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**Research Article**

# EFFECTS OF CONCENTRATION OF $KNO_3$ AND DURATION OF SOAKING ON GERMINATION OF PLANT SEED OF KAWISTA (*Limoniaacidissima* L.)

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**Abstract**

Kawista (*Limoniaacidissima* L.) is a fruit plantthat has many benefits, including its fruit being used as raw material for beverages, food, and medicines. However, its population is limited. The low population of kawista is due to its seeds being difficult to germinate, which can be classified as dormancy. One method to break this dormancy is through a chemical approach, using potassium nitrate ($KNO_3$). A $KNO_3$ solution is considered economical, easy, and safe to use. This solution can break the dormancy of seeds. This study was conducted from July 2024 to September 2024 using a Factorial Completely Randomized Design (CRD). The first factor was the concentration of $KNO_3$, consisting of six levels: Water (0%), 0.25%, 0.5%, 0.75%, 1.00%, and 1.25%. The second factor was the soaking duration, with two levels: 12 hours and 24 hours. This study had 12 treatment combinations, each repeated three times. The data were analyzed using Analysis of Variance (ANOVA). If significant effects were found, a Tuckey test was carried out at a 5% significance level. There was an interaction between the combination of $KNO_3$ concentration and soaking duration on seed viability and vigor in kawista based on the observation parameters. The treatment with 1.00% $KNO_3$ concentration and 12 hours of soaking produced the highest average germination, while the 1.00% $KNO_3$ concentration with 24 hours of soaking resulted in the highest average vigor index.

**Keywords:**Germination, Concentration of $KNO_3$, Duration of soaking, Kawista.

## INTRODUCTION

Kawista or wood apple (*Limoniaacidissima* L.) is a fruit plant from the Rutaceae family (citrus). It is naturally found in India and Ceylon (Hiwale, 2015). Kawista is spread across Southeast Asia, including Myanmar, Indochina, and Indonesia. It was introduced and naturalized in Indonesia in areas such as Sumatra, Java, Madura, Bali, and West Nusa Tenggara (Nurdiana*et al.,* 2016). This plant can be consumed directly and used in food and beverage processing, such as syrups, dodol, jelly, and jam. Kawista is also beneficial as a treatment for diarrhea and dysentery (Panda *et al.,* 2013). Population of Kawista last recorded in 2016 in Rembang Regency was 876 trees, producing 450.2 tons of fruit, with a productivity of 513.93 kg per tree (BPS,2019). The main factor contributing to the rarity of Kawista plants is that its seeds are difficult to germinate. This is evident from the germination percentage of Kawista, which ranges from 15% to 62% without any treatment (Hiwale, 2015). This is due to the seeds having a thick, hairy coat and physiological dormancy typical of the Rutaceae family, making the germination process quite challenging. These factors suggest that Kawista seeds are likely to experience dormancy (Sabarad *et al.,* 2023). $KNO_3$ is the most commonly used chemical and is recommended by the International Seed Testing Association (ISTA) to break seed dormancy and promote seed germination in various plants. However, the concentration used varies for different types of seeds, depending on the characteristics of the particular seed.$KNO_3$ can be used to replace the function of light and temperature to accelerate oxygen absorption into seeds.

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Additionally, $KNO_3$ plays a role in the physiological processes of plant growth and development, including flower and fruit formation, photosynthesis, water transport, and enzyme activity (Suratmi, *et al.,* 2022). $KNO_3$ is believed to enhance the effectiveness of gibberellin in seed germination. Gibberellic acid (GA3) is an important organic compound in the germination process, as it plays a key role in regulating seed germination (Kartika *et al.,* 2015).The effect of soaking seeds in a $KNO_3$ solution is determined by the concentration of the solution. An initial treatment with a $KNO_3$ solution can stimulate germination in almost all types of seeds; however, if the concentration is not appropriate, it can damage the seed's germination potential. This can occur if the concentration is too high, causing toxicity to the seeds, and if the concentration is too low, the seeds may not grow and could experience prolonged dormancy (Sari and Purnamaningsih, 2020).The soaking duration can help break dormancy by altering the seed coat's impermeability, making it permeable to water and oxygen, which are essential for germination. If the soaking duration is optimal, the seeds will absorb the substance effectively, allowing them to germinate optimally (Lubis *et al.,* 2018). Based on these issues, research was conducted on the effects of $KNO_3$ concentration and soaking duration to improve the germination of kawista seeds.

## MATERIALS AND METHODS

### Time and Place of Research

The research was conducted in July - September 2024 at land in MPU Tantular Museum, Buduran District, Sidoarjo Regency, East Java, Indonesia.

### Materials and Tools

The materials for this research include kawista seeds, $KNO_3$ powder, water, polybag25 x 25 cm, NPK 16:16:16 fertilizer, sand, soil and compost. The tools in the research were germination boxes, beaker glass, bottle, stirrer, hand sprayer, ruler and analytical scale.

### Research Methods

This research used a Completely Randomized Design (CRD) which had 2 factors, the concentration of $KNO_3$ as first factor and duration of soaking as second factor. The concentration of $KNO_3$ treatment consisted of 6 levels, 0%, 0,25%, 0,50%, 0,75%, 1,00% and 1,25%. Meanwhile, the shoot pruning treatment consisted of 2 levels, 12 hours and 24 hours. The combination of the two treatments produced 12 combinations and was repeated 3 times for each treatment combination, so there were 36 experimental units. Germination observations were made for 50 seeds per treatment, and 3 samples from each treatment were observed during the early growth observation.

### Observation Parameters and Data Analysis Models

The observation parameters in this research included germination rate, vigor index, plant height, number of leaves, and root length. Research data was collected and analyzed using Anova in Microsoft Excel. If the calculated F result is greater than the F table of 5%, the treatment have a significant difference and continued Tukey Test 5%.

### Research Implementation

The research began by preparing the planting medium consisting of soil, sand, and compost in a 1:1:1 ratio, which was mixed and sterilized. The planting medium was then placed in a germination tray, and 50 planting holes were made in each tray. The seeds were soaked in a $KNO_3$ solution with concentration and soaking duration according to the treatment. The seeds were planted in the prepared planting holes in the germination tray. The planted seeds were maintained, and germination observations were made daily for 30 days. Three germinated seed samples were then transplanted after germination observation into polybags with the same planting medium composition, and early growth observations were made every 7 days for 8 weeks.

## RESULTS AND DISCUSSION

### Germination rate

Therewas very significant interaction between the concentration of $KNO_3$ and duration of soaking on the parameter germination rate according to Anova analysis. The single treatment of $KNO_3$ concentration has a very significant effect, while the duration of soaking has a significant effect. The average germination rate due to the effects of $KNO_3$ concentration and soaking duration is presented in Table 1.The treatment of $KNO_3$ concentration 1.00% and soaking duration 12 hours resulted in the highest average germination rate of kawista, which was 85.33%. However, treatment of $KNO_3$ concentration 1.00% and soaking duration 12 hours did not show a significant difference from all other treatments except for treatment of $KNO_3$ concentration 1.25% and soaking duration 12 hours. Treatment of $KNO_3$ concentration 1.25% and soaking duration 12 hours resulted in the lowest average germination rate of kawista, which was 20.67%.

**Table 1. Average of germination rate due to the effect of concentration of $KNO_3$ and duration of soaking**

| Germination rate | | | | | | |
|---|---|---|---|---|---|---|
| Duration of soaking | Concentration of $KNO_3$ | | | | | |
| | 0% | 0,25% | 0,50% | 0,75% | 1,00% | 1,25% |
| 12 hours | 82,00b | 63,33 b | 74,67 b | 69,33 b | 85,33 b | 20,67 a |
| 24 hours | 71,33b | 74,67 b | 72,00 b | 79,33 b | 84,67 b | 71,33 b |
| Tuckey 5% | 31,36 | | | | | |

*Numbers followed by the same letter in the same column and treatment show no significant difference based on Tukey Test 5%; ns = not significant.

### Vigor index

There was very significant interaction between the combination of concentration of $KNO_3$ and duration of soaking on the parameter vigor index according to Anova analysis. The single treatment of $KNO_3$ concentration has a very significant effect, while the duration of soaking has a significant effect. The average vigor index due to the effects of $KNO_3$ concentration and soaking duration is presented in Table 2.Thetreatment of 1.00% $KNO_3$ concentration and 24 hours soaking duration produced the highest average vigor index of kawista at 3.80, but the 1.00% $KNO_3$ concentration and 24 hours soaking duration treatment was not significantly different in all treatments except for the1.25% $KNO_3$ concentration and 12 hours soaking duration treatment. The treatment of 1.25% $KNO_3$ concentration and 12 hours soaking duration resulted in the lowest average vigor index of kawista, which was 0.90.

**Table 2. Average of vigor index due to the effect of concentration of $KNO_3$ and duration of soaking**

| Vigor index | | | | | | |
|---|---|---|---|---|---|---|
| Duration of soaking | Concentration of $KNO_3$ | | | | | |
| | 0% | 0,25% | 0,50% | 0,75% | 1,00% | 1,25% |
| 12 hours | 3,35 b | 3,18 b | 3,16 b | 2,90 b | 3,00 b | 0,90 a |
| 24 hours | 3,07 b | 2,80 b | 3,51 b | 3,01 b | 3,80 b | 2,92 b |
| Tuckey 5% | 1,58 | | | | | |

*Numbers followed by the same letter in the same column and treatment show no significant difference based on Tukey Test 5%.

### Plantheight

There was no interaction between the combination interaction between the concentration of $KNO_3$ and duration of soaking on the parameter plant height according to Anova analysis on Anova analysis. Likewise, the single factors of concentration of $KNO_3$and duration of soaking also did not produce a significant effect on the average plant height of kawista as presented in Table 3.The treatment of combination interaction between the concentration of $KNO_3$ and duration of soaking did not produce a significant effect on the average plant height of kawista. The single treatment of $KNO_3$ concentration at 1.0% produced the highest average value of kawista height at 7 DAP to 49 DAP, namely with consecutive values of 4.63cm, 5.01 cm, 5.77 cm, 6.67 cm, 8.68 cm, 10.91 cm and 13.51cm, but at 56 DAP the $KNO_3$ concentration at 0% had the highest average height value of 16, 94 cm and the lowest average value of kawista height is $KNO_3$ concentration at 0.25% at 7 DAP with 4.13 cm, $KNO_3$ concentration at 0% at 14 DAP with 4.6 cm, $KNO_3$ concentration at 0.25% at 21 DAP and 28 DAP with values of 5.07 cm and 6.00 cm respectively,

**Table 3. Plant height due to treatment with concentration of $KNO_3$ and duration of soaking**

| Treatment | Plant Height (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Concentration of $KNO_3$ | DAP | | | | | | | |
| | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 |
| 0% | 4,22 | 4,6 | 5,21 | 6,29 | 8,25 | 10,51 | 13,27 | 16,94 |
| 0,25% | 4,13 | 4,49 | 5,07 | 6,00 | 7,85 | 10,05 | 12,36 | 15,23 |
| 0,50% | 4,27 | 4,67 | 5,12 | 6,05 | 7,87 | 9,99 | 12,99 | 16,48 |
| 0,75% | 4,42 | 4,8 | 5,29 | 6,17 | 7,81 | 9,51 | 11,98 | 14,49 |
| 1,00% | 4,63 | 5,01 | 5,77 | 6,67 | 8,68 | 10,91 | 13,51 | 16,50 |
| 1,25% | 4,30 | 4,65 | 5,31 | 6,22 | 8,00 | 9,92 | 12,39 | 15,70 |
| Tuckey 5% | ns | ns | ns | ns | ns | ns | ns | ns |
| Duration of soaking | | | | | | | | |
| 12 hours | 4,31 | 4,65 | 5,16 | 6,07 | 7,81 | 9,84 | 12,33 | 15,45 |
| 24 hours | 4,34 | 4,75 | 5,43 | 6,40 | 8,34 | 10,45 | 13,17 | 16,33 |
| Tuckey 5% | ns | ns | ns | ns | ns | ns | ns | ns |

ns = not significant

**Table 4. Number of leaves due to treatment with concentration of $KNO_3$ and duration of soaking**

| Treatment | Number of leaves (leaves) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Concentration of $KNO_3$ | DAP | | | | | | | |
| | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 |
| 0% | 3,50 | 3,89 | 4,72 | 6,00 | 7,44 | 8,67 | 10,50 | 12,38 |
| 0,25% | 3,38 | 4,00 | 4,44 | 5,89 | 7,05 | 8,61 | 10,11 | 12,11 |
| 0,50% | 3,38 | 3,72 | 4,50 | 5,89 | 7,27 | 8,72 | 10,78 | 12,56 |
| 0,75% | 3,22 | 3,61 | 4,33 | 5,61 | 7,00 | 8,22 | 9,83 | 11,11 |
| 1,00% | 3,50 | 3,89 | 4,56 | 5,78 | 7,61 | 8,67 | 10,05 | 11,83 |
| 1,25% | 3,16 | 3,61 | 4,38 | 5,78 | 7,11 | 8,22 | 10,00 | 11,50 |
| Tuckey 5% | ns | ns | ns | ns | ns | ns | ns | ns |
| Duration of soaking | | | | | | | | |
| 12 hours | 3,29 | 3,78 | 4,44 | 5,78 | 7,25 | 8,38 | 10,09 | 11,70 |
| 24 hours | 3,42 | 3,79 | 4,53 | 5,90 | 7,24 | 8,64 | 10,33 | 12,12 |
| Tuckey 5% | ns | ns | ns | ns | ns | ns | ns | ns |

ns = not significant

$KNO_3$ concentration at 0.75% at 35 DAP to 56 DAP with values of 7.81 cm, 9.51 cm, 11.98 cm and 14.49cm respectively. The single treatment of 24 hours duration of soaking produced the highest average value of kawista height from 7 DAP to 56 DAP with consecutive values of 4.34 cm, 4.75 cm, 5.43 cm, 6.40 cm, 8.34 cm, 10.45 cm, 13.17cm and 16 cm, 33 cm and at duration of soaking 12 hours produced the lowest average value of kawista height from 7 DAP to 56 DAP with consecutive values of 4.31 cm, 4.65 cm, 5.16 cm, 6.07 cm, 7.81 cm, 9.84 cm, 12.33 cm and 15.45 cm.

## Number of leaves

There was no interaction between the treatment combination of concentration of $KNO_3$ and duration of soaking on the average number of leaves based on the result of Anova calculation. Likewise, the single factors of concentration of $KNO_3$ and duration of soaking also did not produce a significant effect on the average number of leaves of kawista as presented in Table 4. The single treatment of $KNO_3$ concentration 1.0% and 0% produced the highest average value number of leaves of kawista at 7 DAP and 14 DAP with a value of 3.5 leaves and 3.89 leaves, concentration of $KNO_3$ at 0% at 21 DAP and 28 DAP with a value of 4, 72 leaves and 6 leaves, concentration of $KNO_3$ at 1.00% at 35 DAP with a value of 7.61 leaves, concentration of $KNO_3$ at 0.50% at 42 DAP, 49 DAP and 56 DAP with values respectively of 8.72 leaves, 10.78 leaves and 12.56 leaves. The average value of the lowest number of kawista leaves is concentration of $KNO_3$ at 1.25% at 7 DAP with 3.16 leaves, concentration of $KNO_3$ at 0.75% and1.25% at 14 DAP with 3.61 leaves, concentration of $KNO_3$ at 0.75% at 21 DAP, 28 DAP and 35 DAP with consecutive values of 4,33 leaves, 5.61 leaves and 7 leaves, concentration of $KNO_3$ at 0.75% and concentration of $KNO_3$ at 1.25% at 42 DAP with a value of 8.22 leaves and concentration of $KNO_3$ at 0.75% at 49 DAP and 56 DAP with a value of 9.83 leaves and 11.11 leaves respectively.

## Root length

There was no interaction between the combination interaction between the concentration of $KNO_3$ and duration of soaking on the parameter root length according to Anova analysis on Anova analysis. Likewise, the single factors of concentration of $KNO_3$ and duration of soaking also did not produce a significant effect on the average root length of kawista as presented in Table 5.

**Table 5. Average of root length due to the effect of concentration of $KNO_3$ and duration of soaking**

| Treatment | Rootlength (cm) |
|---|---|
| Concentration of $KNO_3$ | |
| 0% | 14,86 |
| 0,25% | 13,99 |
| 0,50% | 13,36 |
| 0,75% | 13,63 |
| 1,00% | 14,78 |
| 1,25% | 14,94 |
| Tuckey 5% | Ns |
| Duration of soaking | |
| 12 hours | 13,95 |
| 24 hours | 14,57 |
| Tuckey 5% | Ns |

ns = not significant.

The single treatment of $KNO_3$ concentration at 1,25% produced the highest average value of kawista root length of 14.94 cm and at concentration 0.50% produced the lowest average value of kawista root length of 13.36 cm. The single treatment of duration of soaking at 24 hours produced the

highest average value of kawista root length, namely 14.57 cm and at12 hours produced the lowest average value of kawista root length, namely 14.57 cm.

## DISCUSSION

### Effect of combination of concentration of $KNO_3$ and duration of soaking on the germination plant seed of kawista

The treatment combination between $KNO_3$ concentration and soaking time has a very different response, especially on the germination factor of kawista seeds. This can be seen from the results of the analysis of variance (Anova) which shows a very significant interaction in several combined treatments of $KNO_3$ concentration and soaking time, especially on the parameters of germination rate and vigor index. $KNO_3$ has the ability to soften the seed coat so as to facilitate the entry process of water (imbibition) and oxygen and nutrients in $KNO_3$ can accelerate enzyme activity. The nitrogen element in $KNO_3$ is also useful for stimulating the growth of stems, branches, leaves as well as cell division, cell enlargement and slowing down the ripening of seeds (extending the vegetative period). Potassium nutrients function as activators of essential enzymes in photosynthetic reactions and are needed in leaf formation (Laisbuke, 2022). The concentration of $KNO_3$ needs to be adjusted carefully if the concentration used is too high it will be toxic, while the concentration that is too low becomes less effective (Saputra *et al.,* 2017).Soaking time is related to water absorption. Soaking the seeds causes water imbibition into the seeds so that the seed water content increases which will stimulate the activity of gibberellins and enzymes to initiate and carry out the germination process (Irwan *et al.,* 2024). If the seeds are soaked in the right time interval, the seeds will germinate well. In addition to water entering the imbibition process, dissolved oxygen, potassium and nitrogen elements in the use of $KNO_3$ will enter the seed soaking process which also accelerates the germination process with the right $KNO_3$ concentration and soaking time. Germination can be a benchmark for the ability of seeds to grow normally.

The germination test is conducted to determine the potential of seeds that can germinate from a group or unit weight of seeds. The germination rate in the combination of $KNO_3$ concentration of 1.00%and12 hours soaking duration treatment produced the highest average germination rate of kawista which was 85.33%. The vigor index is the ability of seeds to grow normally under sub-optimal environmental conditions (Kolo and Tefa, 2016). A high vigour index indicates the speed of seed germination is also high and more resistant to unfavorable environmental conditions. The combination treatment in 1.00% $KNO_3$ concentration and 24 hours soaking time produced the highest average vigor index of kawista at 3.80.However, the combined treatment of $KNO_3$ concentration and soaking time had no significant effect on early plant growth factors such as plant height, number of leaves and root length. This may be due to the amount of nutrients in the soil and the food reserves supplied are relatively the same so that the growth is relatively the same (Purnama *et al.,* 2021).In addition, the availability of nitrogen and potassium elements contained in $KNO_3$ in the soaking treatment is not enough to meet the needs of nutrients for plants, because the more plants grow, the need for nutrients increases (Qibthiyah *et al.,* 2024).

### Effect of concentration of $KNO_3$ on the germination plant seed of kawista

The treatment of $KNO_3$ concentration showed different responses, especially on the germination of kawista seeds. This can be seen from the results of the analysis of variance (Anova) which shows a significant effect on several treatments of $KNO_3$ concentration combinations on the parameters of germination rate and vigor index.$KNO_3$ concentration treatment of 1.00% gives the best effect on the viability and vigor of kawista seeds shown in the parameters of germination rate and vigor index giving the highest value.$KNO_3$ is used as a presowing treatment to increase seed germination due to its ionic salt content of nitrate ions ($NO_3^-$) and potassium ions ($K^+$). Nitrogen can assist seeds in synthesizing proteins, thus affecting seed quality (Thongtip *et al.,* 2022).The mechanism of action of $KNO^3$ in addition to softening the seed coat to facilitate imbibition and increase metabolic activity in seeds, the provision of $KNO_3$ can increase GA4 content and reduce ABA (Absisic Acid) levels causing a decrease in the ratio of ABA and Gibberellin, so that germination will increase (Vidal *et al.,* 2018). However, the single treatment of $KNO_3$ concentration had no significant effect on early plant growth factors such as plant height, number of leaves and root length.

### Effect of duration of soaking on the germination plant seed of kawista

Soaking time treatment gives different responses, especially on the germination of kawista seeds. This can be seen from the results of the analysis of variance (Anova) which shows a significant effect on several treatments of germination duration on the parameters of germination rate and vigor index. The results showed that 24 hours of soaking had the effect of increasing the parameters of germination rate and vigor index compared to 12 hours of soaking. This is because the longer soaking can increase the absorption of water, dissolved oxygen and additives together during the imbibition process to a certain extent. Soaking time that is too short can cause the completion of metabolic processes or inhibit enzyme activity in increasing seed germination or growth vigor (Saini *et al.,* 2017). Imbibition is also influenced by seed wall thickness, seed size, seed age, seed surface area and intermolecular space, seed protein and starch components, water concentration, hydrostatic pressure and temperature (Idrus and Fuadiyah, 2021). However, the single treatment of duration of soaking had no significant effect on early plant growth factors such as plant height, number of leaves and root length.

### Conclusion

There was interaction between the combination of concentration of $KNO_3$ and duration of soaking on the germination plant seed of kawista by observation parameters. The Treatment with1.00%$KNO_3$ concentration and12 hours soaking duration produced the highest average germination and 1.00% $KNO_3$ concentration and 24 hours soaking time treatment produced the highest average vigor index.The combined treatment of $KNO_3$ concentration and soaking time had no significant effect on early plant growth factors such as plant height, number of leaves and root length.

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