^{ab} \pm 4.72$	$45.24^{ab} \pm 8.54$
Zinc (Zn) (μg)	$406.39^{a} \pm 90.74$	$109.98^{cd} \pm 2.57$	$218.17^{b} \pm 28.26$	$167.72^{bc} \pm 27.29$	$139.26^{bcd} \pm 7.17$	$57.46^{d} \pm 3.14$	$103.39^{cd} \pm 9.75$	$176.27^{bc} \pm 33.14$
Manganese (Mn) (μg)	$1262.89^{b} \pm 272.94$	$697.61^{c} \pm 10.29$	$2372.48^{a} \pm 287.12$	$1261.19^{b} \pm 197.49$	$504.60^{c} \pm 28.83$	$466.33^{c} \pm 21.51$	$2080.88^{a} \pm 218.26$	$963.83^{b} \pm 184.06$
Iron (Fe) (μg)	$1043.86^{a} \pm 229.53$	$473.64^{bc} \pm 6.14$	$817.59^{a} \pm 92.39$	$746.82^{ab} \pm 125.68$	$467.86^{bc} \pm 25.81$	$391.81^{c} \pm 17.91$	$776.40^{ab} \pm 84.16$	$776.38^{ab} \pm 149.14$
Bore (B) (μg)	$1085.62^{bc} \pm 246.50$	$762.24^{cd} \pm 14.51$	$1450.75^{ab} \pm 194.09$	$1318.29^{ab} \pm 221.44$	$359.18^{d} \pm 20.38$	$459.04^{d} \pm 18.46$	$1798.16^{a} \pm 190.40$	$1416.71^{ab} \pm 266.22$
Copper (Cu) (μg)	$143.63^{a} \pm 31.98$	$48.79^{c} \pm 0.59$	$118.99^{ab} \pm 15.15$	$80.01^{bc} \pm 13.01$	$76.25^{c} \pm 3.99$	$59.14^{c} \pm 2.29$	$60.28^{c} \pm 5.88$	$71.84^{c} \pm 12.95$
Chlorine (Cl) (μg)	$8279.70^{a} \pm 1744.20$	$5254.16^{b} \pm 63.98$	$2380.30^{c} \pm 277.06$	$1829.42^{c} \pm 252.43$	$2027.92^{c} \pm 123.15$	$902.18^{c} \pm 35.80$	$5194.18^{b} \pm 517.45$	$1987.79^{c} \pm 380.71$
Sodium (Na) (μg)	$5526.71^{a} \pm 1158.93$	$3523.61^{b} \pm 80.35$	$1591.39^{c} \pm 193.27$	$1193.58^{c} \pm 193.84$	$1340.21^{c} \pm 71.53$	$601.11^{c} \pm 24.54$	$3463.25^{b} \pm 362.32$	$1333.07^{c} \pm 258.34$

T: Control, FC: Substrate amended with goat manure, TR: substrate amended with rose distillation waste and C: substrate amended with compost).
Means followed by the same letter are not statistically different at $p < 5\%$ according to the Tukey test.

or amended with waste rose with respectively 63.3% and 73.3% (Table 3).
Application of different treatments exhibited a highly significative ($p < 0.001$) effect on the growth of apical internodes (GAI) of *Rosa damascena*. Without inoculation, the application of rose distillation waste and compost increased GAI when compared to the control that had a similar effect to substrate amended with goat manure. With inoculation, only substrate amended with goat manure resulted in a significant increase of GAI (1.33 ± 0.69 cm).
At the leaf surface level, the largest leaves were in seedlings planted in substrate amended with rose distillation waste and control that are fortified with mycorrhizae and rhizobacteria with an expending leaf surface of 135.42 ± 44.14 cm^2 and 131.74 ± 41.75 cm^2 respectively (Table 3). A highly Significant ($p < 0.001$) increase in leaf surface was observed on *Rosa damascena* seedlings planted in control and waste rose amended substrate inoculated with mycorrhizae and PGPR.
Based on the chemical analysis of our study, a high phosphorous content was noticed in the goat manure (FC) amended substrates, which reflected the result of high shoot heights of roses' seedlings in the same substrates. An increase in stem length at higher (N) and (P) was also reported in 2021 by El-Sharnouby et al. (2021) in *Rosa damascena* var. trigentipetala. In addition, substrate amended with goat manure (FC) had excessively high levels of phosphorus and assimilable potassium. This excess of phosphorus does not cause toxicity to the plants because most of the soils adsorb the overdoses of phosphorus, therefore, phosphorus is not present in the soil at a level that could cause direct toxicity. Nevertheless, it could directly affect plant growth through disruption of the uptake of trace elements, such as iron, zinc, and copper (Barker and Pilbeam, 2015). Phosphorus is an essential element in the photosynthesis of plants (Barker and Pilbeam, 2015). Therefore, it is normal to obtain enhancement in plant growth in mycorrhizal substrates since these latter contain more phosphorous. Consequently, a significant difference was observed among substrates inoculated by mycorrhiza. The roses planted in substrate containing the rose distillation waste and inoculated with mycorrhizae and PGPR have shown interesting results, at the level of all the studied parameters especially in phosphorous level uptake and the related plant growth when compared to other substrates with mycorrhizae inoculum. Hence, it is important to point out the remarkable effect of (P) amount on various mycorrhizal fungus reactions over different organic amendments. The substrates in the control, amended with waste rose and compost are therefore favored since they have reasonable P contents, relative to goat manure amended substrate, and present more efficient vegetative development of *Rosa damascena*.
The degradation of rose distillation waste among other substrates was an extremely slow process (Onursal and Ekinci, 2015). This work focused on the use of floral waste as a substrate in plant potting and how it can be managed by converting it into added value products as a beneficial root and rhizosphere colonizing capacity that generates benefit. The results of this study also demonstrated that these wastes can serve as a viable alter-native to chemical fertilizers, effectively enhancing the growth of damask rose seedlings. The increased nutrient content in terms of N, P, and K qualifies the waste as a nutrient rich biofertilizer. Rose distillation waste has been shown to have a significant effect on vegetative and generative growth. in fact, according to a study carried out by Gaurav and Pathade (2011) on five floral species, it was proven that the plants showed a significant length, flowering date, and number of flowers, compared to the control without rose distillation waste (Gaurav and Pathade, 2011). Reflecting on these results, it would be recommended to valorize the rose distillation waste by incorporating mycorrhizae and PGPR to promote growth of *Rosa damascena* seedlings.

Effect of substrate on nutritional status of leaves

The results of the foliar analysis showed a highly significant difference ($p < 0.001$) in the nutrient content of leaves treated by different amendments. The results of heat map represented nutrient dynamics in different inoculated and non-inoculated substrates. Seedlings planted in substrates amended by goat manure and rose distillation waste and not inoculated showed a deficiency in the absorption of macro and oligo elements. The uninoculated control was more efficient in absorbing nutrients than the last two uninoculated treatments (goat manure and rose distillation waste). Seedlings planted in non-inoculated compost were not significantly different in absorbing nutrients compared to those planted in substrate with inoculated compost (Table 4).
Plant growth-promoting rhizobacteria, with their ability to promote plant growth, were widely applied in crops (Trappe, 1977; Selosse et al., 2006). According to a study conducted by (Tariq et al., 2016), rhizobacteria not only promoted plant growth but also participate in increasing row size in Damask rose. *Rosa damascena* growth and nutrition uptake efficiency depended most on biological colonization of the substrate. The results obtained in this study provided clear evidence that mycorrhizae and PGPR colonization improve the vegetative growth of plants. It should be noted that all the substrates treated with mycorrhizae and PGPR, presented interesting results by improving plant growth, first leaves triggering, nutritional status of leaves, and their surface development compared to not treated plants. This could be explained by the fact that mycorrhizae were particularly effective in improving plants' phosphorus nutrition. The fungal hyphae could extract phosphates from the soil more easily than roots, and the hyphae can extend beyond locally depleted areas adjacent to the roots into unexplored soils. Mycorrhizae also helped to take up essential micronutrients such as zinc, copper, and manganese that are needed for plant growth (Umunnakwe et al., 2023).

4. Conclusion

The disposal of large quantities of rose distillation waste poses a significant environmental challenge. However, by using composting processes and incorporating biostimulant inoculation, this waste could be effectively repurposed to enhance the fertilizing qualities of the substrate used in Damask rose organic production. Our findings indicated that the addition of bio-stimulants based on mycorrhizae and PGPR was a highly effective approach, resulting in improved crop growth parameters. For farmers seeking alternative, cost-effective solutions to fertilize seedlings, utilizing floral waste as an amendment could prove to be a lucrative option. Ultimately, our research highlighted the potential for sustainable waste management practices in the context of organic farming, with economic benefits for farmers and a positive impact on the environment.

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Ethical approval
This manuscript does not report on or involve the use of any animal or human data or tissue. So the ethical approval is not applicable.

Author contribution
The authors confirm the study conception and design: S. El Malahi, M. Mokhtari, L M. Idrissi Hassani; data collection: S. El Malahi, L. Irahoui, J. Zim, A. Zayani; analysis and interpretation of results: S. El Malahi, L.Irahoui, B. Zakri, H. Taimourya, M. Ennami; draft manuscript preparation: S. El Malahi, W. Mokhtari, K. Dhassi. The results were evaluated by all authors, and the final version of the manuscript was approved.

Availability of data and materials
Data presented in the manuscript are available via request.

Conflict of interest statement
The authors declare that they are no conflict of interest associated with this study.

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