Combined effects of provenance and NPK-fertilizer on nursery performance of kelor (*Moringa oleifera*) seedlings

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Abstract. Kelor (*Moringa oleifera*) is a superfood that serves potential benefits as a highly nutritious food. The research on kelor in Riau, Indonesia, is still very rare. This study aimed to determine the effect of a combination between provenances and doses of NPK-fertilizer on the growth of kelor seedlings in the nursery. The study was conducted from March to June 2020 in Kuok, Kampar regency, Riau. It was a factorial trial arranged in a split-plot design with three replications. Two provenances from Riau and NTT were arranged as the main plot. Four level doses of NPK-fertilizer, 5 g, 7 g, 9 g, and 0 g, were arranged as the subplot. Seedling height, diameter, survival, number of leaves, and leaf colour were observed. The interaction between P2 and F2 had the best effect on diameter at 3 MAS, while P2 and F4 had the best effect on survival at 1 MAS. The dose of 5 g NPK tended to have a better effect on the height, the number of leaves, survival, and leaf colour at 1 MAS. To stimulate the diameter growth, additional doses of NPK can be given after the kelor seedling reaches a minimum age of 3 MAS.

Keywords: growth, nutritious, food, moringa

1. Introduction

Kelor (*Moringa oleifera*) is an Indonesian endemic plant. The plant also spread around Asia, Africa, and America [1, 2]. Meanwhile, the origin of moringa distribution was estimated from Agra and Oudh, in the northwest region of India, south of the Himalayan Mountains [3, 4, 5]. Different places use different names for this plant. In Indonesia, it has many local names, such as kelor (Jawa, Sunda, Bali, Lampung), kerol (Buru); marangghi (Madura), moltong (Flores), kelo (Gorontalo); keloro (Bugis), kawano (Sumba), ongege (Bima); hau fo (Timor); marungga (Timor) [6]. Kelor has a 12 m maximum height, and it is categorized as an easy-to-grow plant due to its easy survival.

Kelor can be processed in various forms, such as pharmacy products and in flour form [7]. As a consumable plant, kelor has incredibly high and beneficial nutrition for the human body [8, 9]. The use of kelor for an additive or fortification can maintain the food quality so that it is still preferred by consumers [10, 11].

Riau’s food security index in 2019 occupies the 25th position from 34 Province. Furthermore, few districts in Riau are categorized as susceptible to food insecurity. Approximately 40 percent of the food needed by the people of Riau is supplied from outside Riau. Therefore, supplying food availability is an urgent program in Riau, both for its quantity and quality. Exploration of potential
sources of high nutrients food is required. One of the efforts is to explore kelor as a food source. Kelor is easy to grow in dry and marginal soil.

According to Ezekiel et al. [12], the several kelor provenances that have been tested had real different growth in survival, biomass production, diameter, and height. Fertilization using NPK on kelor also needs different doses in different environments. This study aimed to record the effect of the combination between provenance and NPK-fertilizer on the growth of kelor seedlings in the nursery.

2. Method of the research

2.1. Biological materials
This research was conducted in the nursery from March to June 2020 in Kuok, Kampar regency, Riau. It used two provenances of kelor, Riau, and NTT. Riau’s provenance comes from Dumai, while NTT’s provenance comes from Timor island. The seedling process was carried out in a composite way without being separated per tree. The seeds were sown directly into polybags, each polybag filled with two seeds. If all of the seeds grew up, the best would be selected. The nursery media used were topsoil and rice husk with a 4:1 ratio.

2.2. Nursery experiment
This study was a factorial experiment using a split-plot design. There are three replications with two factors as the treatments, and those were provenance and NPK-fertilizer dose. The details are as follows:

| Provenance: | NPK fertilizer: |
|-------------|-----------------|
| Riau (P1); NTT (P2) | 5 gram (F1); 7 gram (F2); 9 gram (F3); 0 gram (F4) |

Treatment combination from those two factors resulted in eight treatment combinations; each of them had 30 seedlings and was repeated three times so that the total seedlings were 8 x 30 x 3 = 720 seedlings. Fertilization was done once, along with seedlings sowing. All of the nursery activities were made in the permanent nursery that belongs to Forest Plants Fiber Technology Research and Development Institute of Kuok, Kampar Regency, Province of Riau.

2.3. Variable measurement and statistical analysis
The measurements were carried out on the growth variables, included seedling height, diameter (1 cm above the surface of medium), number of leaves, and life percentage. For the leaf colour, it was observed by categorizing it into three colours, namely, green, slightly yellow, and yellow. Measurement and observation were carried out on seedlings at one month and three months after sowing (MAS). Statistical Analysis was using SPSS 23 program. The final result would appear in the form of analysis of variance (ANOVA). Duncan Multiple Range Test (DMRT) was used as the post hoc test.

3. Result dan Discussion
The results of the analysis are presented for seedling's height, diameter, number of leaves, percentage of life, and leaf colour variable, and then discussed separately below. The results of the statistical analysis for all variables are presented in Table 1. The table shows the results of the variance analysis on the growth variables of kelor seedlings at the age of 1 and 3 months after sowing. In Table 1, the calculated F value and its significance value were listed for each variable, according to the source of variation and the time of measurement.

The sources of variation (provenance, fertilizer, provenance*fertilizer) were placed in the first column of the table, and a 5% significance level was used for the analysis. The table was divided into two sections which represented the difference time of measurement; those were one month after seed sowing and three months after seed sowing. Not significance (NS) means the factor used as treatment did not have a significant effect on the growth variable. The diameters of kelor seedlings were only measured once at the age of 3 MAS to avoid damage to weak seedling stems.
Table 1. Summary of analysis of variance for all treatments.

|                  | 1 MAS                                      |                                        | 3 MAS                                      |
|------------------|-------------------------------------------|----------------------------------------|-------------------------------------------|
|                  | Height (F, sig.)                          | Diameter (F, sig.)                     | Num of leaves (F, sig.)                   | % live (F, sig.)                            | Leaf color (F, sig.)                       |
| Provenance       | NS                                        | -                                      | NS                                        | NS                                        | NS                                        |
| Fertilizer       | (28.168, 0.00^d)                          | (9.211, 0.02^d)                        | (24.973, 0.00^d)                          | (13.056, 0.00^d)                           |
| Prov*Fert.       | NS                                        | NS                                     | NS                                        | NS                                        | NS                                        |

3.1. Height

Observation for height variable were carried out carefully, especially at young age, kelor stem were still susceptible to damage by external pressure. There was no significant effect on the height from the interaction between provenance and fertilizer, but fertilizer alone showed significant effect on the height. The result (Figure 1) showed that at 1, and 3 MAS, the combination of treatment P1F1 produced the best height descriptively. At 1 MAS the average height were as follow : P1F1 = 21.97cm, P1F2 = 16.67cm, P1F3 = 14.13cm, P1F4 = 19.90cm, P2F1 = 20.90cm, P2F2 = 18.93cm, P2F3 = 15.27cm, P2F4 = 20.67cm. While at 3 MAS the height respectively were P1F1 = 47.37cm, P1F2 = 34.87cm, P1F3 = 34.28cm, P1F4 = 30.77cm, P2F1 = 46.76cm, P2F2 = 46.29cm, P2F3 = 43.56cm, P2F4 = 32.20cm. Figure 1 shows that F1 and F4 had a better effect on height at 1 MAS, while F1 and F2 had a better effect on the height at 3 MAS.

Figure 1. Seedling height at 1 MAS and 3 MAS.
The results of the post hoc test (Table 2) showed that the fertilization treatment had a significant effect on the height of the seedlings both at 1 MAS and 3 MAS. Meanwhile, the provenance and fertilizer did not show interaction on the height of the seedlings at 1 and 3 MAS, according to the analysis of varians. Statistical analysis descriptively showed the height value for P1F1, and P2F1 treatments at 3 MAS were the highest, as shown in Figure 2. The value was not much different (47.37 and 46.76); however, it was statistically different. The difference was quite stable at 1 and 3 MAS. The result of the post hoc tests using DMRT and REGW at 1 and 3 MAS are presented in Table 2 as follows.

**Table 2. DMRT on the height variable.**

| Methods | Fertilizer | A subset (1 mas) | Fertilizer | A subset (3 mas) |
|---------|------------|------------------|------------|------------------|
|         |            | 1    | 2    | 3    | 1    | 2    | 3    |
| Duncan  | F3         | 14.700\(^*\) | F4         | 31.485\(^a\) |
|         | F2         | 17.800\(^b\) | F3         | 38.918\(^b\) |
|         | F4         | 20.283\(^c\) | F2         | 40.580\(^b\) |
|         | F1         | 21.433\(^c\) | F1         | 47.065\(^c\) |
| Sig.    | 1.000      | 1.000 | .174  | 1.000 | 0.577 | 1.000 |

\(^*\)the same letters are not statistically different

The provenances and the interaction of the treatment combinations had no statistically significant effect on the height variable, at 1 MAS and 3 MAS. However, the single effect of fertilization treatment was a statistically significant difference. The post hoc test of the fertilization effect on seedling’s height is presented in table 2. At 1 MAS, the effect of F1 was not significantly different with F4, the value is the highest. But at 3 MAS, the value of F1 was significantly different from the others, the value is the lowest. Fertilization using low dose of NPK has a significant effect on the height variable of young plants or seedlings [13, 14], but excessive fertilization will actually reduce the plant growth performance [15, 16].

3.2. Diameter

Kelor seedlings diameter measurement was only carried out at 3 MAS, considering that at 1 MAS the stem of kelor seedlings was still too small and the wood tissue has not yet formed, so they were very susceptible to damage. The diameter measurement was carried out at the base of the kelor stem. The measurement result showed the average diameter of each treatments combination is as follows: P1F1 = 4.55cm, P1F2 = 4.10cm, P1F3 = 4.08cm, P1F4 = 3.16cm, P2F1 = 4.32cm, P2F2 = 4.82cm, P2F3 = 4.53cm, P2F4 = 3.17cm. The result are presented in Figure 2, below.

**Figure 2.** Seedlings diameter at 3 MAS.

In Figure 2 it can be seen that the treatment combination P2F2 has the largest average seedlings diameter value (4.82 mm) at 3 MAS. Based on the Anova (Table 1), it showed that the provenance has
no a significant effect, however, the single effect of fertilizer, and it’s combination with provenance, have a statistically significant effect on seedling’s diameter. Based on the post hoc test (Table 3) it is known, only F4 that has significantly different effects from the other 3 treatments (F1, F2, F3). This means that NPK-fertilizer had a significant effect on the kelor seedling’s diameter growth and was significantly different from no fertilization treatment [16, 17]. The post hoc test result is presented in Table 3.

Table 3. DMRT on diameter variables.

| Methods | Fertilizer | Subset (3 mas) |
|---------|-----------|----------------|
| Duncan  | F4        | 3.167*         |
|         | F3        | 4.305b         |
|         | F1        | 4.433b         |
|         | F2        | 4.468b         |
| Sig.    |           | 1.000          |
|         |           | 0.333          |

*the same letters are not statistically different

In Table 3, it can be seen that F4 has the lowest value and was significantly different from other fertilization treatments. NPK fertilization had a significant effect on diameter increasing [18, 14]. The NPK fertilizer provided a quick supply of macronutrients (N, P, K) to the plants as compared to compost, from which the slow release of nutrients took place [19]. However, the statistical analysis also showed that there was a significant effect on the interaction of provenance and NPK fertilization. The performance of these interactions is illustrated in the graph below.

Figure 3. Graph of interaction dynamics between provenance and NPK fertilizer.

In Figure 3 above, it can be seen that without fertilizer, the seedling’s diameter did not change much. However, with fertilizer, there was a significant effect on seed diameter change. These three fertilization effects, F1, F2, and F3, were not significantly different. However, the interaction patterns for F1 with provenance looked different compared to the interaction pattern of F2 and F3 with provenance. The interaction graph between F1 and provenances was decreasing, while F2 and F3 were increasing. This condition indicated that P2 was more responsive in diameter growth to the addition of a dose of NPK fertilizer compared to P1. According to [20], a significant interaction between provenance and NPK fertilization occurred in the N content of the Yellowhorn stem (Xanthoceras sorbifolium Bunge). The addition of NPK fertilization doses also increased the P content of the root and stem of the seedling significantly.
3.3. Number of leaves

Leaves are the plant’s organs where photosynthesis takes place, which is often used in growth parameters [21]. The anatomical character of the leaf has been expressed since the beginning of seedling growth [22]. Kelor vegetative growth was better with NPK-fertilizer for the parameters of height, stem circumference, number of leaves, and number of branches [23]. Although kelor is known to survive in marginal land, the adjustment process takes time and will affect the quantity and quality of kelor leaves. In the early stage of adjustment, generally, kelor leaves will turn yellow and slightly wilt, and then were dropped.

![Graph showing number of kelor leaves at 1 and 3 MAS.](image)

**Figure 4.** Number of kelor leaves at 1 and 3 MAS.

Kelor seedlings that were pruned will produce more leaves and branches, a larger stem diameter, and a shorter height than seedlings without pruning [24, 25]. In Figure 4, the graph shows that the kelor’s leaves number varied at 1 MAS and 3 MAS. The variation of kelor leaves number depends on the environmental influences, growth phases, and cultivation method. In its life cycle, kelor tends to reduce the leaves when there is no pruning carried out to its branches or stems. In general, kelor leaves abscission occurred in old leaves due to age, the influence of environmental conditions, biotic stress, and physiological factor [2]. Kelor leaves abscission mainly due to physiological effects. The old leaves located at the bottom of the tree canopy would begin to turn yellow before the leaf dropped. The yellow colour gets stronger until finally, the leaves would fall by themselves. Nitrogen seems to be the nutrient the most closely related to leaf lifespan (LLS). According to the model proposed by Pornon et al. [26], leaves have a short lifespan in N-depleted soils due to the nitrogen in old leaves having to be translocated for growing new leaves; thus, the increments of nitrogen would increase LLS. The trees that grow in soil rich in nitrogen will have excessive leaf growth, and the old leaves are getting senesce because they are shaded. In this case, the senescence rate is higher due to the easier effort for uptaking nitrogen from the soil. Calcium deficiency, although it is a non-mobile nutrient, also accelerates leaf aging and shedding [27].

| Methods | Fertilizer | A subset (1 mas) | Fertilizer | A subset (3 mas) |
|---------|------------|----------------|------------|----------------|
| Duncan  | F3         | 6.4000<sup>a</sup> | F2         | 6.2817<sup>a</sup> |
|         | F2         | 8.0667<sup>b</sup> | F1         | 6.5000<sup>ab</sup> |
|         | F1         | 8.1167<sup>b</sup> | F3         | 8.0817<sup>ab</sup> |
|         | F4         | 8.7167<sup>b</sup> | F4         | 8.2350<sup>b</sup> |
| Sig.    | 1.000      | 0.207           |            | 0.738          |

*the same letters are not statistically different

The analysis of variance (Table 1) showed that the number of kelor leaves was significantly affected by fertilization treatment. However, the provenance and its interaction with fertilizer had no significant effect on the number of kelor leaves, both at 1 MAS and 3 MAS. Based on the post hoc
tests (Table 4), at 1 MAS, only F3 was significantly different from others, with the least number of leaves. While at 3 MAS, F3 with the most number of leaves was not significantly different from F4 but significantly different from F1 and F2. At 1 MAS, the effect of high-dose fertilization resulted in a decrease in the number of leaves. Excessive NPK fertilization caused wilting and faster leaf fall in kelor seedlings. This phenomenon is in line with the statement of Albornoz [28]; crop losses could be caused by excessive N fertilization. The result of research by Nasrullah et al. [29] showed that excessive NPK fertilization could suppress the growth of the cocoa seedlings due to a decrease in soil pH so that the nutrients become unavailable in the soil. Meanwhile, at 3 MAS, the seedling condition was more ready to receive higher doses so that they were able to stimulate leaf growth. This phenomenon is in line with the result of research by Attia et al. [30], who concluded that the effect of the use of fertilizers on yield, nutrient concentration, total phenol, and antioxidant activity on leaves and seeds of kelor were as follows: NPK fertilizer > organic fertilizer > biotic fertilizer. The result of research by Anamayi et al. [31] also concluded that the effect of NPK fertilizer (15:15:15) with a dose of 5 grams gave the best vegetative growth result compared to fertilizer from cow dung. However, in contrast to the result of research by Dania et al. [17], which showed that fertilizer from chicken manure had the best effect on kelor seedling growth in nurseries.

3.4. Life Percentage
Based on the analysis of variance, fertilization had a significant effect on the life percentage of kelor seedling at 1 MAS, and there were significant interactions between NPK fertilizer and provenance. However, when the seedling reached the age of 3 MAS, the significant difference was only in the fertilizer factor, while the interaction effect between provenance and fertilizer became not significant. The graph of the life percentage of kelor seedlings at 1, and 3 MAS, can be seen in Figure 5.

![Figure 5. Life percentage of kelor seedling at 1 and 3 mas.](image)

In Figure 5, it can be seen that the highest life percentage of kelor seedling was found in the F4 treatment, and the lowest was found in the F3 treatment. This phenomenon is similar to the kelor number of leaves described earlier, the treatment without fertilizer has the best result for the live percentage, and higher fertilizer dose had harmed the live percentage. However, for the live percentage variable, the difference between 1 MAS and 3 MAS was not much different, except for the P1F2 and P2F3 treatments. This condition is in line with the phenomenon that occurred in the leaves number variable, that higher NPK fertilization harms the live percentage of kelor seedlings. This circumstance showed that kelor seedlings were very sensitive to fertilization using inorganic fertilizers. Based on table 1, provenance had no significant effect on the life percentage of kelor seedling, either at 1 MAS or 3 MAS. This condition is different from the result of research by Ezekiel et al. [12], which showed significant survival differences in 14 different provenances.

Furthermore, based on the analysis of variance for the life percentage variable (Table 5), kelor seedlings at 3 MAS were significantly different due to the fertilization factor alone. F4 (without fertilizer) was significantly different from F2 and F3 but not significantly different from F1. The
treatment F4 and F1 (dose of 5 gr) gave the best result for live percentage. The result is in line with the previous analysis that the young seedling of kelor was very sensitive to excessive chemical fertilizer application. The young leaves generally dominated the kelor seedlings; the protein content of young leaves was less than the old ones [21]. In contrast to the next growth phase of kelor in the field, which still showed a positive response to fertilizer application when the plants reached nine years of age [32]. In general, fast-growing species were very responsive to NPK-fertilizer but decreased with age. This phenomenon is in line with the result of research by Singh [33], who reported this phenomenon in *Leucaena leucocephala* which decreased its responses to the applying of NPK-fertilizer at 53 months after planting (4 years 5 months).

| Fertilizer | Subset 1 | Subset 2 | Subset 3 |
|------------|----------|----------|----------|
| Duncan     | 47.225a  | 62.770ab | 78.333bc |
| F3         | 62.770ab | 78.333bc | 93.890c  |
| F2         | 78.333bc |          |          |
| F1         | 83.333bc | 93.890c  |          |
| F4         | 93.890c  |          |          |
| Sig.       | 0.154    | 0.154    | 0.154    |

* the same letters are not statistically different

### 3.5. Leaf colour

Leaf colour is an indicator of plant health that is very easy to observe. It also indicates the quality and quantity of substance or nutrients contained in the leaves. The changes in the quality of kelor leaves can be known based on changes in their colour. The observation of the kelor leaves colour was carried out at 1 MAS dan 3 MAS. Leaf colour was divided into three categories based on visual observation, namely green, slightly yellow, and yellow. And then, each colour was scored 3 for green, 2 for slightly yellow, and 1 for yellow. Based on the analysis of variance (Table 1), it was known that the provenance factor had no significant effect on the leaf's colour at either 1 MAS or 3 MAS. Similarly, the interaction between the provenance and fertilization. However, the fertilization factor alone had a significant effect at 1 MAS but not significant at 3 MAS. This condition can be explained; the leaf color of the young kelor seedlings was quite sensitive to the applying of NPK-fertilizer. However, over time with stronger seedlings development, the application of NPK-fertilizer did not have significantly affect the leaf colour of kelor.

The process of color changing in the plant’s leaves was closely related to the physiological process of aging and leaves shedding in plants. Many research results showed that the aging and leaves shedding were influenced by the availability of N in soil and the power of *sink activity* of plant's organ. A very good explanation was given by Pornon et al. [26], which stated that there was a plastic response of leaves age of the plants toward the availability of the N soil, described by a linear curve. In descending part of the curve, the leaves age of the plants decreased after reaching its peak as the N soil availability increased. This condition can be caused by the presence of old leaves, which were in a downward position. Thus the photosynthesis process was blocked [34, 35] and or changed the physiology of plants by excessed N [26]. The yellowing process of kelor seedling leaves at 1 MAS was due to the presence of excessed N in the seedling medium. With increasing the age of seedling, the need for N from the seedling medium increased so that the N from excessive NPK fertilizer at 1 MAS became a quantity that was no longer excessed which was then used by kelor seedling for its growth at 3 MAS.

### 4. Conclusion

The young kelor seedling (1 MAS) was very responsive to the NPK application. The lower dosage of NPK that is, F1 (5 gr), has a better effect on the height variable, the number of leaves, the life
The percentage, and the leaf colour. The higher dosage of NPK fertilizer (F2 = 7gr dan F3 = 9gr) had a better effect on the seedling’s diameter at 3 MAS. Thus, to stimulate the diameter growth, additional doses of NPK can be given after the kelor seedling reaches a minimum age of 3 MAS. Provenance has no significant effect on all variables, but its interaction with NPK fertilizer has a significant effect on a life percentage at 1 MAS and diameter at 3 MAS.

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