Effect of Seed Coat Removal and Nursery Growing Media on the Growth and Leaf Nutrient Content of Mango Seedling Rootstocks

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ABSTRACT

Research on the growth of containerized mango seedlings at the nursery level has been extensively documented in the literature. However, information on mango leaf nutrient content remain limited. In 2015, a container-based experiment was conducted under a Completely Randomized Design (CRD) with factorial arrangements and three replications to investigate the growth parameters and leaf nutrient content of mango seedlings in response to seed coat removal and different growing media. Seeds of the 'Sindhri' variety were used, divided into two groups: corticated (with seed coat) and decorticated (without seed coat). Each group was planted in one of four growing media: GM_1 (soil), GM_2 [bagasse (70%) + cocopeat (5%) + canal silt (25%)], GM_3 [bagasse (60%) + cocopeat (5%) + canal silt (35%)], and GM_4 [bagasse (20%) + cocopeat (20%) + canal silt (30%) + press mud (30%)]. The analysis indicated that seed coat removal and growing media exhibited non-significant interactions for most parameters, but were significant as main effects. Decorticated seeds outperformed in nearly all growth and leaf nutrient content parameters. Among the growing media, GM_2 demonstrated superior performance in terms of seed germination, germination index, seedling vigor index, seedling height, stem diameter, number of leaves per seedling, and leaf chlorophyll content. However, GM_4 resulted in higher concentrations of nitrogen, phosphorus, potassium, and magnesium in the leaves. In conclusion, decorticated seeds and the GM_2 medium were optimal for seed germination and growth, while GM_4 was most effective in enhancing leaf nutrient content.

Introduction

Mango, a well-known fruit from the family Anacardiaceae, is scientifically known as *Mangifera indica* L. This tropical fruit is considered pivotal and economically significant on a global scale. In Pakistan, mango cultivation ranks second, following citrus (Raza et al., 2017; Badar et al., 2019). Commercially, mango is propagated through grafting, with the rootstock and scion being the two main components that combine to grow as a single plant. Rootstocks are typically produced from mango seeds (seedling rootstocks), upon which the grafting of the desirable variety (scion) is performed. These rootstocks provide the root system to the grafted plant and have a profound influence on the vigor, longevity, and productivity of the scion variety (Kaur, 2017).

Traditionally, rootstocks were grown in soil, but improved plantation technologies have been adopted due to various challenges. Salinization

and the conversion of fertile agricultural areas into non-productive lands are major concerns in Pakistan. Addressing these issues requires a combination of better agricultural practices, appropriate use of fertilizers and pesticides, efficient irrigation techniques, and enhanced soil management strategies (Safdar et al., 2019). These factors significantly impact agricultural production and output (Harhash et al., 2022). Based on improved plantation technology, the trend of mango cultivation has shifted from open field planting to container or polyethylene-grown plants in shade houses, using various growing media as a potential solution to soil-related problems (Khan et al., 2006; Dhaliwal et al., 2004).

For raising seedling rootstocks in containers, growing media is a crucial and basic requirement for containerized seedling production. Selecting the appropriate media components is essential for the proper growth and quality of the seedlings. Various organic materials, such as bagasse, sawdust, and cocopeat, as well as inorganic materials, such as sand, perlite, and canal silt, are used in different ratios for formulating growing media. The composition and formulation of each growing medium may depend on the type of crop, locality, and availability (Donovan et al., 2016). Many commercial growing media are available in advanced countries for raising and establishing healthy seedlings of various crops. However, the availability of commercial media poses a significant challenge for raising mango rootstock seedlings in developing countries.

The delay in mango seed germination is typically attributed to the hard seed coat, which is impermeable to water and gases, as well as to imbalances in growth hormones (Basra et al., 2005). Pre-sowing treatments, such as removing the endocarp (decorticated seed), are considered effective for improving mango seed germination, as reported by Muralidhara et al. (2015) and Pinto et al. (2018). In the present study, both decorticated and corticated seeds were used for plantation. Decorticated seeds have been referred to by different scientists as husked seeds, decaoted seeds, decaoted stones, or seeds with the removal of the endocarp or seed coat—all of which have the same meaning. This study was thus designed to formulate and evaluate the effect of growing media, along with corticated and decorticated seeds, on the growth and leaf nutrient content of seedling rootstocks.

Materials and Methods
The experiment was conducted under the agro-ecological conditions of Tandojam, Sindh, Pakistan (25°25'60"N, 68°31'60"E, 19.5 m altitude) to evaluate the growth and nutritional status of mango seedling rootstocks. A factorial-based Completely Randomized Design (CRD) was employed, incorporating two factors: seed coat removal and growing media.

Seed coat removal
Mango fruits of the 'Sindhri' variety were sourced from a healthy commercial orchard and allowed to ripen. Once fully ripened, the fruits were washed and air-dried at room temperature for one week. After drying, the fruits were divided into two groups: corticated (with the endocarp) and decorticated (without the endocarp). To obtain the decorticated seeds, the endocarp was carefully removed using a sharp knife.

Growing media
Available low-cost materials of the locality viz. bagasse, cocopeat and press mud were used for plantation. The bagasse was taken from Matiari Sugar mill of the province, Sindh, Pakistan. For cocopeat, compressed bricks were purchased and for loosening the bricks of cocopeat, they were immersed in water. After the water was squeezed from the material, the cocopeat underwent the procedure of drying. Following formulations of growing media were prepared.

1. Soil (traditional method) (GM_1)
2. Bagasse (70 %) + Cocopeat (5%) + Canal silt (25%) (GM_2)
3. Bagasse (60%) + Cocopeat (5%) + Canal silt (35%) (GM_3)
4. Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%) (GM_4)

Analysis of growing medium
Three samples from each growing medium were analyzed for various physico-chemical properties. Electrical conductivity (EC) and pH were measured using the saturated media extract method (Warncke and Krauskopf, 1983). Water holding capacity was determined following the method described by Huang and Fisher (2005), while air-filled porosity (%) was measured using the saturation and drainage method (Yahya et al., 1997). Organic matter content (%) was analyzed by the Walkley–Black method (Nelson and Sommers, 1982). All analyses were conducted in the Laboratory of Soil Science, Sindh Agriculture University, Tandojam.

Evaluation of seedling rootstocks in relation to growth parameters

The evaluation of the growth parameters was done weekly and continued for six months. Seed germination was examined every week for up to a month of plantation, and the percentage of the germination was calculated using Larsen and Andreasen (2004).

Germination percentage (GP) = $\Sigma n / N \times 100$

Where n denotes the count of germinated seeds at each count, while N represents the overall number of seeds per treatment. Germination time (d) was calculated using a formula by Ellis and Roberts (1981).

MGT = $\sum Dn / \sum n$

Where n is the number of seeds germinated on day D, and Dn is the number of days as counted from the beginning of germination. Germination index (GI) was calculated by the formula given by the Association of Official Seed Analysts (1983).

GI = Number of germinated seeds/Days of first count + . . . + . . . + Number of germinated seeds/days of last count

For seedling height, five random plants of each treatment were sampled for seedling examination at 30 d intervals. Stem diameter was also recorded with digital vernier caliper at the center, top and bottom of the stem, and mean values were calculated. Random leaves were considered for chlorophyll content analysis using a portable chlorophyll meter via SPAD 502.

Analysis of leaf nutrient content

The total nitrogen content was determined using Kjeldahl's method. For the analysis of phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) in leaf samples, the samples were digested in a 1:5 mixture of perchloric acid ($HClO_4$) and nitric acid (HNO_3) and left overnight. The following day, the samples were digested further on a hot plate until white fumes appeared, following the protocol by Zarcinas et al. (1987) and Estefan et al. (2013). After cooling, the volume of each digest was adjusted to 50 mL. Phosphorus was quantified using the vanadomolybdo-phosphoric acid yellow color method (Cottenie, 1980) with a spectrophotometer. Potassium was analyzed using a flame photometer, as per the method of Knudsen et al. (1982). The concentrations of

calcium and magnesium were determined using the EDTA titration method (Richards, 1954).

Statistical analysis

Data pertaining to the effects of four growing media and two seed coat removal treatments, along with three replications, were analyzed using Statistix Software (Ver. 8.1). The least significant difference (LSD) test was employed to compare treatment mean values for determining treatment superiority.

Results

The study evaluated the effects of the interaction between seed coat removal and growing media, as well as the independent (main) factors, on various growth and nutrient content parameters of mango seedling rootstocks. Most parameters assessed were found to be non-significant in their interaction, with the exception of seed germination-related observations. Consequently, the results were discussed based solely on the main effects (P<0.05).

Decorticated seeds demonstrated superior performance across all recorded parameters, including seed germination (75.8%), germination time (20.41 d), germination index (10.85), seedling vigor index (3406.9), seedling height (32.85 cm), stem diameter (8.74 mm), leaves per seedling (10.51), chlorophyll content (50.07 relative greenness), leaf nitrogen content (1.115%), phosphorus content (0.139%), potassium content (0.854%), calcium content (2.634%), and magnesium content (0.337%), compared to corticated seeds (Figs. 1-11).

The growing media responded differently across all growth and nutrient content parameters. The growing medium GM_2 demonstrated superior results in seed germination and seedling growth parameters. However, the outcomes varied for leaf nutrient content. The data presented in Figures 1, 2, 5, and 12 show that GM_2 exhibited the highest seed germination rate (78.85%), the earliest germination time (19.58 d), maximum seedling height (33.96 cm), and highest leaf calcium content (2.712%) compared to the other growing media treatments. In contrast, GM_1 had the lowest seed germination rate (67.09%), took the longest time to germinate (24.25 d), had the shortest seedling height (27.45 cm), and the lowest leaf calcium content (1.505%).

Figures 3 and 4 indicate that the germination index (11.33 and 10.1) and seedling vigor index (3359.8 and 3407.8) were more stable for GM_2 and GM_4, reflecting better germination and seedling growth. In contrast, GM_1 recorded the lowest germination index (9.88) and seedling

vigor index (2894.3). Additionally, GM$_2$ produced the largest stem diameter (9.44 mm), the highest number of leaves per seedling (10.75), and the

greatest chlorophyll content (50.11 g). These results, however, were comparable to those obtained from the GM$_3$ medium (Figs. 6-8).
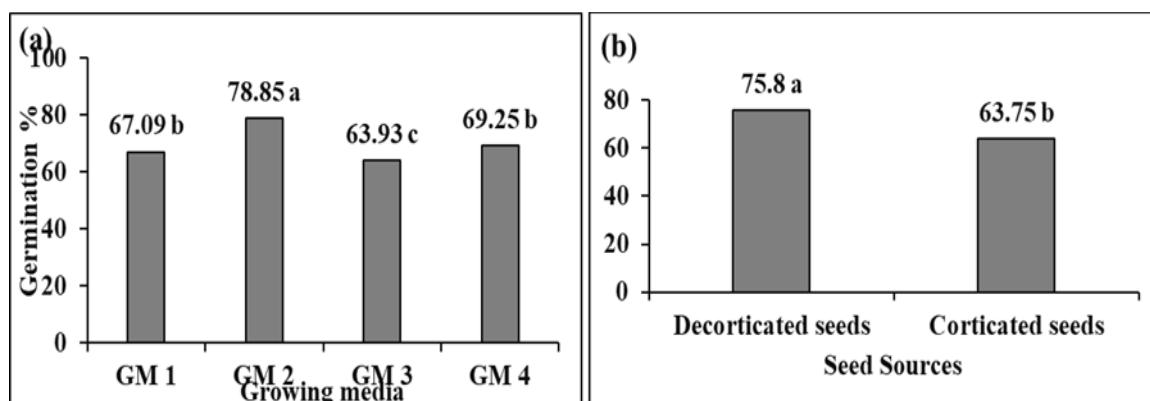

Fig. 1. Seed germination (%) of the seedling rootstocks of mango in response to varying growing media (a) and Seed coat removal (b). GM$_1$- Soil (traditional method), GM$_2$- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM$_3$- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM$_4$- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).
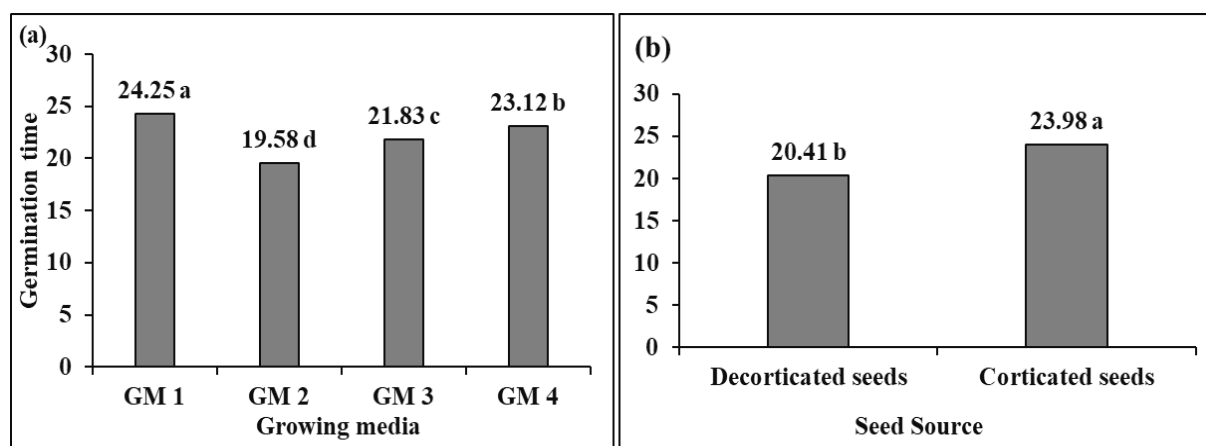

Fig. 2. Seed germination time of mango in response to varying growing media (a) and seed coat removal (b). GM$_1$- Soil (traditional method), GM$_2$- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM$_3$- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM$_4$- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).
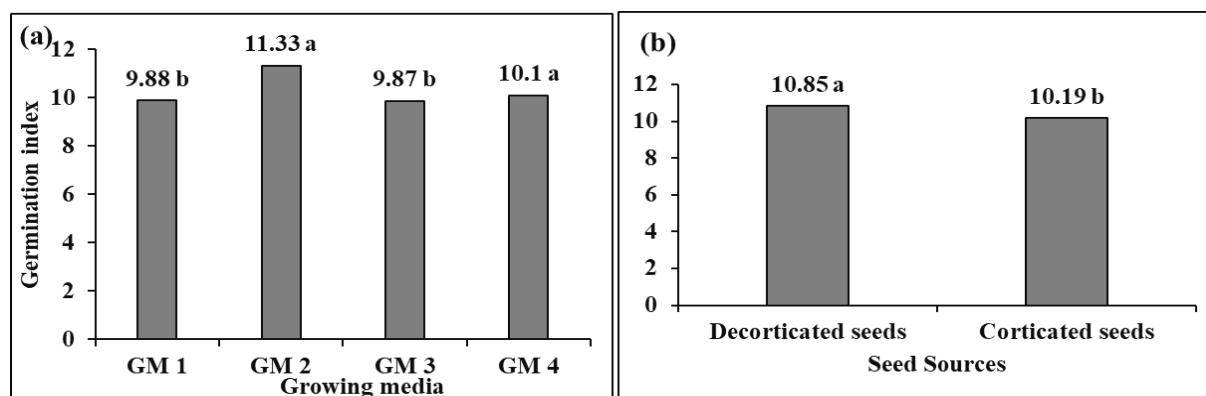

Fig. 3. Germination index of mango seedlings in response to varying growing media (a) and seed coat removal (b). GM$_1$- Soil (traditional method), GM$_2$- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM$_3$- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM$_4$- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).
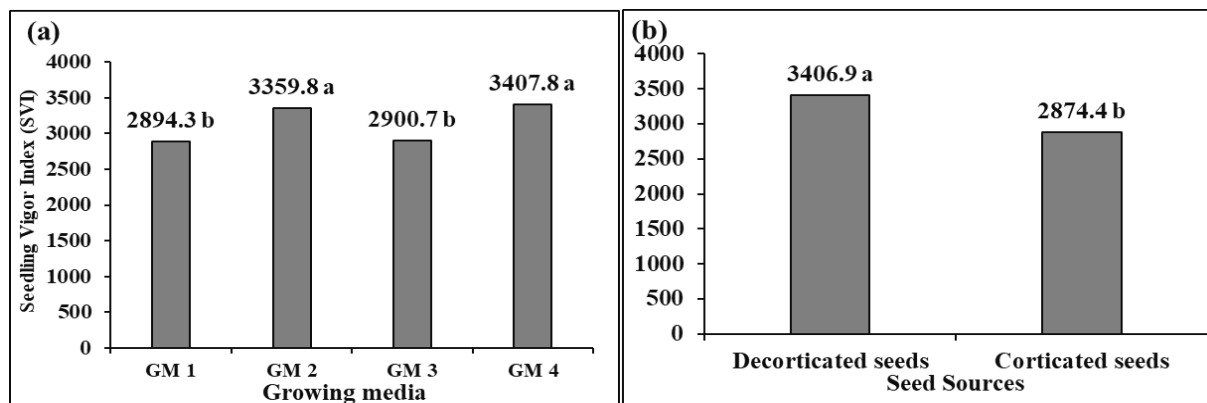
Fig. 4. Seedling vigor index of mango seedling rootstocks in response to varying growing media (a) and seed coat removal (b). GM1- Soil (traditional method), GM2- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM3- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM4- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).
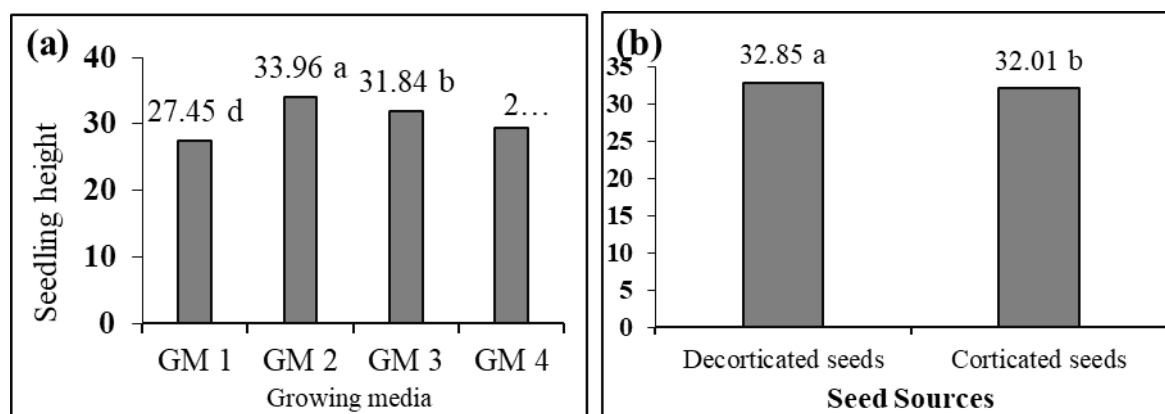

Fig. 5. Seedling height of the mango seedling rootstocks in response to varying growing media (a) and seed coat removal (b). GM1- Soil (traditional method), GM2- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM3- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM4- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).
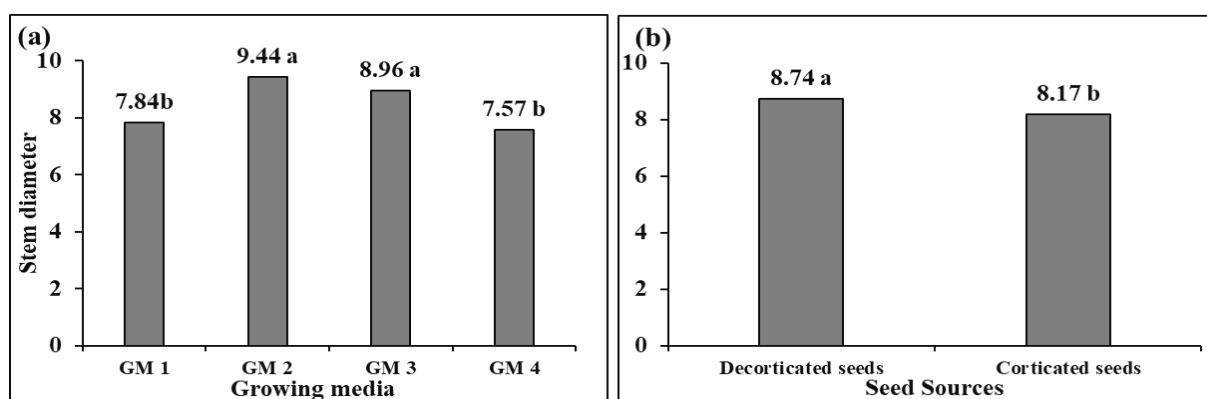

Fig. 6. Stem diameter of mango seedling rootstocks in response to varying growing media (a) and seed coat removal (b). GM1- Soil (traditional method), GM2- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM3- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM4- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).
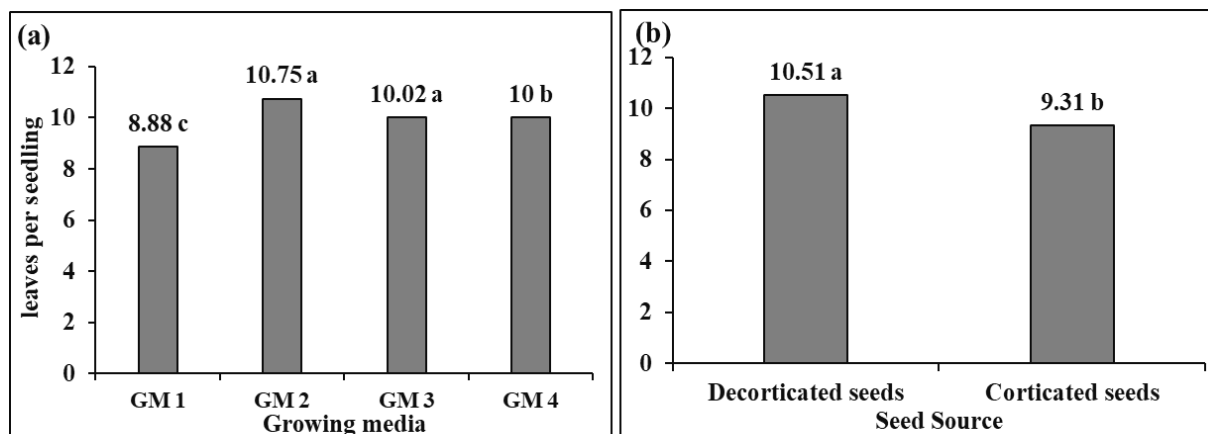
Fig. 7. Leaves per seedling rootstock of mango in response to varying growing media (a) and seed coat removal (b). GM$_1$- Soil (traditional method), GM$_2$- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM$_3$- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM$_4$- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).
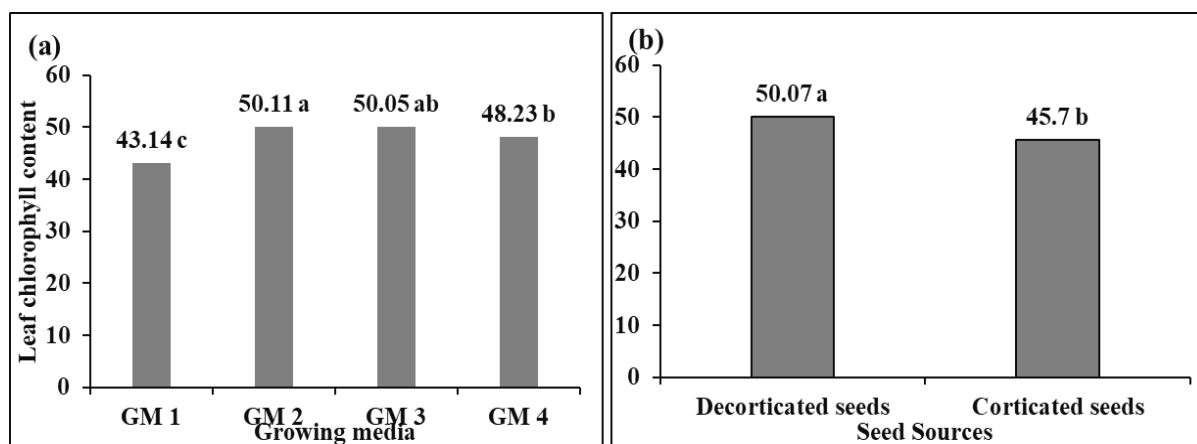

Fig. 8. Leaf chlorophyll content of mango seedling rootstocks in response to varying growing media (a) and seed coat removal (b). GM$_1$- Soil (traditional method), GM$_2$- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM$_3$- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM$_4$- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).
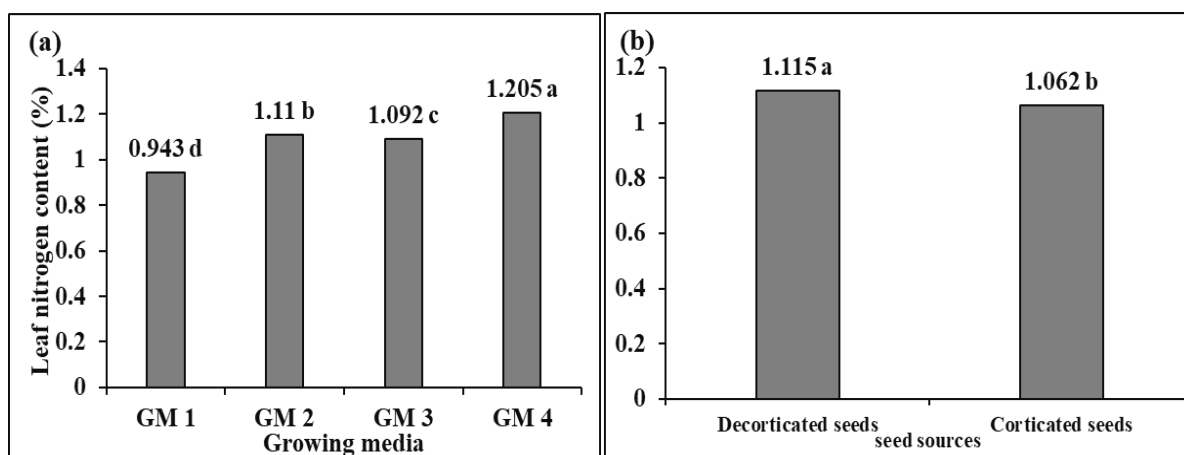

Fig. 9. Leaf nitrogen content (%) of mango seedling rootstocks in response to varying growing media (a) and seed coat removal (b). GM$_1$- Soil (traditional method), GM$_2$- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM$_3$- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM$_4$- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).

Fig. 10. Leaf phosphorous content (%) of mango seedling rootstocks in response to varying growing media (a) and seed coat removal (b). GM$_1$- Soil (traditional method), GM$_2$- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM$_3$- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM$_4$- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).
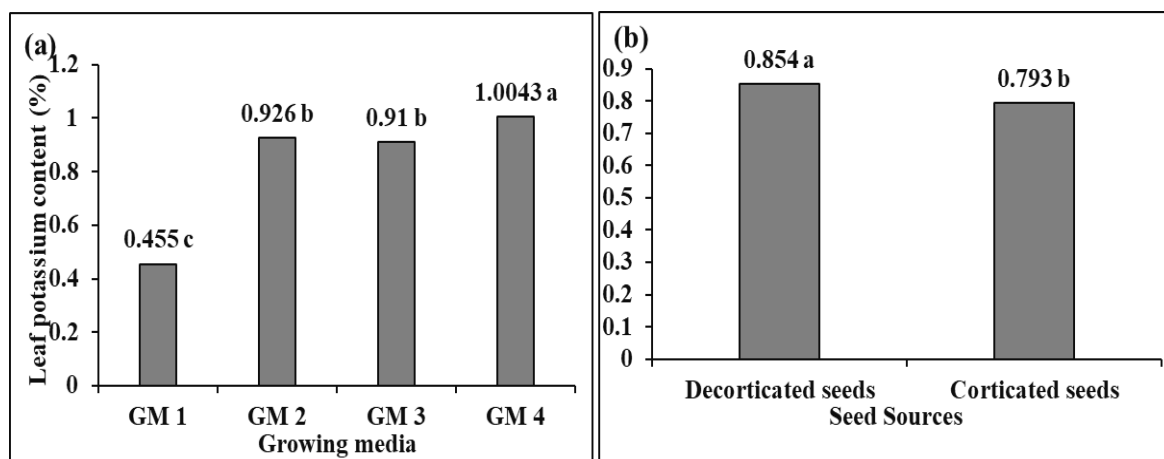
Fig. 11. Leaf Potassium Content (%) of mango seedling rootstocks in response to varying growing media (a) and seed coat removal (b). GM$_1$- Soil (traditional method), GM$_2$- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM$_3$- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM$_4$- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).
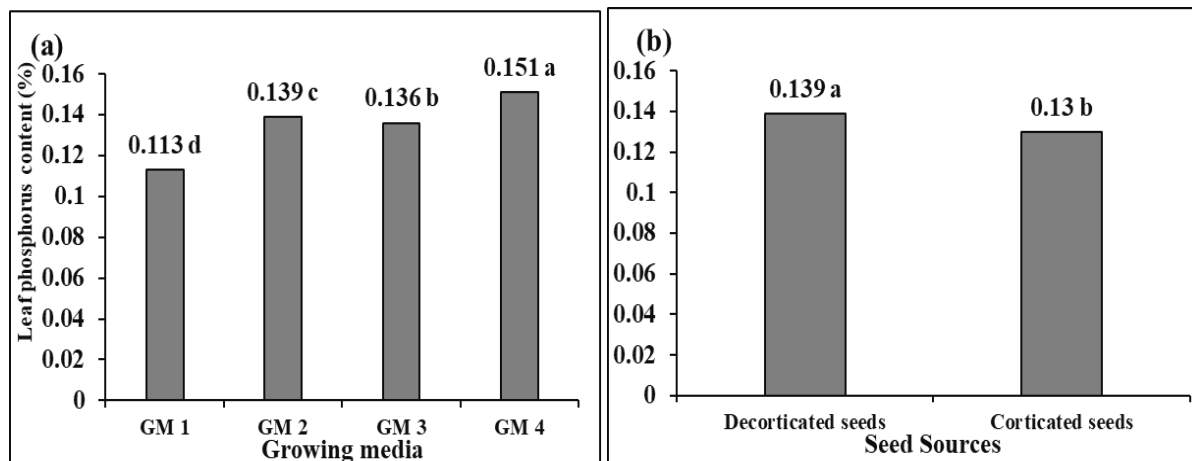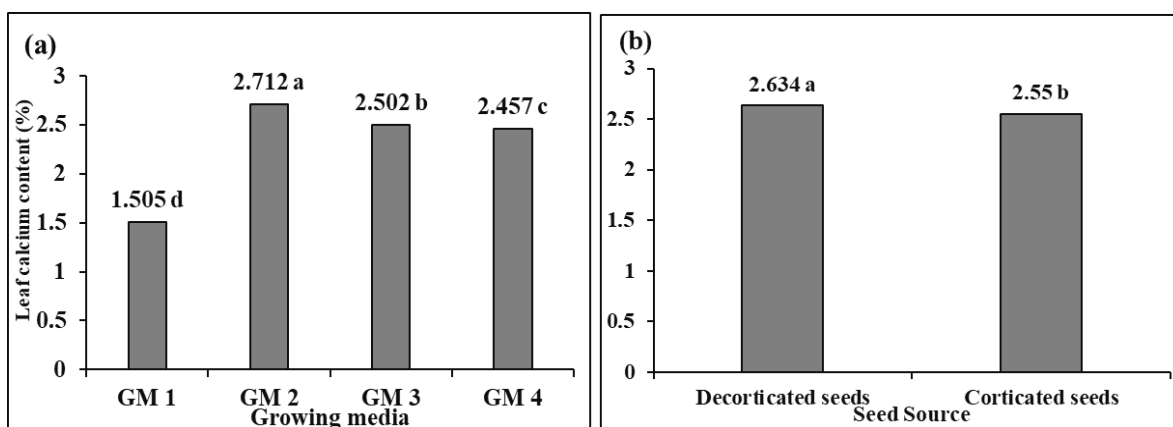

Fig. 12. Leaf calcium content (%) of mango seedling rootstocks in response to varying growing media (a) and seed coat removal (b). GM$_1$- Soil (traditional method), GM$_2$- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM$_3$- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM$_4$- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).

The leaf nutrient content showed a distinctly different response compared to seed germination and seedling growth parameters (Figs. 9-13). Nutrient levels, including nitrogen (1.205%), phosphorus (0.151%), potassium (1.0043%), and magnesium (0.387%), were observed to be higher in GM$_4$, all falling within the critical ranges reported by various researchers. Even the lowest values for nitrogen (0.943%), phosphorus (0.113%), potassium (0.455%), and magnesium (0.212%) remained within the critical range. Notably, calcium content deviated from this pattern, showing superior values in GM$_2$ compared to other growing media.

Fig. 13. Leaf magnesium content (%) of mango seedling rootstock in response to varying growing media (a) and seed coat removal (b). GM$_1$- Soil (traditional method), GM$_2$- Bagasse (70%) + Cocopeat (5%) + Canal silt (25%), GM$_3$- Bagasse (60%) + Cocopeat (5%) + Canal silt (35%), GM$_4$- Bagasse (20%) + Cocopeat (20%) + Canal silt (30%) + Press mud (30%).
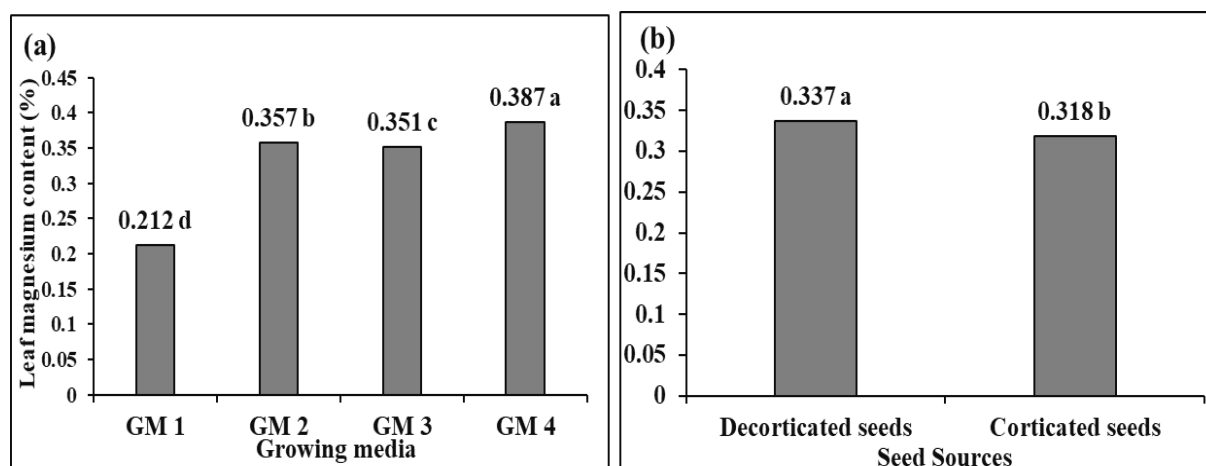
Discussion

Growing media play a pivotal role in improving the physical and chemical properties of the soil, enhancing root penetration and nutrient uptake (Khan et al., 2016). Various materials are used to create suitable media for raising containerized mango seedlings, combining organic materials like peat, sawdust, rice hulls, coconut husk, leaf manure, bark, sugarcane waste, and inorganic materials like sand, canal silt, perlite, and vermiculite (Aklibasinda et al., 2011; Donovan et al., 2016; Gulcu et al., 2010; Indriyani et al., 2011; Khan et al., 2008; Mhango et al., 2008; Wilson et al., 2001). In this study, different growing media were used to sow decorticated and corticated seeds of Sindhri mango, producing distinct results. GM$_2$, consisting of 70% bagasse, 5% cocopeat, and 25% canal silt, was identified as the best medium in terms of seed germination, germination index, seedling vigor index, number of leaves, stem diameter, seedling height, and chlorophyll content. The compactness of GM$_2$, attributed to the inclusion of bagasse and cocopeat, may have contributed to its efficacy, as these materials possess favorable physical and chemical properties for seedling nurseries (Anonymous, 2016; Sarkar et al., 2005).

Bagasse is known to contain significant amounts of iron, manganese, calcium, magnesium, silicon, and phosphorus, making it a valuable substitute for organic matter (Dotaniya et al., 2016). Similarly, cocopeat has good nutritional qualities and is effective as a growing medium, particularly for mango seedlings, due to its high potassium content and excellent water retention capacity (Abad et al., 2002; Chand et al., 2011; Deepak et al., 2018). In contrast, Khan et al. (2017) reported better seed germination using a medium composed of bagasse, sand, and pine bark. Memon et al. (2017) found that seed germination and germination time were unaffected by varying media, using press mud in different proportions with bagasse, canal silt, and cocopeat. Farooq et al. (2018) achieved better results with a medium of 50% bagasse, 25% canal silt, and 25% cocopeat for grape plants. Ul Haq et al. (2017) found a seedling survival rate of 94% using a medium of 70% bagasse, 25% silt, and 5% coconut fiber, while the highest stem girth and seedling height were also observed with this combination. Memon et al. (2017) recorded higher seedling height (31.3 cm), stem diameter (7.2 mm), and number of leaves (21.3) in four-month-old seedlings using a medium of 30% press mud, 30% silt, 20% bagasse, and 20% coconut fiber.

Decorticated seeds yielded better germination results and germinated more quickly. This is likely due to the removal of the stony endocarp, which often inhibits or delays seed germination

(Deepak et al., 2018; Pinto et al., 2018). Removing the endocarp promotes faster germination and the emergence of more upright seedlings, which improves graft quality. While removing the thin tissue covering the cotyledons has no significant effect on germination success, Muralidhara et al. (2015) reported earlier and higher germination rates from decorticated mango seeds.

Seedling rootstocks were analyzed for their leaf nutrient content and compared to critical levels reported by various researchers (Table 1). The results for nitrogen fell within the critical ranges, as reported by different scientists. The phosphorus and potassium levels also fell within the established ranges, with the exception of the values reported by Stassen et al. (1999) for phosphorus, which were much higher (1.45%) than other critical ranges. Calcium levels were within the critical range, except for the values reported by Young and Sauls (1981), who suggested a range of 3.00–5.00%. Similarly, Stassen et al. (1999) reported much higher magnesium levels than those found in other studies. In this study, magnesium levels ranged from 0.212% to 0.387%. These variations may be attributed to differences in tree age and plant growth stage, as nutrient analyses are typically conducted on established orchard trees, while this study focused on seedlings (Zuazo et al., 2006; Puranik et al., 2017; Faria et al., 2016).

Zuazo et al. (2006) evaluated the nutrient content of grafted and ungrafted mango rootstocks, grafting two mango varieties (Keitt and Osteen) onto Gomera 1 and Gomera 3 rootstocks. They recorded higher nitrogen (2.07%) and phosphorus (0.15%) levels in the G3-Keitt combination compared to other scion-rootstock combinations, while potassium (0.64%) was higher in the G1-Keitt leaves. Sukthumrong et al. (2000) observed nitrogen levels of 0.8-1.9%, phosphorus at 0.1-0.8%, potassium at 0.1-1.3%, calcium at 0.6-3.5%, and magnesium at 0.2-0.7% in leaf samples from Nam Dok Mai mango. For non-grafted rootstocks, Zuazo et al. (2006) found that Gomera 1 had higher nitrogen (1.40%), phosphorus (0.07%), and potassium (0.41%) compared to Gomera 3.

Table 1. Critical levels/range of nutrient content in mango leaves as reported in the literature.

Nutrient element	Samra et al. 1978	Young and Koo (1971) Young and Sauls (1981)	Catchpole and Bally (1995)	Robinson et al. (1997)	Stassen et al. (1999)	Poffley and Owens, 2005
Nitrogen (%)	0.95-1.45	1.00-1.50	0.80-1.90	1.00-1.50	1.25	0.8-1.2
Phosphorus (%)	0.03-0.12	0.09-0.18	0.12-1.30	0.080-0.18	1.45	0.08-0.18
Potassium (%)	0.40-0.77	0.50-1.00	0.40-2.50	0.30-1.20	0.10	0.4-1.2
Calcium (%)	1.74-3.45	3.00-5.00	1.50-2.80	2.00-3.50	0.80-1.05	1.5-2.8
Magnesium (%)	0.22-0.75	0.15-0.47	0.20-0.40	0.15-0.40	2.08	0.2-0.4

Conclusions

In conclusion, decorticated seeds demonstrated superior performance in terms of seed germination, growth parameters, and leaf nutrient content of the seedlings. This improvement is attributed to the removal of the seed coat, which typically inhibits germination and seedling growth. Regarding the growing media, the best results for seed germination and growth parameters were observed in GM$_2$, consisting of bagasse (70%), cocopeat (5%), and canal silt (25%). However, higher leaf nutrient content was achieved in GM$_4$, which comprised bagasse (20%), cocopeat (20%), canal silt (30%), and press mud (30%).

Conflict of Interest
The authors indicate no conflict of interest in this work.

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