

## BIOCHAR AND SILVER NANOPARTICLES FOR ENHANCING GERMINATION OF LEMON AND MANGO SEEDS

### *BIOCHAR E NANOPARTÍCULAS DE PRATA PARA MELHORAR A GERMINAÇÃO DE SEMENTES DE LIMÃO E MANGA*

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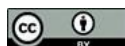
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## Abstract

In the present investigation, several fruit seed germinations have been aided by the innovative use of nanomaterials and biochar obtained from organic sources. In order to address issues with seed viability, it was anticipated that a more intelligent use of such compounds in producing fruits would be possible. The effects of nanomaterials and biochar on fruit seed germination were studied, focusing on their interaction with mango and lemon seeds. A soil-less petri dish bioassay was conducted, utilizing varying concentrations of silver nanoparticles (AgNPs) and olive pomace-derived biochar. Results indicated a positive impact of biochar on mango seed germination, whereas the additions of the same concentrations of both treatments showed a negative effect in the case of lemon, which was unexpected. Principal Component Analysis (PCA) results show a consistent physiological trend: treatments that increased germination percentages also decreased mean germination time and increased germination rate. However, cc, whereas Lemon showed greater dispersion in treatment scores, indicating greater susceptibility to treatment effects. This study opens a new door for investigating the effect of the novel applications of AgNPs and biochar in different fruit seed germinations, taking into account that each fruit species requires individual experimental studies to determine the promising additions of both treatments.

**Keywords:** Seed Germination. Biochar. Metallic and Organic Nanoparticles. Mango. Lemon. and Soilless Petri Dish.

## Resumo

*Nesta investigação, a germinação de diversas sementes de frutas foi auxiliada pelo uso inovador de nanomateriais e biochar obtidos de fontes orgânicas. Visando solucionar problemas relacionados à viabilidade das sementes, antecipou-se a possibilidade de um uso mais inteligente desses compostos na produção de frutas. Os efeitos de nanomateriais e biochar na germinação de sementes de frutas foram estudados, com foco em sua interação com sementes de manga e limão. Um bioensaio em placa de Petri sem solo foi conduzido, utilizando concentrações variáveis de nanopartículas de prata (AgNPs) e biochar derivado de bagaço de azeitona. Os resultados indicaram um impacto positivo do biochar na germinação de sementes de manga, enquanto a adição das mesmas concentrações de ambos os tratamentos apresentou um efeito negativo no caso do limão, o que foi inesperado. Os resultados da Análise de Componentes Principais (ACP) mostram uma tendência fisiológica consistente: os tratamentos que aumentaram as porcentagens de germinação também diminuíram o tempo médio de germinação e aumentaram a taxa de germinação. No entanto, o tratamento com AgNPs apresentou maior dispersão nos escores dos tratamentos, indicando maior suscetibilidade aos efeitos dos tratamentos. Este estudo abre novas perspectivas para a investigação do efeito de aplicações inovadoras de nanopartículas de prata (AgNPs) e biochar na germinação de sementes de diferentes frutos, considerando que cada espécie frutífera requer estudos experimentais específicos para determinar as adições mais promissoras de ambos os tratamentos.*

**Palavras-chave:** Germinação de sementes. Biochar. Nanopartículas metálicas e orgânicas. Manga. Limão. Placa de Petri sem solo.

## 1 INTRODUCTION

One of the most important stages of fruit life is reproduction. As a result, in plant populations, genes that influence fruit seed germination have been those most strongly selected. Germination is the natural process that terminates seed dispersal and thus affects the location and timing of the plant growth stage (Penfield, 2017, Qaoud *et al.*, 2017, Maleki *et al.*, 2023). It is well-known that several methods have been used to promote seed germination including chemical, biological, and mechanical approaches

(**Barabanov et al., 2018**). One of the fastest-growing trends in contemporary nanotechnology is the production and use of nanosized particles of various substances; silver nanoparticles (AgNPs) are among the most widely used manufactured nanoparticles in consumable products, where their antimicrobial qualities are being utilized more and more (**Cornier et al., 2017, Solaiman et al., 2017, Ahmed et al., 2023**). Only a small number of studies have recently examined the impact of AgNPs on higher plants (**Yin et al., 2012; Thuesombat et al., 2014; Cvjetko et al., 2017; Yang et al., 2022; Thwala et al., 2021**), but they have consistently demonstrated that AgNPs have a variety of effects (either positive or negative) on plant growth and seed germination.

The pyrolysis process, which produces biochar (BC), is a very stable kind of carbon that is created when organic compounds are burned without oxygen (**Bolan et al., 2022**). Biochar may be stable and stay in the soil for a number of years, according to certain reports (**Ascough et al., 2009, Leng et al., 2019**). It has recently been employed as a soil amendment that can increase soil fertility and serve as a carbon sink on agricultural land (**Chan et al., 2007**). However, BC might include undesirable materials including metals, phenolic compounds, and hydrocarbons (PAHs), which could be extremely dangerous to humans, microorganisms, and plants (**Thies and Rillig, 2009**). Because they contain vital nutrients for plants, several chemicals in BC have the ability to either promote or inhibit seed germination and seedling growth (**Gaskin et al., 2008**). According to reports, biochar can either boost plant growth and yield (**Jaiswal et al., 2020**) or decrease it (**Huang and Gu, 2019**). Furthermore, the impact of biochar on the initial phases of plant development, including seed germination, has not been well studied. As far as we are aware, no prior research has attempted to use AgNPs and biochar together to encourage seed germination for regional fruits in Egypt. Two distinct seed kinds-lemon and mango-were chosen as the best model system for germination issues and have never been used to nanomaterial or biochar uses. For example, Ismailia city is known as the most famous city for Mango production. However, framers, there, are suffering from a lower germination percentage that could reach 50% because of their desiccation-sensitive and lower viability (**Corbineau et al., 1986**). For first assessments of BC and silver nanoparticle utilize soilless environments, petri dish biological assays are an easy and quick technique. It was anticipated that the effects of BC and silver NPs on the plant's early germination rate would vary; adding that, compared to a complex bio-assay based on soil that takes time (perhaps three years of growth), interactions with different soil

qualities, and other components, biological assays in petri dishes are a simpler initial screening method, and more labor-intensive tasks. Thus, the primary goal of this study was to use a petri dish soil-less bioassay to evaluate the potential addition of both treatments and ascertain if AgNPs and biochar may accelerate the germination rate of mango and lemon seeds.

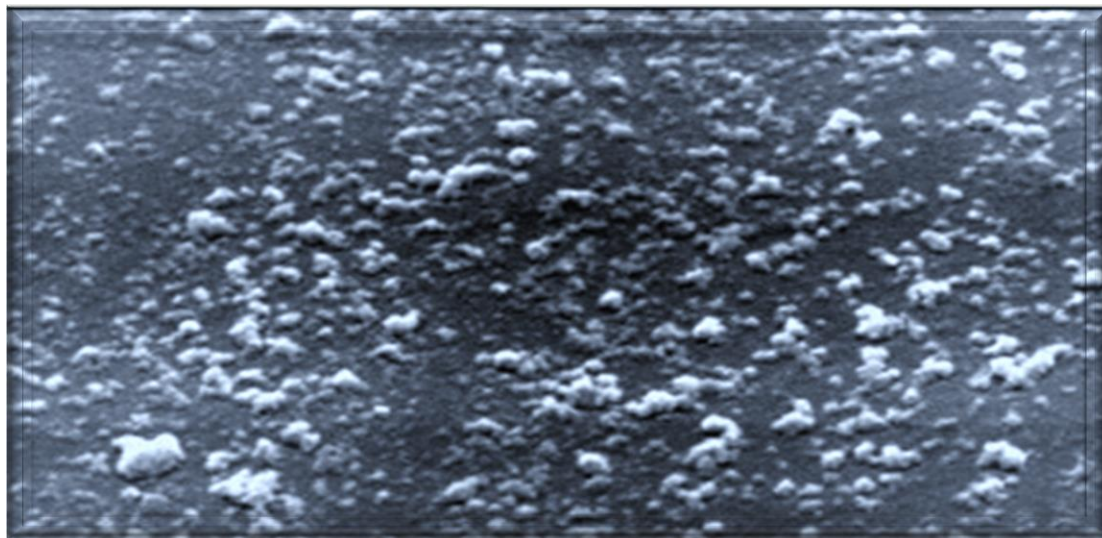
## 2 MATERIALS AND METHODS

### 2.1 Silver nanoparticle preparation

The Agriculture Research Centre (ARC) in Giza, Egypt, is where the silver nanoparticles were acquired. The synthesis of the AgNP followed **Vigneshwaran *et al.* (2006)**. In short, the green chemistry method was used to create silver nanoparticles by dissolving 1.0 g of soluble starch in 100 ml of deionized water. After adding one milliliter of 100 mM AgNO<sub>3</sub>, it was thoroughly mixed. This combination was autoclaved for five minutes at 121 °C and 15 psi of pressure. Silver nanoparticle production was indicated by the bright yellow hue of the resultant nanoparticle suspension solution (**Vigneshwaran *et al.*, 2006**). Silver nanoparticles with a diameter of less than 100 nm and a concentration of 100 mg<sup>l</sup>-1 were imported from ARC. As shown in Figure 1, AgNP supplied by ARC was confirmed to be smaller than 100 nm, in size using a scanning electronic microscope (SEM; Quanta 450 FEG-ESEM, FEI Company).

**Figure 1**

*Silver nanoparticles' SEM image, which verifies that they are less than 100 nm, Marzouk (2017).*

**2.2 Characterization and preparation of biochar**

Biochar was created using leftover olive pomace from a trial oil harvester at Arish University's Faculty of Environmental Agricultural Sciences. A specific amount of dry air substance was contained in a closed vessel and heated to 450 °C for 60 minutes in a muffled oven, per **Vijayanand *et al.* (2016)**. The morphological properties of the generated biochar were analyzed at a scanning electronic microscope (SEM; Quanta 450 FEG-ESEM, FEI Company). Figure 2 shows seamless surfaces with different sizes of permeability. The pore diameters, which ranged from tens of nanometers to micrometers, were irregular. The chemical characteristics of the produced biochar were determined by measuring its pH in water using a pH meter (Model pH 209, HANNA Instruments, UK) in water: solid ratio of 1: 2.5. Atomic absorption spectrophotometry (SHIMADZU AA-6800) was used to evaluate the total metallic contents in acid degraded biochar (aqua regia; a 3:1 mixture of concentrated HCl and HNO<sub>3</sub>). Oxidizable dichromate was used to determine the organic content of biochar (**Walkley and Black, 1934**). The chemical properties of the biochar made from olive pomace were compiled in Table 1.

### 2.3 Seed germination experiment

Mango (*Mangifera indica* L.) Keitt variety (n = 6) and Lemon (*Citrus Limon* L. *Burm.*) Banzahir variety (n = 8) were planted in Petri Containers (30 and 8.5 cm diameter, for mango and lemon seed, respectively) on three layers of distilled water-moistened filter paper. At quantities of 0, 1, 5, 10, 20, and 40 mg/L, silver nanoparticles were introduced. According to (Yin *et al.*, 2012), these concentrations were chosen to span a broad spectrum of AgNPs. As a control treatment, distilled water was utilized to observe how applications of biochar and nanoparticles affected seed germination. The water-holding capacity of the biochar and the need for the maximum biochar rate (5 g) were used to determine how much water (20 mL) should be added to the filter paper for both treatments. For every rate of AgNPs and biochar, the same volume of water was added to the petri dish.

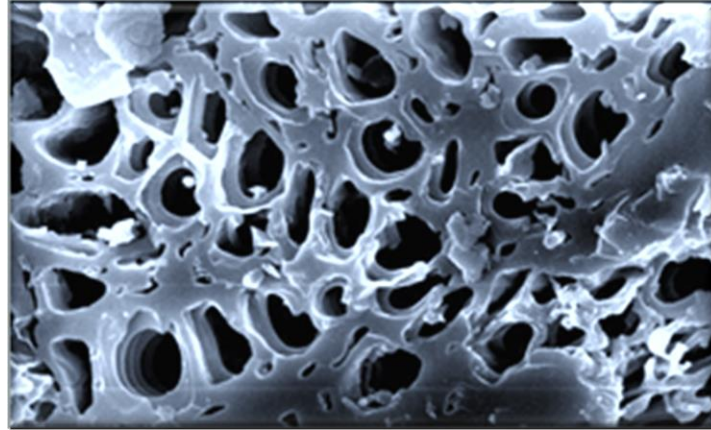
**Table 1**

*Synopsis of the chemical properties of biochar made from olive pomace*

Biochar	
pH (1:2.5)	8.81
OM (%)	50.87
Zn (g/kg)	0.021
Fe (g/kg)	0.39
Na (g/kg)	0.047
Mg (g/kg)	2.981
K (g/kg)	13.51
Ca (g/kg)	22.7

**Figure 2**

*Marzouk (2017) produced an imaging electronics microscopy of biochar at 450 degrees Celsius).*



Following the design outlined by **Morrison and Morris (2000)** for mango and lemon, respectively, biochar was put in triplicates to each plate at each of the rates: 0, 0.5, 1, 2, 3, and 5.0 g/petri dish (each 1 g/petri= 20 ton/ha on a volume base at 10 cm soil depth) during two successive seasons of 2024 and 2025 in September. Each plate contained 6 and 8 seeds. A replicate was defined as a single petri plate. Once all Petri dishes were sealed with coverings and maintained for the required duration at 30 °C in the dark, the germination percentages were calculated. Following planting days, the following formula was used to determine the germination percentage:

The germination percentage, or germinability (G), is determined by dividing the number of seeds that germinated (n) by the total number of seeds (n<sub>t</sub>).

$$G = \frac{n}{n_t} \times 100 \quad (1)$$

The following expression is used to determine the mean germination time.

$$\bar{t} = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i} \quad (2)$$

where:

n<sub>i</sub> is the number of seeds that germinated in the i<sup>th</sup> time (not the total number, but the number that corresponded to the i<sup>th</sup> observation); k is the last time of germination; and t<sub>i</sub> is the time between

the beginning of the experiment and the  $i^{\text{th}}$  observation (day in the example). The reciprocal of the mean germination period is used to get the mean germination rate.

## 2.4 Statistical analysis

The statistical analysis was carried out based on the mean results of the two study success seasons. Data were subjected to the analysis of variance and a completely randomized design was used (Steel and Torrie, 1980). Analysis of variance and mean comparison (LSD, at 5%) were done by MSTAT-C program version 7 (1990).

Principal Component Analysis (PCA) was performed on the findings for the germination parameters under study using an online PCA calculator (<https://www.statskingdom.com/pca-calculator.html>). Based on three crucial germination characteristics of both Lemon and mango seeds-germination percentage and germinability (G), mean germination time (MGT), and mean germination rate (MGR)-the PC was utilized to ascertain the degree of variation in treatments. The 12 treatments underwent PCA in a favorable trend. To ascertain the relative distinguishing power of the axes and the characters that correspond to them, eigenvalues were retrieved from PC (Pradhan *et al.* 2015). The treatments were grouped in a bi-plot figure and compared with the cluster analysis.

## 3 RESULTS

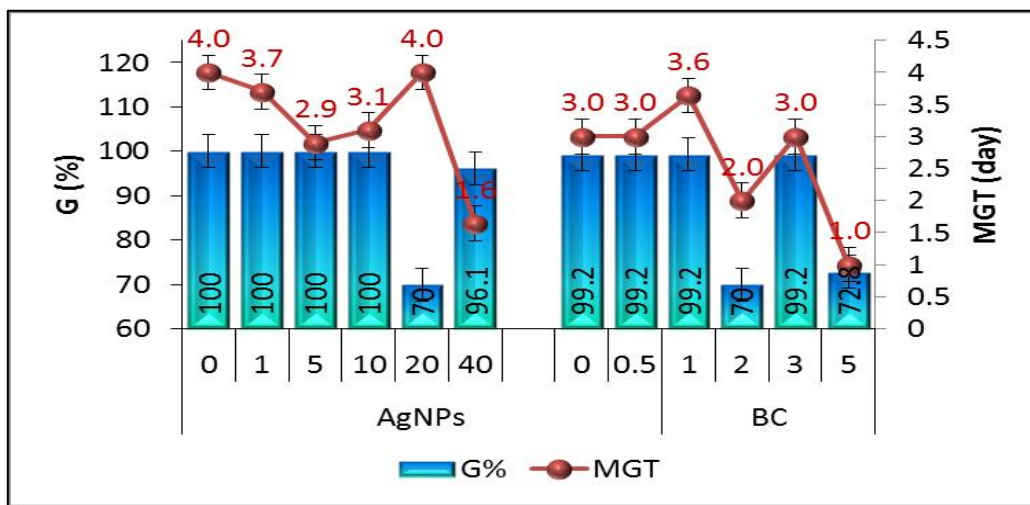
### 3.1 Effect of silver nanoparticles and biochar on germination of Mango seed:

Fresh mango seeds produced embryos that germinated incredibly quickly; nearly all of them did so in two to four days. With the treatment of Ag nanoparticles, germination increased steadily; the overall germination rate (%) was  $94.4 \pm 13.6$  ( $\pm$ standard deviation), while for biochar applications it was  $88.9 \pm 17.2$ . After five days, the control's germinability reached 100%, whereas the other treatments only needed two to four days. After the first day of applying  $40 \text{ mgL}^{-1}$  of AgNPs and 5g Petri dish-1 of biochar, respectively, the first germination (appeared germination) was seen. At  $20 \text{ mg L}^{-1}$  of Ag NPs as well as both 2 and 5 g/petri biochar application rates, the lowest germinability rate was noted. However, for both Ag NPs and biochar, MGT (day) was found to be  $3.22 \pm$

0.81 and  $2.61 \pm 0.95$  per day, respectively (Fig. 3). At 40 mgL<sup>-1</sup> of Ag NPs and 5% biochar additions, the maximum MGT was achieved. For both treatments, the values were two and one day, respectively.

### Figure 3

*Germinability (G, %) and mean germinate time (MGT, day) for Mango seeds treated at varying rates with AgNPs and biochar (BC). Standard errors of triplicates are shown by error bars.*

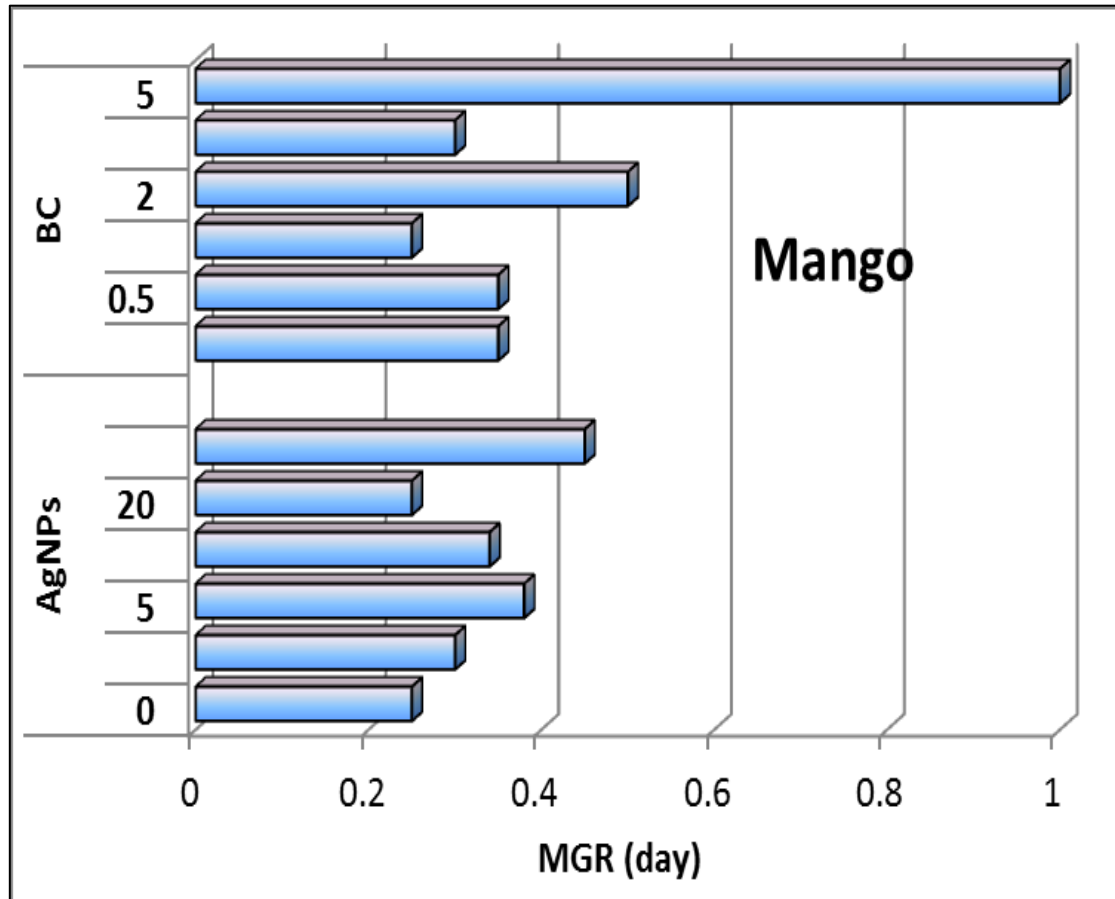


When it comes to this issue, biochar appears to have performed better than AgNPs. Furthermore, a statistical examination of the germination coefficient of variation (CV) across several treatments revealed significant heterogeneity in the biochar treatment scenario. For biochar, the overall value was  $31.9 \pm 16\%$ , but for AgNPs applications, it was  $24.3 \pm 16.8\%$ . That indicates a certain degree of consistency in the outcomes of AgNPs applications.

Figure 4, which display MGR, translate all of these findings. For AgNPs and biochar treatments, the overall mean numbers were  $0.33 \pm 0.09$  and  $0.46 \pm 0.27$  day<sup>-1</sup>, respectively. This indicates that the application rates of AgNPs and biochar increased mango germination by roughly 33% and 45% each day, respectively. The results also demonstrate the superiority of the highest dosages for both treatments (see plate 1 and Fig. 4) as a result of MGT results (Fig. 3). The overall conclusion of this section is that the applications of biochar at the highest levels demonstrated more promising outcomes than AgNPs in terms of mango germination capabilities. Using two coated substances, *i.e.*, PVPAgNPs and GAAgNPs,

**Figure 4**

*Mean germinate rate (MGR, day) for Mango seeds treated at varying rates with AgNPs and biochar (BC).*



The student t-paired test revealed non-significant differences between AgNPs and biochar treatments for % germination ( t value = -0.54; p = 0.61), MGT ( t value = 1.87; p = 0.12), and MGR ( t value = -1.61; p = 0.17), when comparing the effects of AgNPs and applying biochar on mango seed germination attributes.

### Figure 5

The highest level of biochar (left) and AgNPs (right) application effects on Mango (above) and Limon (below) germination behavior (images were taken at the same time after sowing date, (digital photographs Sony DLSAR A2, Japan, (<https://imagej.net/Welcome>))

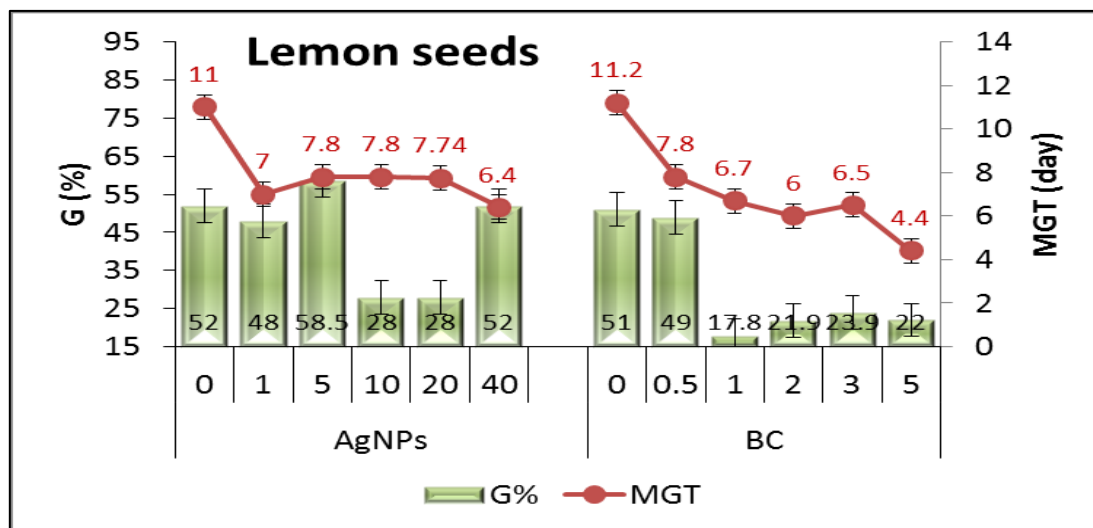


### 3.2 How biochar and silver NPs affect lemon seed germination

Unlike Mango, Lemon showed low germination of embryos from fresh seeds (Fig. 6). Germination became progressively lower with biochar application. The general germination rate (%) showed  $44.4 \pm 12.6$  ( $\pm$ standard deviation) compared to  $30.9 \pm 15.9$  for biochar applications (Fig. 6). The germinability reached 57.1% in the 5 mg AgNPs L<sup>-1</sup> after 6 days.

**Figure 6**

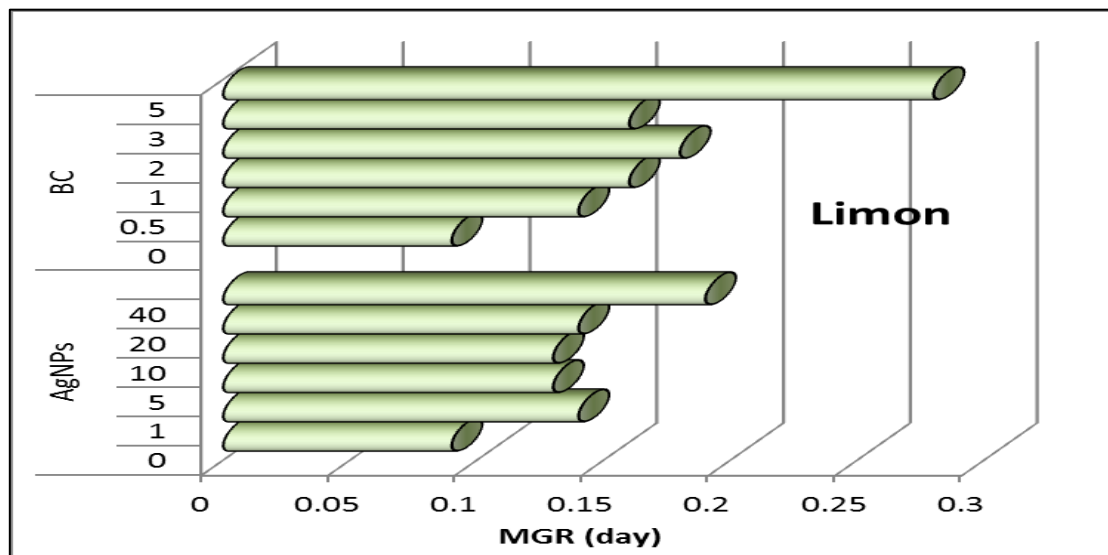
*Germinability (G, %) and mean germinate time (MGT, day) for Lemon seeds treated at varying rates with AgNPs and biochar (BC). Standard errors of triplicates are shown by error bars.*



The lowest germinability rate was observed at 20 mg L<sup>-1</sup> (AgNPs) and both 1 and 2 g/petri biochar application rates. However, mean germination time (MGT) was observed to be 7.96 ± 1.55 and 7.11 ± 2.16 per day for both AgNPs and biochar respectively (Fig. 6). Surprisingly, the highest MGT was obtained at control for both treatments. This might be attributed to the toxicity effect of both treatments on lemon seed's early germination stage. Regarding this issue, it appears that neither treatment had any impact when compared to the control. Furthermore, there was a lot of variation in the percentage CV of germination across the various treatments. For AgNPs, the overall value was 45.5 ± 16.8%, whereas for biochar applications, it was 36.1 ± 7%. This implies that both applications may produce rather erratic results.

**Figure 6**

Mean germinate rate (MGR, day) for Lemon seeds treated at varying rates with AgNPs and biochar (BC).



All these findings were also reflected in Fig. 7 which shows MGR. For AgNPs and biochar treatments, the overall mean values were  $0.14 \pm 0.03$  and  $0.17 \pm 0.07$  day<sup>-1</sup>, respectively. Compared with mango results, the MGRs for Lemon seeds were very low (see Fig. 5 and Fig. 7). This means both treatments show insignificant effects on the germination rate of lemon seeds compare with controls.

#### PCA Biplot:

Table 2 displays the results of a cluster analysis based on all treatments with biochar (BC) and silver nanoparticles (AgNPs) divided into four groups. Table 3 displays the average result of all variables in each cluster.

In Lemon, the 2<sup>nd</sup> cluster (II) has four treatments accounting 33.33% of the total 12 treatments followed by both 3<sup>rd</sup> and fourth clusters (25% each) as well as both the 1<sup>st</sup> one having two [40 mg/L AgNPs and 5 g/petri-dish BC] treatments accounting 16.67% of total treatments as shown in Table 2. As for Mango, the 1<sup>st</sup> cluster has six treatments accounting 50% of the total 12 treatments followed by the 2<sup>nd</sup> cluster (25%), fourth cluster (8.33%) as well as both the 3<sup>rd</sup> one having two [40 mg/L AgNPs and 5 g/petri-dish BC] treatments accounting 16.67% of total treatments

**Table 2**

*Clustering patterns of the all biochar (BC) and silver nanoparticles (AgNPs) treatments based on germination traits*

Clusters	Treatments		
	No.	%	Included
<b>Lemon</b>			
I (Y1 >= 0, Y2 >= 0)	2	16.67%	AgNPs (40 mg/L) and BC (5 g/petri dish)
II (Y1 >= 0, Y2 < 0)	4	33.33%	AgNPs (20 mg/L) and BC (1, 2, 3 g/petri dish)
III (Y1 < 0, Y2 < 0)	3	25.00%	AgNPs (10 mg/L), Zero AgNPs or BC
IV (Y1 < 0, Y2 >= 0)	3	25.00%	AgNPs (1, 5 mg/L) and BC (0.5 g/petri dish)
<b>Mango</b>			
I (Y1 >= 0, Y2 >= 0)	6	50.00%	AgNPs (1, 5, 10 mg/L) and BC (0, 0.5, 3 g/petri dish)
II (Y1 >= 0, Y2 < 0)	3	25.00%	AgNPs (0, 20 mg/L) and BC (1 g/petri dish)
III (Y1 < 0, Y2 < 0)	1	08.33%	BC (2 g/petri dish)
IV (Y1 < 0, Y2 >= 0)	2	16.67%	AgNPs (40 mg/L) and BC (5 g/petri dish)

As shown in Table 3, the treatments (40 mg/L AgNPs and 5 g/petri dish BC) of cluster I (Lemon) and IV (Mango) were prevalent in 66.7% and 100%, respectively compared to the rest clusters. However, it had the highest in cluster desirable mean values for MGR and MGT for Lemon as well as germinability for mango, suggesting that the most successful treatments in this group can be widely applied to future germination studies to maximize germination speed and rate, whereas clusters I (Mango) and IV (Lemon) especially AgNPs (1, 5 mg/L) and 0.5 g/petri dish BC was significantly equal to the cluster mean values in both MGT and MGR, by about 66.67% in which the best desirable Germinability (G%) in both mango and Lemon.

**Table 3**

*Each cluster's average of the variables and the variation between the means of each group and the overall mean*

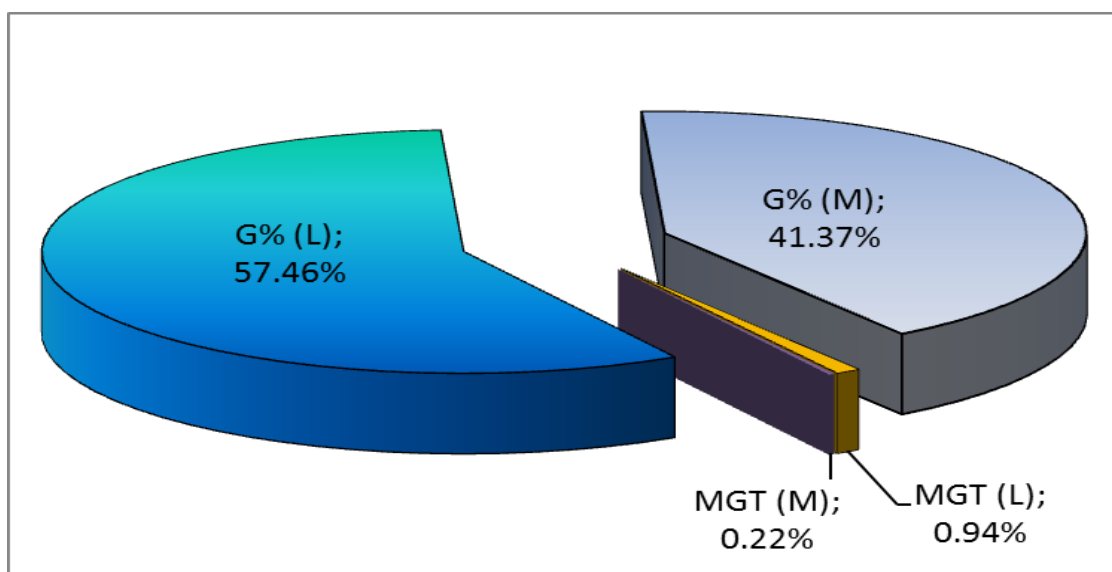
	I		II		III		IV	
	Value	diff. (%)	Value	diff. (%)	Value	diff. (%)	Value	diff. (%)
<b>Lemon</b>								
G	37	-1.79%	22.9	-39.22%	43.67	15.90%	51.83	37.58%
MGT	5.4	-28.27%	6.74	-10.54%	10	32.83%	7.53	0.07%
MGR	0.24	54.10%	0.16	4.92%	0.1	-32.24%	0.14	-10.38%
<b>Proportion contribution of enhanced traits</b>								
High	66.67%		0.00%		0.00%		33.33%	
Medium	33.33%		66.67%		33.33%		66.67%	
Low	0.00%		33.33%		66.67%		0.00%	
<b>Mango</b>								
G	99.6	8.09%	89.73	-2.61%	70	-24.03%	96.1	4.30%
MGT	3.12	6.92%	3.88	33.10%	2	-31.39%	1.64	-43.74%
MGR	0.34	-14.41%	0.25	-36.44%	0.5	27.12%	0.45	14.41%
<b>Proportion contribution of enhanced traits</b>								
High	33.33%		0.00%		66.67%		100.00%	

Medium	66.67%	33.33%	0.00%	0.00%
Low	0.00%	66.67%	33.33%	0.00%

On the other hand, in terms of the germination associated traits, the current study found that the proportional effect of Lemon seed germination (%) towards the variation was 57.46% (Fig. 8), followed by that of mango seed germination (%) at 41.37%, MGT for Lemon at 0.94%, and MGT for mango at 0.22%. Meanwhile, the proportional contributions of MGR for both Lemon and mango do not skip 0-0.01%. Consequently, depending on the germination parameters, the trait of germinability (G%) would be a crucial parameter for choosing different treatments.

### Figure 8

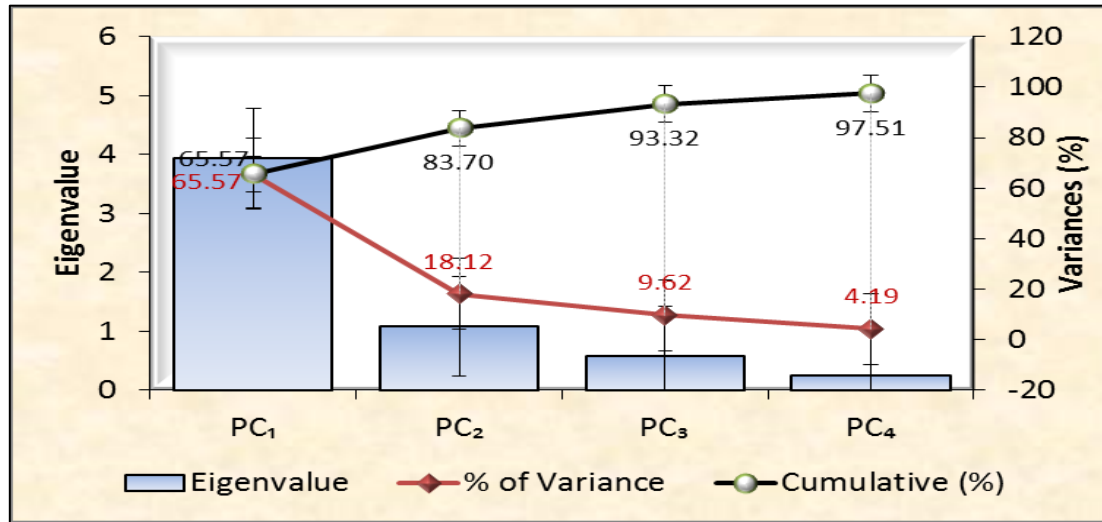
*Graphical representation of the proportionate contribution of studied germination parameters toward different treatments*



The first two components were taken into consideration in the principal component analysis, and their combined eigenvalue was 83.7% (Fig.9). The linear combination of the six variables under study yields the first and second main components. (MGR, G% and MGT for both mango and Lemon seeds) and both explained 65.57 % and 18.12 % of the variance, respectively.

**Figure 9**

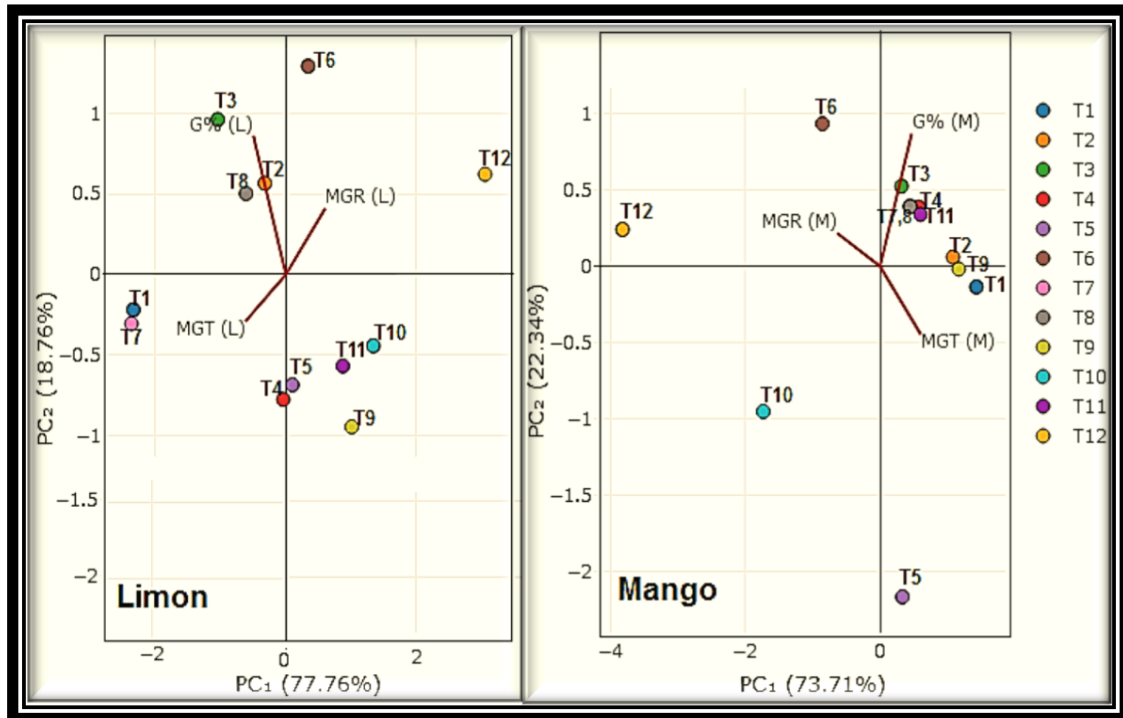
*Eigenvalue scree plot with both accumulated and explained variability (%) for germination related parameters of 12 treatments.*



The multivariate relationships between treatments involving the application of biochar (BC) and silver nanoparticles (AgNPs) and their effects on germination variables in the two fruit crop seeds (Lemon, L. and Mango, M.) are summarized in the principal component analysis (PCA) biplot (Fig. 9 and Table 4). Based on the 3 variables of each crop under study, the Biplot-PCA results (Fig. 10) showed that there were significant differences between the treatments under study, which could have an impact on germination and growth performance.

**Figure 10**

The Principal Component Analysis (PCA) Biplot illustrates how the treatments of biochar (BC) and silver nanoparticles (AgNPs) were distributed among Lemon (L) and Mango (M) seeds according to their mean germination rate (MGR), mean germination time (MGT), and germination percentage (G%).



### *Lemon (L) seeds*

96.52% of the variation was described by the first two principal components, PC1 (77.76%) and PC2 (18.76%). Rapid germination and high germination percentage are strongly positively correlated, according to the Biplot, which showed that the G% and MGR vectors were directed in the same direction. MGT, on the other hand, was projected in the other direction, indicating that it has a negative association with both G% and MGR. AgNPs (5, 10, 20, and 40 mg/L) treatments were placed next to the G% and MGR vectors, indicating that these treatments significantly improved germination speed and performance. On the other hand, treatments like BC at 0, 0.5, and 1 g/petri were found on PC1's negative side, indicating their correlation with slower germination times and lower vigor. As a result, PC2 made a small contribution to treatment differential based on G% magnitude, but PC1 mainly reflected a gradient from slow to quick germination.

**Mango (M) seeds**

96.05% of the variation in Mango (M) was explained by the first two components (PC1 = 73.71%, PC2 = 22.34%). Similar to Lemon (L) seeds, the loading pattern showed a positive correlation between G% and MGR and an inverse correlation with MGT, suggesting that larger germination percentages were associated with faster germination. Nonetheless, compared to Lemon seeds, the characteristic vectors were more closely matched, suggesting a greater interaction among germination traits. While BC at 1 and 5 g/petri were separated toward the negative side of the axes, indicating slower and less uniform germination, AgNPs (0, 1, 5, 10 mg/L) treatments were grouped in the direction of G% and MGR, indicating improved germination performance. Compared to Lemon (L), the total dispersion of treatments was narrower in mango (M), indicating more stability and less variance in response to treatments.

**Table 4**

*Summarized results of the multivariate relationships between treatments and their effects on germination variables*

Aspect	Lemon	Mango
Explained variance	96.52% (PC1 = 77.8%)	96.05% (PC1 = 73.7%)
Trait relationships	G% and MGR strongly positive; both opposite to MGT	Same relationship but even stronger correlation
Trait independence	- Vectors more spread - traits somewhat independent	- Vectors closer - traits highly interrelated
Treatment effects	T3–T6 improved germination; T7–T9 slower	T1–T4 improved germination; T9, T12 slower
Overall sensitivity	- Greater variability - seeds more responsive to treatment differences	- Lower variability - seeds more stable but slightly less responsive

**Comparative Description:**

For both crops, the PCA results show a consistent physiological trend: treatments that increased germination percentages also decreased mean germination time and increased germination rate. However, mango (M) displayed a more compact pattern, indicating more consistent performance across treatments, whereas Lemon (L) displayed a wider dispersion of treatment scores, indicating more susceptibility to treatment effects. Mango (M) exhibits greater stability and resilience under the applied treatments, while Lemon (L) appears to be more responsive yet varied.

### *Connection to PCA and Correlation*

The clustering pattern confirms what you would anticipate from correlation or PCA results: similar treatment groups are shaped by variables that move together, such as germination time (MGT) for both plant species and germination percentage for mango seeds.

## 4 DISCUSSION

It is suggested that organic biochar and nanomaterials will be the materials of the future, especially in the agricultural field. Many issues could be resolved by using these materials more wisely in crop production. However, before making a broad suggestion, a complete understanding of how biochar and nanoparticles interact with plants is needed. One more technique that scientists frequently use to determine germination speed is mean germination time (MGT) (**Chen *et al.*, 2013, Zhang *et al.*, 2014**). Fresh mango seeds produced embryos that germinated incredibly quickly; nearly all of them did so in two to four days. With the treatment of Ag nanoparticles, germination increased steadily; the overall germination rate was  $94.4 \pm 13.6$  ( $\pm$ standard deviation), while for biochar applications it was  $88.9 \pm 17.2$ .

**Pražak *et al.* (2020)** discovered that quick and uniform germination was the immediate benefit of low doses of AgNPs (0.25, 1.25 mg dm<sup>-3</sup>). Although the greatest concentration of AgNPs tested showed a substantial antibacterial impact and was helpful during the seed germination phase, it was likely unfavorable for the plants at later stages of development. However, for both Ag NPs and biochar, MGT was found to be  $3.22 \pm 0.81$  and  $2.61 \pm 0.95$  per day, respectively (Fig. 3). At 40 mg/L of Ag NPs and 5% biochar additions, the maximum MGT was achieved. For both treatments, the values were two and one day, respectively. According to published statistics, both treatments were preferable for raising MGT, with average values ranging from two to four days (**Corbineau *et al.*, 1986**). **Yin *et al.* (2012)** studied the effects of varying levels of AgNPs (0, 1, 10, 40 mg/L) on the germination and growth of various species of plants utilizing an assay without soil. They discovered that although 40 mg Ag/L GAAGNP exposure dramatically decreased the MGR of 3-species and increased in one, PVPAgNPs did not affect germination. According to their findings, the cytotoxic effect on seed

germination should be considered alongside the coating materials, AgNP particle size, and plant species.

Unlike Mango, lemon showed low germination of embryos from fresh seeds, unexpectedly, biochar shows a lower effect compared to the control. This may be due to the antagonism between lemon seed compound and biochar properties. This observation needs more studies to answer this questionable finding. The nutrients that were present in the generated biochar together with the phenolic compound, as indicated in Table 1, may also corroborate this finding. Because biochar is composed of organic leftovers from olive pomace, it may contain unwanted molecules called phenolic compounds, which could lower or even eliminate the germination rate when compared to the control (**Thies and Rillig, 2009**). Because they contain vital nutrients for plants, specific substances in biochars have the ability to either promote or restrict seed germination and seedling growth (**Gaskin et al., 2008**). Generally speaking, the obtained results gained from applied AgNPs and biochar on mango seed showed that biochar has a superior positive effect on mango seed germination properties. This could be attributed mainly to the existence of nutrient contents of biochar (Table 1). However, the same addition rates of both treatments could make a negative effect in the case of lemon seed germination. One of the major problems to citrus seed production, especially lemon, is the quick loss of seed germinability. According to **Bajpai et al. (1963)**, lemon seeds held for 15 days lost 50% of their germination viability, and after 3 months, they were completely non-viable. Numerous researchers have shown that the primary cause for low storage ability in lemon seeds is their shriveling sensitivity (**Saipari et al., 1998**). Therefore, the lower concentrations of both treatments could provide a positive effect on lemon seed germination rates. This study opens a new door for investigating the effect of the novel applications of silver nanoparticles and biochar in different fruit seed germinations taking into account that each fruit species require individual experimental studies to determine the promising additions of both treatments.

According to **Parveen and Rao (2015)**, pre-sowing *Pennisetum glaucum* seeds with AgNPs at levels of 20 and 50 mg dm<sup>-3</sup> for two hours stimulated the germination rate of the seeds. These results are consistent with the present results, despite the fact that the amount of AgNPs used to treat the mango and Lemon seeds were lower. The results of the seed germination test, however, showed that AgNPs at very low concentrations ( $\leq 10$  mg dm<sup>-3</sup>) greatly enhance the mean germination time, seed germination index, seed

vigour index, and seed germination ability. These findings are consistent with those of **Hojjat (2015) and Michalek *et al.* (2018)**. In both ideal and stressful settings for this process, **Rajjou *et al.* (2012), Chen and Arora (2013), Panyuta *et al.* (2016), and Mahakham *et al.* (2017)** have likewise found a favorable effect of low levels of AgNPs on seed germination. The process by which AgNPs improve seed germination has not been sufficiently elucidated.

At least three mechanisms are suggested by the findings of earlier research. Most likely, the first one occurs at the point of controlling gene expression. Silver nanoparticles activate genes for aquaporin's, membrane-spanning proteins that facilitate the passage of gasses, water, and reactive oxygen species (ROS) across biological membranes (**Maurel *et al.*, 2015**). The **second** occurs when gibberellins activate the production of gibberellins and activate hydrolytic enzymes, such as lipases, proteases, and  $\alpha$ -amylase (**Feregrino-Perez *et al.*, 2018**). High-molecular-weight organic molecules are hydrolyzed by these enzymes to produce low-molecular-weight chemicals that serve as respiratory substrates for embryo cells. Furthermore, increases in monosaccharide and other low-molecular-weight compounds increase cells' osmotic ability, which speeds up the absorption of more seed components and storage resources. **Mahakham *et al.* (2017)** observed that germination of seeds primed in an AgNPs solution activated  $\alpha$ -amylase and improved water absorption. AgNPs' **third** mode of action might entail altering the correlation between the embryo cells' antioxidant system activity and the degree of oxidative stress (**Kibinza *et al.*, 2011 & Mahakham *et al.*, 2017**). AgNPs increase the synthesis of reactive oxygen species (ROS), including hydrogen peroxide and hydroxyl radicals, which activates the antioxidant enzymes catalase and superoxide dismutase in cells. These changes condition the right amount of ROS in embryo cells, creating what is known as "Oxidative window," which is necessary to trigger signal pathways that initiate seed germination. The direct influence of AgNPs and/or their physical and biochemical activation of the seed germination phase also accelerate the physiological phase of this process, which involves the differentiation and growth of seedlings.

Regarding the biochar used in this work, the increased rate of Lemon and mango germination was comparable to that of **Hilioti *et al.* (2017)**, who found that seeds containing 5% castor stalk biochar germination was higher than that of the control. Furthermore, the rising rate of Lemon and mango germination with increasing biochars is consistent with the findings of **Kanwal *et al.* (2018)**, who found that adding 1% and

2% biochar made from sawdust and dry leaf debris increased the germination of wheat seeds.

The impact of biochar on plants may vary based on the kind of biochar, the type of plant, and the pace of application (**Kanwal et al., 2018 and Rawat et al., 2019**). According to **Ogunremi et al. (2023a&b)**, plants can react differently to different types of biochars. In their study, they found that *Zea mays* interacted better with all types of biochars than *Pennisetum glaucum*, which only interacted with rice and sorghum biochars.

Early seedling growth and seed germination could be significantly impacted by the availability of certain minerals, which could explain the improved seed germination seen in the current study (**Das et al., 2020**). This factor can be linked to **Das et al. (2020)**, who found that biochar boosted the minerals in the soil, hence improving *Zea mays* and Pearl mille seed germination and early growth. **Bu et al. (2020)** noted that the rate of seed germination was reduced when biochar was applied at 1% and 2%, which is consistent with the possibility of a fluctuating mango germination rate seen in the current study. Additionally, **Li et al. (2017)** found that, in comparison to the control, tomato germination was unaffected by lower concentrations of oak biochar (between 1% and 4%). The detrimental reaction of plants to seed germination and early seedling growth may also be caused by biomass-derived biochars that include toxic chemicals (**Thomas & Gale 2015**).

**Marzouk (2017)** looked into how biochar and silver (Ag) nanoparticles (NPs) affected the seed germination and early seedling development rate. The findings revealed that all treatments had substantially greater seed germination rates than the control. Although they showed increasing values of seed germination as opposed to the control, higher concentrations of AgNPs and biochar initially inhibited seed germination compared with the lesser amounts. However, the maximum germination values were obtained when either 2 g of petri dish biochar or 20 mg AgNPs l-1 were added. The empirical model for root growth, however, confirms that compared to the seed first treated with AgNPs, the seed initially treated with 2 g/petri dish biochar showed superior root growth. Therefore, it is recommended to apply both treatments (biochar and AgNPs) at lower concentrations to improve seed germination.

Overall, the PCA shows that while excessive concentrations had a negative impact on these parameters, moderate levels of AgNPs and BC improved germination properties (greater G% and MGR, lower MGT) in both Lemon and mango seeds. Additionally, the

grouping pattern indicates that Lemon (L) seeds responded to treatments somewhat more strongly and consistently than mango (M) seeds, suggesting that there may be genotypic difference in tolerance or responsiveness to treatments with nanomaterials and biochar.

## 5 CONCLUSIONS

Trees that are economically significant for agroforestry use are mango and lemon. As a result, we should consider improving its seed germination processes through the use of innovative ways. Mango and lemon seed germination metrics, including germinability, mean germination time, and mean germination rate, were increased by varying the quantities of AgNPs and biochar generated from olive pomace. In the instance of mango seed, the results demonstrated a notable improvement in seed germination characteristics when compared to the control of both AgNPs and biochar. When compared to other treatments, the MGR and MGT data indicated that biochar at the highest concentration (5 g Petri dish<sup>-1</sup>) produced encouraging effects. Nonetheless, the maximum mango seed germination values were also seen at a concentration of 40 mg AgNPs l<sup>-1</sup>. The presence of nutrients in biochar may be the reason for the increased germination of mango seeds following its addition. Lemon seed germination was negatively impacted by the addition of AgNPs and biochar, which is in contrast to the outcomes of mango seed germination. In fact, the toxicity of AgNPs and the presence of certain phenolic compounds in biochar may affect the germination of lemon seeds. The method by which AgNPs and biochar treatments improve lemon germination actually need more research. To determine the effect of various soil characteristics on AgNPs and biochar behaviors in interaction with mango and seed, the present findings should be further examined under soil conditions.

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### Authors' Contribution

All authors contributed equally to the development of this article.

### Data availability

All datasets relevant to this study's findings are fully available within the article.

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