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INDUCING THE VIABILITY OF DETERIORATED JATROPHA SEEDS THROUGH MATRICONDITIONING TECHNOLOGY AND *Pseudomonas fluorescens* AS BIOLOGICAL AGENT

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KEYWORDS
Jatropha; matriconditioning; *Pseudomonas fluorescens*; seeds

ABSTRACT
Jatropha curcas is a prospective vegetable oil or biodiesel and various derivative products as well as phytoremediation (plants cleaning the environment) and phytomining (plants that mine metals such as gold, nickel etc.). However, the commodity often experiences problems in the supply of seeds. The seed used usually has decreased their viability after going through a certain storage period. Therefore, matriconditioning technology and the application of the biological agent *Pseudomonas fluorescens* were used to increase the viability and vigor of these seeds. The aim of the study was to determine the interaction between the duration of the matrioditioning treatment and the application of *Pseudomonas fluorescens* to increase the viability and vigor of Jatropha seeds and to determine the optimal duration of the matrioditioning treatment and the dosage of *Pseudomonas fluorescens* in increasing the viability and growth performance of sprouts and seedlings. Field experiments would be carried out in the greenhouse of the Faculty of Agriculture, Universitas Jember. This experiment was arranged using a factorial design (two factors) with a randomized block design (RBD) repeated 3 times. The result showed that the interaction effect of matriconditioning time and dose of *Pseudomonas fluorescens* was significantly different on the observed variables of growth speed, vigor index and T50. Combination of 24 hours matriconditioning treatment and dose of *Pseudomonas fluorescens* 100 ml l^{-1} produced the best treatment on variables representing seed vigor (growth rate and vigor index).

INTRODUCTION
Jatropha curcas is a prospective plant, as a source of biofuel and various kinds of its derivative products. For the development, the seeds of the plant experience deterioration after going through a storage period and decrease in quantity due to biotic factors (Rustam & Audina, 2018). To induce the viability of Jatropha seeds which have experienced deterioration, a special technology or seed treatment is needed, in order to increase the vigor and viability of the seeds so that they can be used as planting material. Invigoration technique is an example for seed treatment before planting which aims to increase the value of seed viability and vigor, indicated by increasing germination and seed performance. An invigoration technique that has been proven successfully in increasing the viability of some seeds is matriconditioning technology. The induction is a controlled hydration treatment depending on the moist solid media used, has a low matrix potential and negligible osmotic potential (Khan, 1992).

Invigoration of matriconditioning can stimulate seed metabolism in germination; on the other hand, the radicle emergence can be delayed and is proven to improve seed performance (Sucahyono, 2013). Seeds that have been given the matriconditioning treatment have metabolic conditions in the seeds that are ready to germinate, so that the percentage of germination power and the speed of growth of sprouts increases. In a research on soybean plants, this technique was able to increase seed viability with increased germination parameters and growth rates (Priyanto, 2017). In addition, the combination

of invigoration with the addition of biological agents to *nyamplung* seeds can increase seed viability by up to 42% (Yuniarti, 2020).

The biological agents commonly used as seed treatments are from the PGPR (Plant Growth Promoting Rhizobacteria) group, one of which is *Pseudomonas fluorescens*. The secondary metabolites produced by *Pseudomonas fluorescens* are useful for stimulating seed germination, in the form of the hormones auxin indole acetic acid (IAA) (Istiqomah et al., 2017), cytokinins (Kapoor & Kaur, 2016) and gibberellins (Tefa, 2015). Siderophore compounds as bioprotectants against pathogens are also produced by *Pseudomonas fluorescens* from metabolic processes. The use of the biological agent *Pseudomonas fluorescens* on the invigoration of testa hard (teak) type seeds was able to increase the maximum growth potential and germination rate by 32.65% (Afa, 2008).

Based on the above background, matriconditioning technology and the application of the biological agent *Pseudomonas fluorescens* have to be studied to induce Jatropha seeds whose decreased viability, especially in study of the length of matriconditioning treatment and the density of *Pseudomonas fluorescens* in order to increase optimally the viability of Jatropha seeds. In fact, each seed has a different character and sensitivity to the seed treatment above. Therefore, this study aims to determine the effect of the interaction between matriconditioning duration and *Pseudomonas fluorescens* dose and to determine the optimal matriconditioning duration and *Pseudomonas fluorescens* dose to increase the viability and growth performance of Jatropha shoots and seedlings.

RESEARCH METHOD

Place and Time

Field research was carried out in the greenhouse of the Faculty of Agriculture, The University of Jember. Laboratory research activities are carried out at the Laboratory of Genetics, Microbiology, and Biotechnology, Faculty of Teaching and Education; Soil Chemistry and Fertility Laboratory; and the Seed Laboratory of the Faculty of Agriculture, University of Jember. The research time starts from June to November 2022.

Materials and Tools

The important ingredients to be used are the Jet 1 Agribun variety from the Research Institute for Sweeteners and Fiber Plants (BALITTAS). *Pseudomonas fluorescens* isolate was obtained from the Observation Laboratory of Food Plant Pest and Horticultural Pests in Tanggul, Jember. While important tools needed in testing free fatty acids, electrical conductivity, *Pseudomonas fluorescens* culture and testing bacterial density.

Experimental design

Two-factor research had been carried out using Factorial RAK with 3 repetitions. The first factor was the long matriconditioning treatment consisting of 4 levels, namely 12 hours, 24 hours, 36 hours and 48 hours. The second factor was the density of *Pseudomonas fluorescens*, consisting of 3 levels, namely 0 (without *Pseudomonas fluorescens* application), dose of 100 and 200 $ml.l^{-1}$.

Research Implementation

Seed Deterioration Testing

Analysis of free fatty acids and electrical conductivity (EC) was carried out to find out the deterioration of the seeds. Analysis of free fatty acid levels used the method used by Hasanuddin (2010). The EC test was based on the method used by Zanzibar (2016).

Culture of the biological agent Pseudomonas fluorescens

Fluorescence test was carried out to see that the isolate was a bacterium (Pf) *Pseudomonas fluorescens*. The bacterial isolate of *Pseudomonas fluorescens* introduced from the Laboratory of Monitoring Pests of Food Plant Diseases and Horticulture of the Embankment. Then, the bacterial culture was carried out using NB media (Nutrient Broth). The complete method was adopted from the method used by Scales *et al*. (2014).

Determination of Bacterial Density

The method used is TPC (Total Plate Count) spread plate to determine the density of *Pseudomonas fluorescens* bacteria (Yunita et al., 2021).

Matriconditioning Treatment and Application of Pseudomonas fluorescens

Mixing of materials was carried out on plastic clips for each treatment and stored for 12 hours, 24 hours, 36 hours and 48 hours at room temperature. Each treatment units used a comparison of seeds, husk charcoal media, bacterial solution/suspension (97.6 g : 48.8.1 g : 48.8 ml). Sterilized roasted husks and seeds were added according to the comparison on the plastic clip labeled for each treatments. The treatments consisted of a control (without bacteria) and a bacterial solution prepared and mixed with 1:10 distilled water (density 100 $ml.l^{-1}$) as much as 200 ml l^{-1} and a bacterial solution prepared as much as 200 ml. The solution was poured according to each treatments, and stored according to the matriconditioning old treatment level.

Planting

Planting was carried out after the matriconditioning treatment and the application of the biological agent *Pseudomonas fluorescens* were completed. The media attached to the seeds is cleaned using clean water. Sand that is ready to use before planting is watered so that the media becomes moist. The seeds are planted to a depth of 2-3 cm in an upright position.

Observational Variables

Preliminary observation variables that would be carried out to assess the deterioration of the seeds used as above include fatty acid levels and electrical conductivity; while the observations after the experiment were as follows:

1. Growth rate was calculated based on the ISTA standard (ISTA, 2014):

$$\text{Growth rate (\%/Etmal)} = \sum_{i=0}^{i=n} \text{\% KN/etmal}$$

2. The vigor index was calculated based on the Ilyas (2012) method with the formula:

$$\text{Vigor index} = \frac{\Sigma \text{ normal sprouts at first observation}}{\Sigma \text{ planted seeds}} \text{ x } 100\%$$

3. Simultaneous growth is calculated by the formula:

$$\text{Simultaneous growth } = \frac{\Sigma \text{ normal sprouts on day} - 11}{\Sigma \text{ planted seeds}} \text{ x } 100\%$$

4. Germination capacity (GC) (not attacked by pests or diseases) was calculated based on the ISTA standard (2014) with the formula:

$$\text{\% Normal sprouts} = \frac{\Sigma \text{ KN I} + \text{KN II}}{\Sigma \text{ planted seeds}} \text{ x } 100\%$$

5. Seed growing time was calculated by the formula:

$$\text{WTB} = \text{ti} + \frac{(\text{n50\%} - \text{ni})}{\text{nj} - \text{ni}} (\text{tj} - \text{ti})$$

6. Maximum potential growth was calculated based on normal sprouts and abnormal sprouts until the end of the observation, which was 14 days of observation (Sutopo, 2010).
7. Sprout height
   Measurement of the height of dicotyledonous sprouts was based on the height of the tip of the growing point to the base of the stem.
8. Normal sprout dry weight
   Normal dry weight of sprouts was a measure of potential viability which described the amount of food reserves available so that when conditions were in an appropriate environment they could grow and develop properly (Sadjad, 1989).
10. Abnormal sprouts (infected by pests or diseases)
    This parameter was used to find out whether the given treatment affected the percentage of abnormal seeds or infected diseases that grew significantly.

The research data were statistically analyzed using ANOVA. If the treatment had a significant effect, the data were further analyzed using Duncan's Multiple Range Test (DMRT) with a 95% confidence level as well as correlation and regression analysis to determine the optimal level.

RESULTS AND DISCUSSION

Research Results

Seed biochemical analysis tests were carried out to obtain information on seed viability estimation before being used as material for invigoration experiment. Estimation of seed viability was carried out by finding the free fatty acid (FFA) and electrical conductivity (EC) values of the seeds.

Results of free fatty acid test analysis on several seed lots

Analysis of free fatty acid levels from the extraction of seed fat could be used as an indicator of seed viability. The results of the analysis on seeds with varying viability yielded different percentages of seed fat content.

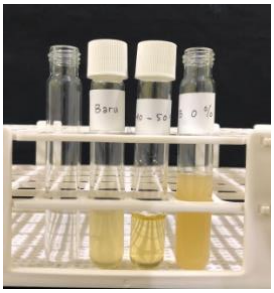

Figure 1. Results of analysis of the fat content of jatropha seeds

The percentage of free fatty acids from the yield could be used as an illustration of the viability of jatropha seeds. The longer the seeds are stored, the free fatty acids of the seeds will increase (Table 1).

Table 1. Result of Jatropha seed free fatty acid test

Seed Lots	% Fat	% FFA
80% GC Seeds	38,9	2.40
GC Seeds 50.67%	37,7	4.94
GC Seeds 0%	32,4	16.07

The content of fatty acids in seeds could be used as a determinant of the estimation of seed viability and vigor values. Free fatty acid titration results on seeds using KOH showed a change in the colour of the fat to pink (Figure 2).

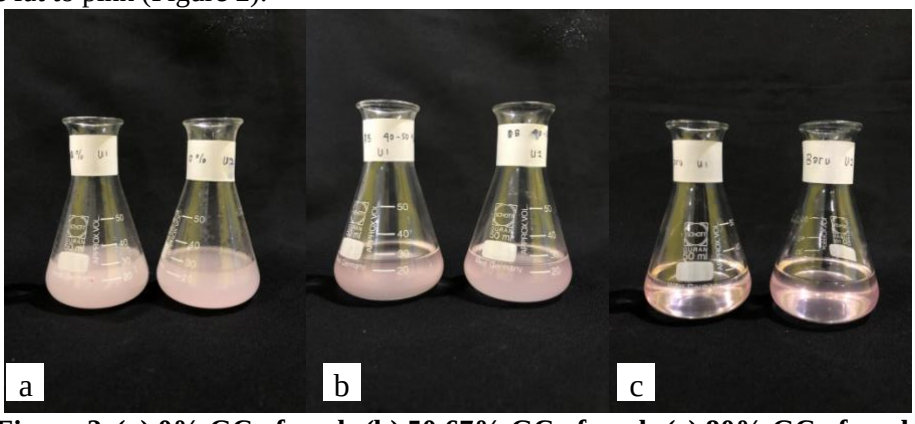

Figure 2. (a) 0% GC of seeds (b) 50.67% GC of seeds (c) 80% GC of seeds

The use of KOH in the jatropha seed fat titration process can neutralize the free fatty acids as indicated by the change in the colour of the fat to pink. The more KOH solution needed to neutralize 1 g of fat, the lower the seed viability. Jatropha seed viability and vigor decreased more quickly than other seeds with carbohydrate (starch) content, due to the high content of unsaturated fat in jatropha seeds.

The fat undergoes oxidation which would affect the viability and vigor of the seeds. The occurrence of the oxidation process of unsaturated fats produced free radicals in the seeds which trigger a decrease in the viability and vigor of jatropha seeds.

Oil content in seeds was influenced by many external factors including the seed production process, storage period, and the environmental seed storage. The influence of internal factors that affected the fat content contained in jatropha seeds is plant genetics. The change of fat into free fatty acids would be quickly damaged if the seeds were not immediately used for germination, so that the available energy reserves in the seeds were reduced. The available energy reserves in the seeds affected the value of seed viability and vigor.

The results of the analysis of the electrical conductivity of jatropha seeds

The EC test method is a simple test for estimating seed quality that has been suggested by ISTA (International Seed Testing Association) for several types of seeds. The electric conductivity test has the principle that seeds will experience a reaction when electrified which is affected by cell membrane leakage due to changes in seed permeability. Seeds that have low viability and vigor will release a lot of elements present in the seeds in the form of elements K, Cl, amino acids and sugars. These elements will accumulate in the liquid used to soak the seeds. Seeds with lower viability and vigor during the EC test will show higher conductivity values.

The conductivity values of several seed lots presented in (Table 2) can be used as an illustration of the viability of jatropha seeds by looking at the leakage of the cell membranes in the seeds.

Table 2. Results of the electrical conductivity (EC) test of jatropha seeds

Seed Lots	Electrical Conductivity (µmhos $cm^{-1}g^{-1}$)
80% GC of seeds	44.88
GC of seeds 50.67%	56,38
GC of seeds 0%	78,46

The difference in the conductivity value indicates that the longer the jatropha seeds are stored the conductivity value increases. The conductivity test results of Jatropha seeds used as research planting material were 56.38 µmhos $cm^{-1}g^{-1}$. The seed lot with 80% viability has a conductivity value of 44.88 µmhos $cm^{-1}g^{-1}$, lower 11.5 µmhos $cm^{-1}g^{-1}$ compared to the GC of seed lot of 50.67%. Lower 33.58 88 µmhos cm-1g-1compared to 0% GC of seed lots.

The higher the conductivity value of Jatropha seeds the lower the viability of the seeds, indicating that the seed lot had cell leakage and low permeability. Seeds that experienced deterioration showed decreased cell membrane integrity and cell wall permeability. Cell leakage in seeds occured as a result of decreased cell membrane integrity, resulting in the dissolving of food reserves in seeds when soaking in imbibition solutions. The decrease in the integrity of the cell membrane was affected by seed storage time, followed by a decrease in the value of seed viability and its vigor. The decrease in the integrity of the cell membrane was caused by the respiration activity of the seeds and the effect of free radicals. Free radicals caused damage to the cell membrane that interferes with electron transport, spreading free electrons that join with free radicals resulting in damage to the integrity of the germ cell membrane.

Damage to cell membranes and decreased cell integrity in seeds have an impact on differences in seed metabolic activity during imbibition. Uncontrolled hydration during seed imbibition caused cell membrane damage. The imbibition process on seeds that experience deterioration with the characteristic of cell membrane damage could be given controlled hydration, so that germination performance could be improved.

The accuracy of the estimation of the electrical conductivity test was affected by the moisture content of the seeds, the temperature of immersion in the liquid, the duration of immersion, the dirt of the seeds and the mechanical damage to the seeds. The permissible moisture content was in the range of 11-17%, with a soaking time of 16-24 hours, comes from pure seed lots and was not mechanically damaged. Temperature, soaking time, mechanical damage and seed impurities could affect the

conductivity value of the tested seeds, so it was necessary to standardize the EC test method on jatropha seeds.

Experiment Results

Based on the results of analysis of variance withα= 5%, the F-value was obtained to determine the effect of matriconditioning invigoration and the application of the biological agent of *Pseudomonas fluorescens* on various observed variables on jatropha seeds in Table 3.

Table 3. Summary of F-count results of the analysis of variance of the observation variables

No	Observational Variables	Compute F Value		
		Old Matriconditioning (A)	*Pseudomonas fluorescens* (B)	Interaction (A×B)
1.	Growth Rate	14,185 **	62,030 **	2,712 *
2.	Vigor Index	14,698 **	61,899 **	2,726 *
3.	Simultaneous Growth	3,867 *	12,419 **	1.133 ns
4.	Germination Capacity	3,867 *	12,419 **	1.133 ns
5.	T50	429,302 **	171,963 **	65,705 **
6.	Maximum Growth Potential	2,825 ns	7,596 **	0.908 ns
7.	Plant Height	0.078 ns	0.429 ns	0.073 ns
8.	Normal Sprout Dry Weight	0.443 ns	0.067 ns	0.298 ns
9.	Abnormal Sprouts	1.698 ns	1.070 ns	0.920 ns

(*) = Significantly different
(**) = Very significantly different
(ns) = Insignificantly different

Based on Table 3, it showed that there was an interaction in the old treatment matriconditioning invigoration and the biological agent of *Pseudomonas fluorescens* on the observed variables of growth rate, vigor index and T50. The effect of a single factor in the matriconditioning treatment showed significantly different values for the variables of simultaneity of growth and germination. The matriconditioning treatment had no significant effect on the maximum growth potential, plant height, dry weight of normal and abnormal sprouts. The effect of a single factor treatment of *Pseudomonas fluorescens* was highly significant to the variable of growth simultaneity, germination capacity and maximum growth potential. The treatment of *Pseudomonas fluorescens* had no significant effect on plant height, dry weight of normal and abnormal sprouts.

Results of Regression Correlation Analysis between Observational Variables

Correlation is a measure of the level of closeness and direction of the relationship between two variables (R). The regression coefficient is a measure of the magnitude of change in one variable related to one experimental unit and another variable. Correlation analysis was carried out on the growth rate, vigor index and T50 variables on the jatropha seed germination variable. Correlation and regression analysis between variables could be seen in Table 4.

Table 4. Summary of R values and t-counts of regression coefficients and correlations

No.	Observational variable		r	t-count
	X	Y		
1	Growth Speed	Germination	0.95	9.43 *
2	Vigor Index	Germination	0.95	9.40 *
3	T50	Germination	0.43	1.48 ns

The results of regression analysis and correlation based on the T test (comparison of t-count with t-table shows that there was a significant correlation between the growth rate and vigor index variables

on seed germination, t-count> t-table. There was no significant correlation between the variable of T50 on jatropha seed germination (t-count < t-table), the value of t-table compared to the t-count used was 2.22814. The interpretation of the correlation coefficient (r) as follows: 0.00-0.20 very low, value 0.20-0.40 low, value 0.40-0.70 sufficient, high correlation 0.700-0.90, value> 0.90 very high.

Relationship between growth speed and seed germination

The relationship between the observed variable growth rate and seed germination from the results of correlation and regression analysis was presented in Figure 3.

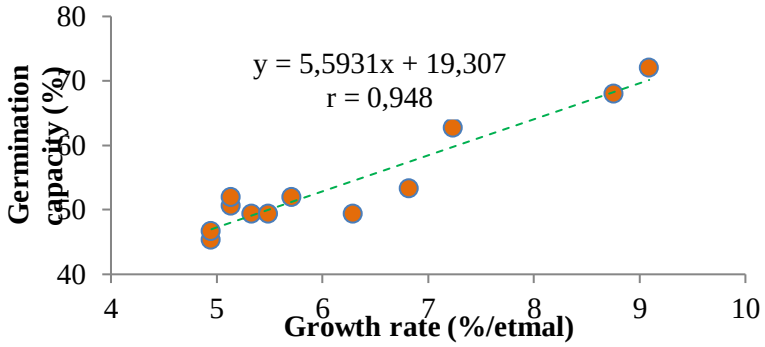

Figure 3. Correlation of growth rate variable to seed germination capacity

Figure 3 showed that the growth rate and germination variables had a unidirectional relationship with a very high degree of closeness. The regression equation shown was y = 5.5931x + 19.307 which meant that every increase in growth rate of 1%/etmal would be followed by an increase in seed germination capacity of 5.5931%. This correlation indicated that any increase in seed vigor, represented by growth rate, would be followed by an increase in viability, represented by the variable seed germination. Seed viability and vigor were a unit of seed quality which had the same cycle graph forming a sigmoid-shaped curve. The curve meant that the decrease in quality that occurs in seeds was preceded by a decrease in vigor and was followed by seed viability.

Correlation between vigor index variables and seed germination

The relationship between the observation variable vigor index and seed germination from the results of the correlation and regression analysis was presented in Figure 4.

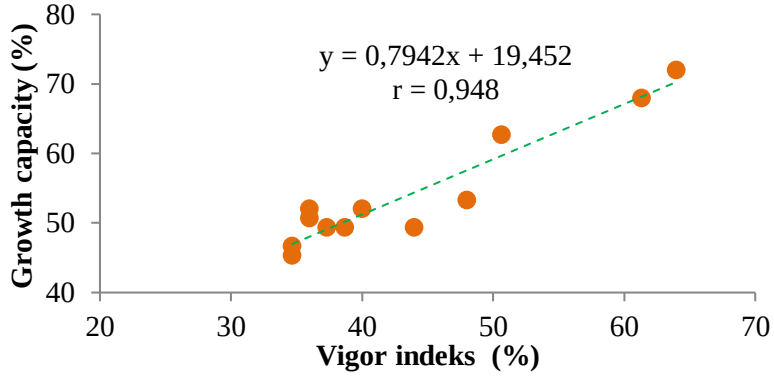

Figure 4. Correlation of the vigor index variable on seed germination

Figure 4 showed that the vigor index variable on seed germination had a unidirectional relationship with a very high degree of closeness. The regression equation shown was y = 0.7942x + 19.452 which meaned that every 10% increase in the vigor index would be followed by a 7.94% increase in seed germination. This correlation indicated that any increase in seed vigor represented by the vigor index would be followed by an increase in viability represented by the variable seed germination. The increase in seed viability in the form of germination had a close relationship with the value of the vigor index, so that the high value of germination could be known from the first count germination performance.

Effect of Interaction of Matriconditioning and *Pseudomonas fluorescens* in Increasing Jatropha Seed Viability and Vigor

Based on analysis of variance of all observed variables, it was shown that the effect of the interaction between the duration of matriconditioning and the dose of *Pseudomonas fluorescens* was significantly different from the observed variables of growth velocity, vigor index and T50.

Growth Rate (Kct)

The effect of matriconditioning invigoration time and biological agent *Pseudomonas fluorescens* on growth speed variables was presented in Table 5.

Table 5. The effect of the interaction of matriconditioning and the application of the biological agent *Pseudomonas fluorescens* on increasing seed vigor on the parameter of growth rate of Jatropha seeds

Pf dose (ml^{l-1})	*Matriconditioning* (hours)			
	12	24	36	48
0	5.14 ± 0.57 ab	5.7 1 ± 0.00 ab	6.82 ± 0.57 c	4.95 ± 0.66 a
100	7.24 ± 0.33 c	8.76 ± 0.66 d	9.09 ± 0.98 d	6.29 ± 0.57 bc
200	5.14 ± 0.57 ab	5.33 ± 0.33 ab	5.49 ± 0.66 ab	5.71 ± 0.66 a

The numbers followed by the same letters were not significantly different according to Duncan's test at the 5% level of significance.

The relationship between the duration of matriconditioning treatment and the dose of *Pseudomonas fluorescens* on the growth rate (Kct) of jatropha seeds was also presented in Figure 5.

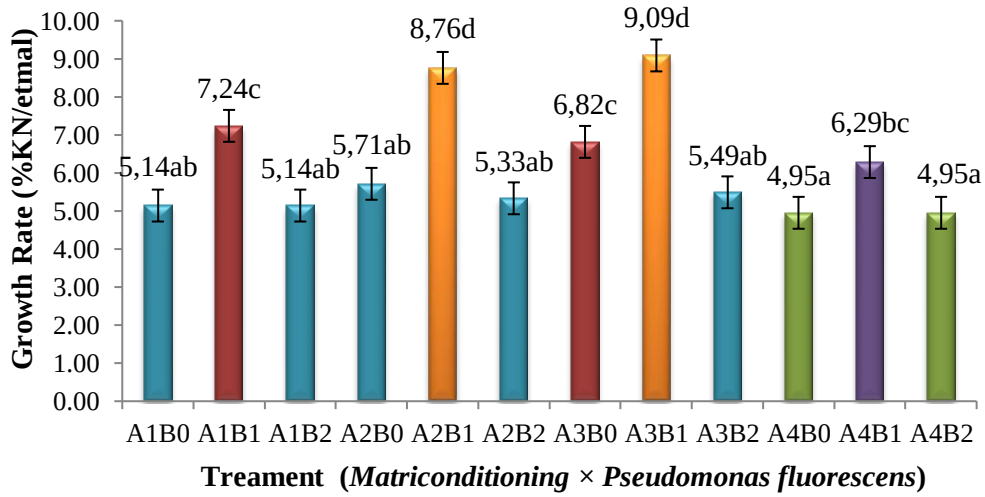

Figure 5. Effect of matriconditioning duration interaction treatment and doses of *Pseudomonas fluorescens* on the growth rate of jatropha seeds

The combination treatment that produced the highest Kct occurred in the 36-hour matriconditioning treatment at a dose of *Pseudomonas fluorescens* 100 ml l^{-1} for9.09%/etmal, but not significantly different from the 24-hour matriconditioning combination treatment at the dose of *Pseudomonas fluorescens* 100 ml l^{-1} of 8.76%/etmal. The same matriconditioning time at the level of 24 hours produces mark The Kct was significantly different at the doses of Pseudomonas fluorescens. Same dose of *Pseudomonas fluorescens* 100 ml l^{-1} yield value Kct which was not significantly different at 36 hours and 24 hours matriconditioning time.

The combination of treatments that produced the lowest growth rate values occurred in the 48-hour long matriconditioning treatment, at a dose of 0 ml of *Pseudomonas fluorescens* and 200 ml l^{-}

[1].The best treatment suggested in this experiment was based on the parameter of increasing Kct, namely the combination of 24 hours of matriconditioning and doses of *Pseudomonas fluorescens* 100 ml l^{-1}.The value of growth speed was obtained from observing the growth of Jatropha seed sprouts in units of the percentage of normal sprouts per etmal. The higher the value of Kct produced in this study meant that the seed vigor had increased.

Vigor Index (VI)

The effect of matriconditioning invigoration time and biological agent *Pseudomonas fluorescens* on the vigor index variable was presented in Table 6.

Table 6. The effect of the interaction of matriconditioning and the application of the biological agent *Pseudomonas fluorescens* on increasing seed vigor on the parameter of Jatropha seed vigor index

Pf dose (ml.l^{-1})	*Matriconditioning* (hours)			
	12	24	36	48
0	36±4.00 ab	40±0.00 ab	48±4.00 c	34.67±4.62 a
100	50.67±2.31 c	61.33±4.62 d	64±6.93 d	44±4.00 bc
200	36±4.00 ab	37.33±2.31 ab	38.67±4.62 ab	34.67±4.62 a

The numbers followed by the same letters were not significantly different according to Duncan's test at the 5% level of significance.

Display of the relationship between matriconditioning duration and *Pseudomonas fluorescens* dose on the vigor index value of Jatropha seeds was presented in graphical form which could be seen in Figure 6.

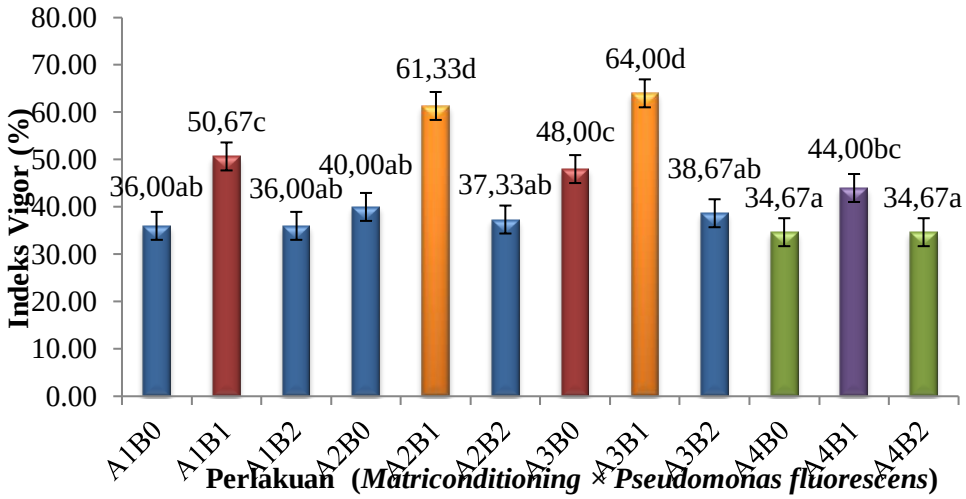

Figure 6. Effect of matriconditioning duration interaction treatment and *Pseudomonas fluorescens* dose on the vigor index of Jatropha seeds

The combination treatment that produced the highest vigor index was found in the 36-hour matriconditioning treatment at a dose of *Pseudomonas fluorescens* 100 ml l^{-1} for 64% but not significantly different from the 24-hour matriconditioning combination treatment at the dose of *Pseudomonas fluorescens* 100 ml l^{-1} for 61.33%. The same matriconditioning time at the 24 hours level resulted in significantly different vigor index values at the doses of *Pseudomonas fluorescens*. Same dose of *Pseudomonas fluorescens* 100 ml l^{-1} yield value vigor index which was not significantly different at 36 hours and 24 hours matriconditioning time. The combination of treatments that produced the lowest vigor index value occurred in the 48-hour long matriconditioning treatment, at a dose of 0

ml of *Pseudomonas fluorescens* and 200 ml l^{-1}. The best treatment suggested in this experiment was a combination of 24 hours of matriconditioning and doses of *Pseudomonas fluorescens* 100 ml l^{-1}.

T50 (50% Seed Growing Time)

The effect of matriconditioning invigoration time and the biological agent *Pseudomonas fluorescens* on variable T50 was presented in Table 7.

Table 7. The effect of the interaction of matriconditioning and the application of the biological agent *Pseudomonas fluorescens* on increasing seed vigor in the parameter T50 of Jatropha seeds

Pf dose (ml.l^{-1})	*Matriconditioning* (hours)			
	12	24	36	48
0	11.92±0.00 d	9.68±0.00 c	7.04±0.00 ab	0.00±0.00 e
100	7.95±1.02 b	7.53±0.89 b	6.31±1.48 a	0.00±0.00 e
200	10.53±0.00 c	0.00±0.00 e	0.00±0.00 e	0.00±0.00 e

The numbers followed by the same letters were not significantly different according to Duncan's test at the 5% level of significance.

Display of the relationship between matriconditioning duration and *Pseudomonas fluorescens* dose on the vigor index value of Jatropha seeds is presented in graphical form which could be seen in Figure 7.

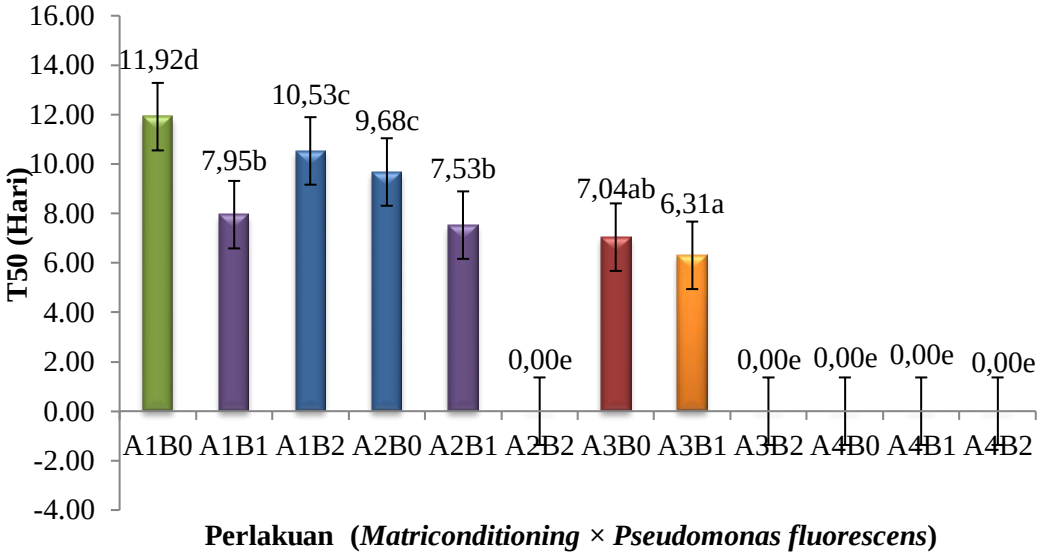

Figure 7. Effect of matriconditioning duration interaction treatment and *Pseudomonas fluorescens* dose on the T50 value of jatropha seeds

The combination treatment that produced the fastest T50 occurred in the 36-hour matriconditioning treatment at a dose of *Pseudomonas fluorescens* 100 ml l^{-1}. The T50 value of the interaction effect on the 36-hours matriconditioning treatment and the combined dose of *Pseudomonas fluorescens* 100 ml l^{-1} with a value of 6.31 was better than the old matriconditioning hour treatment without *Pseudomonas fluorescens* administration, but there was no significant difference between the two treatments. The same matriconditioning time at the 36 hours level resulted in a T50 value that was not significantly different at the dose of *Pseudomonas fluorescens* 100 ml l^{-1} and without *Pseudomonas fluorescens*. Same dose of *Pseudomonas fluorescens* 100 ml l^{-1} generate valueT50 which was significantly different in the old matriconditioning treatment. A value of 0 (zero) on the results indicated

that the combination of these treatments did not achieve a minimum germination of 50%. The best treatment suggested to increase the T50 value in this experiment was a combination of 36 hours of matriconditioning without the application of *Pseudomonas fluorescens*.

The Effect of a Single Factor of Matriconditioning Old Treatment in Increasing Viability and Vigor of Jatropha Seeds

Simultaneous Growth (Kst)

The results of the analysis of variance in (Table 3) showed that the matriconditioning duration had a significant effect on the growth simultaneity value (Kst) of *Jatropha curcas* seeds. Based on the DMR follow-up test, the highest Kst value for the matriconditioning duration was achieved at the 36 hours level, namely 58.22%. Further test results showed that the matriconditioning duration among 12 hours, 24 hours and 36 hours resulted in a Kst that was not significantly different, respectively 55.11%, 56.44%, 58.22%. The Kst value of the 12, 24 and 36 hours matriconditioning duration was significantly different from the 48 hour matriconditioning treatment which had the smallest Kst value of 47.11%. The negative impact of the matriconditioning treatment occurred at the 48 hours level resulting in a Kst value of 47.11%, which was followed by a decrease in the value of seed germination.

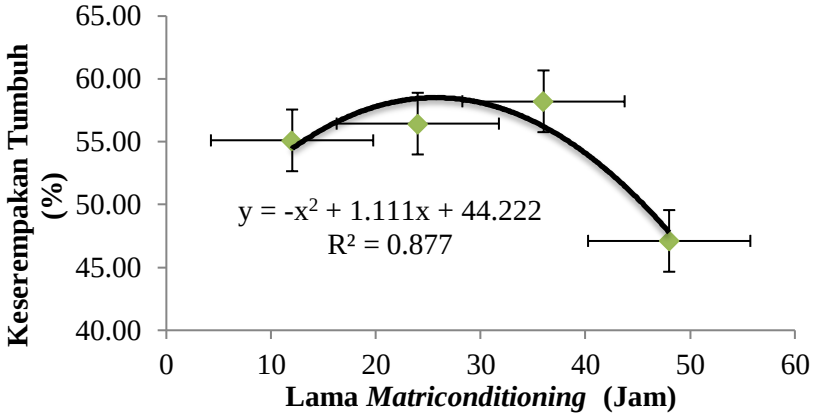

Figure 8. The effect of long matriconditioning treatment on the uniformity of growing Jatropha seeds

The results of the orthogonal polynomial test produce a regression equation (y) = -x2 + 1.111x + 44.222 with a coefficient of determination (R^2) = 0.877. Based on the regression equation, it showed that the optimal value for the 25.72 hours long matriconditioning treatment resulted in an optimal Kst of 58.57%. The 24 hour matriconditioning time was the best time to affect the simultaneity of Jatropha seed growth, although it was not significantly different from the 12 hour and 36 hour matriconditioning time. The matriconditioning time of 24 hours was close to the time that produces the optimal value of growing simultaneity. The high value of growing simultaneously indicated that matriconditioning was able to stimulate seed metabolism so that it could grow simultaneously in the planting area.

Germination Capacity (GC)

The prolonged matriconditioning treatment had a significant effect on the germination rate of Jatropha seeds as shown by the results of the analysis of variance in (Table 3). Based on the DMRT follow-up test, the highest germination capacity of matriconditioning was achieved at the 36 hours level, namely 58.22%. Further test results showed that the matriconditioning duration between 12 hours, 24 hours and 36 hours resulted in a GC that was not significantly different respectively 55.11%, 56.44%, 58.22%. The increase in the GC value for the matriconditioning time of 12 hours, 24 hours and 36 hours against the GC test value without treatment was 4.44%, 5.77%, 7.55%, respectively. The GC values of the 12, 24 and 36 hours matriconditioning duration were significantly different from the 48 hour matriconditioning treatment which had the smallest GC value of 47.11%.

Based on the DMRT test on the observation variable germination had the same value as the simultaneity of growth, because the addition of sprouts ends in calculating the simultaneity of growth (11 DAP). The results obtained from the study, although not significantly different at the level of matriconditioning time of 12 hours, 24 hours and 36 hours, proved that matriconditioning was able to

increase the germination value of jatropha seeds. The increase in GC values in this study is in line with Mariani and Andi (2021), research on soybean seeds resulted in an increase in GC values of up to 13% compared to no matriconditioning treatment. The 48-hours matriconditioning treatment caused the GC value to decrease by 3.56% compared to the GC test value without treatment. The increase in GC value was an indicator that prolonged matriconditioning treatment could increase seed viability. The relationship between matriconditioning and long-term germination of Jatropha seeds could be seen in Figure 9.

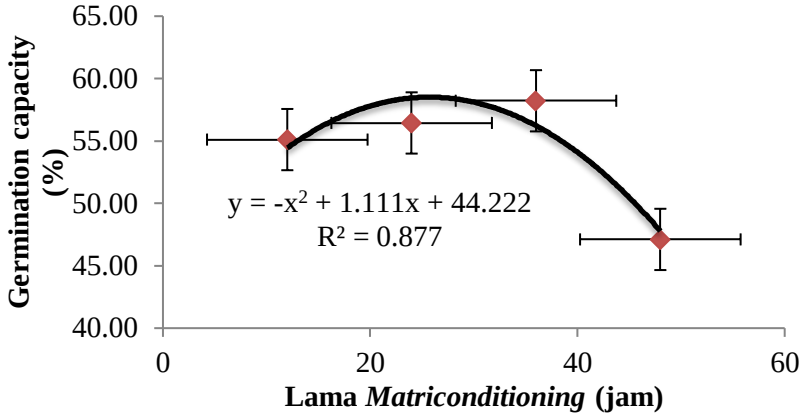

Figure 9. Effect of long matriconditioning treatment on jatropha seed germination capacity

The results of the orthogonal polynomial test produced a regression equation (y) = -x2 + 1.111x + 44.222 with a coefficient of determination (R^2) = 0.877. Based on the regression equation, it showed that the optimal value for the long matriconditioning treatment was 25.72 hours, resulting in an optimal GC of 58.57%. The 24 hours matriconditioning time was the best time to affect the simultaneity of Jatropha seed growth, although it was not significantly different from the 12 hour and 36 hours matriconditioning time. The matriconditioning time of 24 hours was close to the time that produces the optimal value of growing simultaneity.

Effect of Single Factor Treatment of *Pseudomonas fluorescens* Dosage in Increasing Jatropha Seed Viability and Vigor

Simultaneous Growth (Kst)

The results of the analysis of variance in (Table 3) showed that the treatment dose of *Pseudomonas fluorescens* had a significant effect on the growth simultaneity (Kst) value of Jatropha curcas seeds. Based on the DMRT follow-up test, *Pseudomonas fluorescens* dose treatment 100 ml l^{-1} produced the highest Kst value, compared to other treatments with a Kst value of 63%. Kst value of *Pseudomonas fluorescens* dose treatment100 ml l^{-1} significantly different from the control treatment and at the dose of *Pseudomonas fluorescens* 200 ml l^{-1} which had a GC value of 50.33% and 49.33% respectively. Kst value of *Pseudomonas fluorescens* dose treatment100 ml l^{-1} significantly different with dose 200 ml l-1 and control. *Pseudomonas fluorescens* dose treatment100 ml l^{-1} was able to increase the Kst value by 12.67% compared to the control.

Pseudomonas fluorescens dose treatment200 ml l^{-1} produced values that were not significantly different from the control, and produced the smallest Kst value. *Pseudomonas fluorescens* dose treatment 200 ml l^{-1} negatively affected the Kst value, decreasing the Kst value by 1% compared to the Kst control. The decrease in the value of the simultaneity of growth was suspected to be the dose of *Pseudomonas fluorescens* 200 ml l^{-1}, had a toxic effect on seeds in the germination process. The relationship between the dose of *Pseudomonas fluorescens* and the simultaneous growth of Jatropha seeds could be seen in Figure 10.

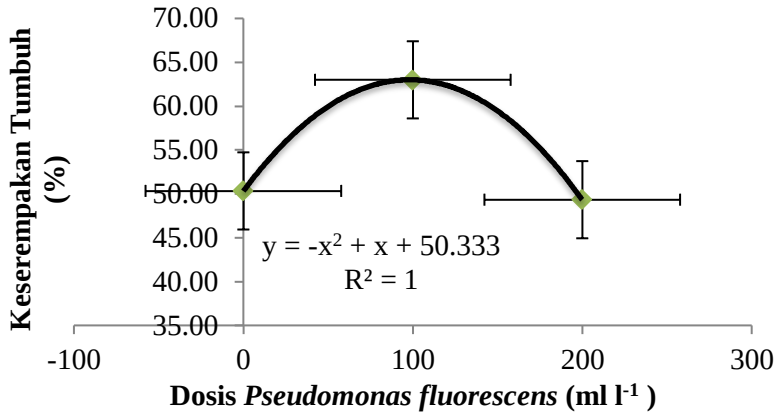

Figure 10. Effect of *Pseudomonas fluorescens* dose treatment on the uniformity of Jatropha seed growth

The results of the orthogonal polynomial test produced a regression equation (y) = -x2 + x + 50.333 with a coefficient of determination (R^2) = 1. Based on the regression equation, it showed that the optimal value was at the treatment dose of *Pseudomonas fluorescens* 99.35 ml l^{-1} produces an optimal value of Kst 63.16%. *Pseudomonas fluorescens* 100 dose ml l^{-1} be the best dose in affecting the simultaneous growth of Jatropha seeds compared to other doses. Dose 100 ml l^{-1} *Pseudomonas fluorescens* approached the time that produced the optimum value of the growing synchrony of Jatropha seeds. According to Sutariati et al. (2014), the application of *Pseudomonas fluorescens* used in matriconditioning invigoration had an effect of increasing the Kst value by 19% compared to matriconditioning without *Pseudomonas fluorescens* on upland rice seeds.

Germination Capacity (GC)

The results of the analysis of variance in (Table 3) showed that the dose of *Pseudomonas fluorescens* had a significant effect on the germination rate of jatropha seeds. Based on DMRT follow-up test with *Pseudomonas fluorescens* dose treatment 100 ml l^{-1} produces the highest DB value, compared to other treatments with a germination rate value of 63%. Germination rate value of *Pseudomonas fluorescens* dose treatment 100 ml l^{-1} significantly different from the control treatment, and at the dose of *Pseudomonas fluorescens* 200 ml l^{-1} which has a germination rate value of 50.33% and 49.33% respectively. The dose of *Pseudomonas fluorescens* was able to increase germination rate values by 12.67% compared to control.

Pseudomonas fluorescens dose treatment200 ml l^{-1} produces the smallest germination rate value, not significantly different from the control. *Pseudomonas fluorescens* dosage 200 ml l^{-1} had a negative impact on germination rate values, reduced germination rate values by 1% compared to control germination rate and was suspected of having a toxic effect on the seed germination process. The relationship between *Pseudomonas fluorescens* dose treatment and jatropha seed germination could be seen in Figure 11.

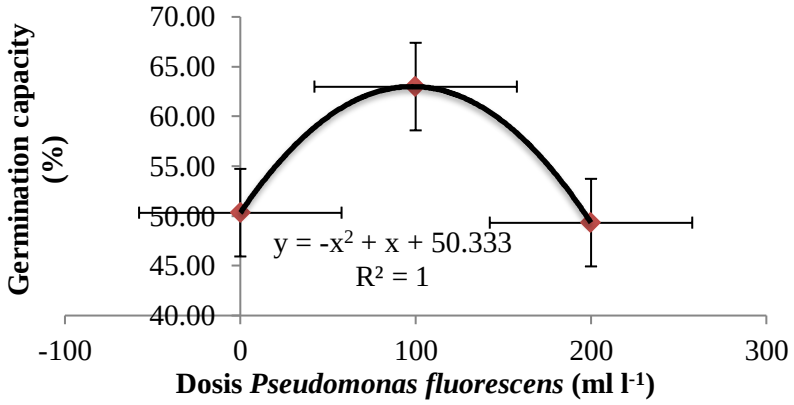

Figure 11. Effect of *Pseudomonas fluorescens* treatment on jatropha seed germination

The results of *orthogonal polynomial test* produced a regression equation (y) = -x2 + x + 50.333 with a coefficient of determination (R^2) = 1. Based on the regression equation it showed the optimal value at the treatment dose of *Pseudomonas fluorescens* of 99.35 ml l^{-1} produces an optimal GC value of 63.16%. *Pseudomonas fluorescens* 100 dose ml l^{-1} in the exact treatment was the best dose in influencing jatropha seed germination compared to other doses. Dose 100 ml l^{-1} *Pseudomonas fluorescens* was closed to the time that produces the optimal value of jatropha seed germination.

Several studies have stated that matriconditioning was a combination of giving *Pseudomonas fluorescens* density 109 cfu/ml or equivalent to dose of 100 ml l^{-1} in this study, could increase seed viability. According to Sutariati et al. (2014), administration of *Pseudomonas fluorescens* dose of 109 cfu/ml used in matriconditioning invigoration, had an impact on increasing germination rate values by 15% compared to matriconditioning without *Pseudomonas fluorescens* on upland rice seeds. *Pseudomonas fluorescens* dosage 109 cfu/ml used in the matriconditioning invigoration of chili seeds had an impact on increasing germination by 20%.

Maximum Growth Potential (PTM %)

Based on DMRT test, *Pseudomonas fluorescens* dose of 100 ml l^{-1} produced the highest maximum growth potential (PTM), compared to other treatments with a PTM value of 64.83%. Maximum growth potential from *Pseudomonas fluorescens* dose of 100 ml l^{-1} significantly different from the control treatment and at the dose of Pseudomonas fluorescens 200 ml l^{-1} which has a PTM value of 53.58% and 53.50% respectively. PTM value of *Pseudomonas fluorescens* dose of 100 ml l^{-1} significantly different with dose of 200 ml l^{-1} and control. *Pseudomonas fluorescens* dose of 100 ml l^{-1} was able to increase PTM by 11.25% compared to control.

Pseudomonas fluorescens dose of 200 ml l^{-1} produced values that were not significantly different with the control, and produced the smallest Kst value. *Pseudomonas fluorescens* dose of 200 ml l^{-1} had a negative impact on PTM values, reducing PTM values by 1% compared to controls. The increase of PTM was affected by the germination rate of the number of normal sprouts and the number of abnormal sprouts, the dose of *Pseudomonas fluorescens* 200 ml l^{-1} increased the number of abnormal sprouts by 1.99%. The relationship between the dose of *Pseudomonas fluorescens* and the maximum growth potential of Jatropha seeds could be seen in Figure 12.

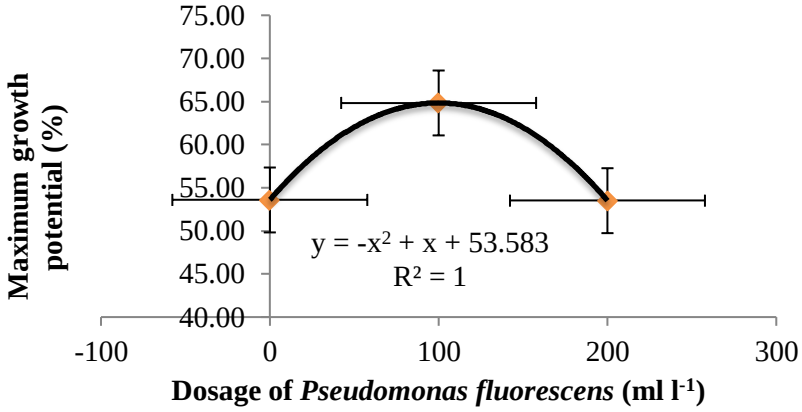

Figure 12. Effect of *Pseudomonas fluorescens* treatment on maximum growth potential of Jatropha seeds

The results of the orthogonal polynomial test produced a regression equation (y) = -x2 + x + 53,583 with a coefficient of determination (R^2) = 1. Based on the regression equation, it showed that the optimal value at the treatment dose of *Pseudomonas fluorescens* is 102.46 ml l-1 produced an optimal PTM value of 65.13%. *Pseudomonas fluorescens* dose of 100 ml l^{-1} was the best dose in influencing the maximum growth potential of jatropha seeds compared to the other doses. Dose of 100 ml l^{-1} *Pseudomonas fluorescens*close to the time that produces the optimal value of the maximum growth potential of Jatropha seeds. Application of *Pseudomonas fluorescens* used in matriconditioning

invigoration had an effect of increasing PTM values by 8% compared to matriconditioning without *Pseudomonas fluorescens* on upland rice seeds (Sutariati et al., 2014).

Discussion

Interaction between treatments of matriconditioning invigoration and application of the biological agent *Pseudomonas fluorescens* significantly affected the observed variables of growth speed, vigor index and T50 (Table 3). The interaction occuring in the growth rate variable (Kct) could explain that the combination of matriconditioning invigoration treatment and the application of the biological agent *Pseudomonas fluorescens* increases the vigor of Jatropha seeds in the form of an increase in the value of growth speed. The combination of 24 hours of matriconditioning and a dose of 100 ml l^{-1} *Pseudomonas fluorescens* was the recommended treatment for use with a Kct value of 8.76%/etmal. Increasing the value of Kct had a positive effect on increasing the viability (GR/GC) of Jatropha seeds, the two observational variables had a strong positive correlation. The increase in growth rate also occurred in the combination of matriconditioning treatment of roasted husk matrix and *Pseudomonas fluorescens* at a dose of 109 cfu ml^{-1} which had a positive effect on upland rice seeds, increasing Kct up to 30 %/etmal (Sutariati et al., 2021).

The interaction that occurs in the vigor index variable (VI) could explain that combination of matriconditioning invigoration and *Pseudomonas fluorescensincrease* Jatropha seeds increased vigor index. The combination of 24 hours of matriconditioning time and a dose of 100 ml l^{-1} *Pseudomonas fluorescens* was the recommended treatment for use with the best VI value of 61.33%. Increasing the value of VI had a positive effect on increasing the viability (germination rate) of Jatropha seeds, the two observational variables have a strong positive correlation. The results of this study are in line with research Sutariati et al. (2021) explained that VI upland rice seeds increased by 52% compared to the control without matriconditioning and combination treatment*Pseudomonas fluorescens*. Other sources mentioned that the combination of *matriconditioning* and *Pseudomonas fluorescens* increased VI in chili seeds up to 17.33% and up to 20% in chili seeds infected with *C. acutatum* (F. A. Permatasari et al., 2016).

The interaction occuring in the variable T50 could explain that combination of matriconditioning invigoration and application of *Pseudomonas fluorescens* accelerate seed growth time by 50% (T50). The combination of 24 hours of matriconditioning without administration of *Pseudomonas fluorescens* was the recommended treatment for use in increasing T50 values. The relationship between the T50 value in influencing the germination value had a very low correlation. Combination matriconditioning and *Pseudomonas fluorescens*also showed significant results in solving postharvest physiological dormancy in upland rice (after ripening). This conclusion was seen based on the T50 value as an indicator of seed dormancy, the T50 value decreased compared to the control (5.04 days) to (4.47 days) after the combination treatment matriconditioning for 48 hours and *Pseudomonas fluorescens* dose of 109 cfu ml^{-1} (Sutariati et al., 2021).

The variables of growth rate and germination had a unidirectional relationship with a very high degree of closeness. Each increase in seed vigor was represented by a growth rate of 1%/etmal would be followed by an increase in seed viability represented by seed germination of 5.59%. The combination of 24 hours of matriconditioning and dose of 100 ml l^{-1} of *Pseudomonas fluorescens* increased Kct, followed by an increase in seed viability (GC).This combination treatment was also able to increase the Kct value in chili seeds by 3.74%/etmal and by 3.94%/etmal in chili seeds infected with *C. acutatum* disease compared to controls (F. A. Permatasari et al., 2016).The correlation between Kct and GC values also occurs in sesame seeds with a correlation value (0.88) in the same direction and closely (Hartati, 2019).

The vigor index variable on seed germination has a unidirectional relationship with a very high level of closeness. Each increase in Jatropha seed vigor is represented by a vigor index of 10%, followed by an increase in seed viability represented by germination of 7.94%. The increase in Jatropha seed viability in the form of germination has a close relationship with the vigor index value, so that the high germination value could be known from the first count germination performance. Increases and decreases in the vigor index are always followed by increases and decreases in germination. This occurs

in several different types of seeds, in papaya (Rosyad et al., 2016), in rice seeds (Zhulfikri, 2018), in cocoa seeds (Rachma et al., 2018). These results are in accordance with (Hartati, 2019).

The results of this study are in line with the opinion of Sadjad et al. (1999), that seeds having a high growth rate have a high level of vigor. Seed viability and vigor are a unit of seed quality which has the same cycle graph, forming a sigmoid-shaped curve (Ilyas, 2012). This curve means that the decrease in quality that occurs in seeds is preceded by a decrease in vigor and is followed by seed viability (Justice and Bass, 1979).

The duration of matriconditioning invigoration significantly affected on growth rate (Kct), vigor index (VI), T50, growth simultaneity (Kst) and germination capacity (GC). However, it had no significant effect on the plant height, maximum growth potential (MGP), normal sprout dry weight (NSDW) and abnormal seeds.

Based on the research result, the 24-hours matriconditioning treatment produced the best Kst value compared to other matriconditioning treatments. It was supported by the results of the polynomial regression equation test which produced the optimal value of Kst closed to the 24 hours matriconditioning treatment. Increasing the value of Kst has a positive effect on increasing the viability (GC) of Jatropha seeds. The matriconditioning invigoration of seeds for each plant species requires a different time depending on the type of seed, size, physical seed and chemical content of the seed. This statement was proven by the matriconditioning treatment for 5 hours on cocoa seeds on the roasted husk matrix, which was the right length of treatment, capable of increase the Kst value by 28.5% (Rachma et al., 2018). Matriconditioning research on peanut seeds resulted in an increase in Kst of 26.67% in the roasted husk matrix with the best treatment time of 12 hours (Muazizah, 2019).The negative impact of the matriconditioning treatment in this study occurred at the 48 hours level resulting in a Kst value of 47.11%, which was followed by a decrease in the value of seed germination.

The 24-hours matriconditioning treatment resulted in the best germination value compared to other matriconditioning treatments. The combination of these treatments was supported by the results of the polynomial regression equation test which produced the optimal GC value close to the 24 hours matriconditioning treatment. Increasing the vigor of jatropha seeds in the form of Kct, vigor index, T50, MGP and Kst, had a positive effect on increasing GC values. Invigorating seed matriconditioning for each plant species required different time to increase seed viability and vigor, depending on the type of seed, size, physical seed and chemical content of the seed. Matriconditioning treatment for 48 hours on upland rice seeds on the burnt husk matrix was the right treatment time, able to increase GC value by 65% (Sutariati et al., 2021). The germination capacity of peanuts could be increased to 26.67% through the matriconditioning invigoration treatment with the roasted husk matrix for 12 hours (Muazizah, 2019).

The negative impact of the matriconditioning treatment occurred at the 48 hour level resulting in a GC value of 47.11%, which was followed by a decrease in the value of seed germination. A significant increase in germination value is an indicator that 24 hours of matriconditioning time could increase the percentage of seed viability. Negative impact of treatment matriconditioning on seed viability could occur due to the length of treatment on the seed. Germination decreased by 2% in the matriconditioning treatment of soybean seeds for 18 hours compared to no matriconditioning treatment while the optimal length of treatment was 12 hours (Mariani & Wahditiya, 2021). Matriconditioning treatment of purple egg plant seeds for 7 days could reduce germination by 15.4%, lower than the best treatment time (Hasan et al., 2018).

Application of *Pseudomonas fluorescens* significantly affected on growth rate (Kct), vigor index (VI), T50, growth simultaneity (Kst) and germination rate (GC). In contrast, it had no significant effect on the plant height, maximum growth potential (MGP), normal sprout dry weight (NSDW) and abnormal seeds.

Based on research results, *Pseudomonas fluorescens* 100 ml l^{-1} treatment produced the highest and best Kst value compared to other treatments, by 63%. The effect of application of this dose of *Pseudomonas fluorescens* increased the Kst of Jatropha seeds by 12.67% compared to the Kst of seeds without *Pseudomonas fluorescens* application. It was supported by the results of the polynomial

regression equation test which yielded an optimal value of Kst closed to the 100 ml l-1 dose of *Pseudomonas fluorescens*. Increasing the value of Kst had a positive impact on increasing the viability (GC) of Jatropha seeds. The need for biological agents in seed treatment needed to be adjusted because they could have positive and negative effects at certain doses on seed vigor and viability. The results of this study were in line with research's Sutariati et al. (2021), the simultaneous growth of upland rice seeds increased by 19% in the addition of treatment *Pseudomonas fluorescens* density 109 cfu ml-1 compared to matriconditioning without application*Pseudomonas fluorescens*.

The treatment of *Pseudomonas fluorescens* 100 ml l^{-1} increased the percentage of germination and maximum growth potential of Jatropha seeds. The highest and best germination percentage achieved by 63%. This treatment increased the GC value of Jatropha seeds by 12.67% compared to control. It also was supported by the results of the polynomial regression equation test which yielded the optimal GC value close to the 100 ml l^{-1} dose of *Pseudomonas fluorescens*. The increase in the GC value followed the increase in the vigor value of the seeds, the Kct value and vigor index in this study had a positive and very close correlation with the viability (GC) value of Jatropha curcas seeds. This research was in line with the exposure of Sutariati et al. (2021), treatment of *Pseudomonas fluorescens* dose of 109 cfu ml-1 on capable upland rice seeds increase GC value 15%.

The treatment of *Pseudomonas fluorescens* 100 ml l^{-1} could produce the highest maximum growth potential and achieve the best by 64.83%, an increase of 11.25% compared to control. This combination of treatments was supported by the results of the polynomial regression equation test which yielded optimal PTM values close to the 100 ml l^{-1} dose of *Pseudomonas fluorescens*. The increase in PTM value was determined by the increase in DB and the percentage of abnormal seeds. The increase in the viability of Jatropha seeds in this study had a positive impact on increasing the maximum growth potential of the seeds. The positive impact on this treatment also occurred in the study of Sutariati et al. (2021), maximum growth potential upland rice seeds increase 8% after treatment *Pseudomonas fluorescens* 109 cfu ml l^{-1} compared to the control.

The matriconditioning treatment in this study increased viability in the form of germination capacity (GC) compared to before treatment, vigor in the form of growth speed (Kct, vigor index, T50) and growth simultaneity (Kst) in Jatropha curcas seeds. Seeds that have been given matriconditioning treatment according to Sucahyono (2013) had metabolic conditions that were ready to germinate, but the appearance of the radicle could be delayed so that the percentage of germination and growth rate of sprouts increases when planted. The process of seed germination occured through several stages, matriconditioning could affect the mechanism and performance of jatropha seed germination. Utilization of Pf also had the potential to prevent disease attacks on plants (Harmaningrum, no year).

The factors influencing success matriconditioning according to Copeland and McDonald (2001), namely conditions during matriconditioning (temperature and light), type and matrix osmotic potential, oxygen availability, length of treatment time, control of pathogen contamination, and method of seed drying. The matriconditioning treatment of Jatropha seeds with the right length of time affected the success of the aim of increasing seed viability and vigor. Pathogen control in the form of matriconditioning integration with the biological agent *Pseudomonas fluorescens* also influences the factors of increasing the viability and vigor of Jatropha seeds.

Seed germination process according to Copeland and McDonald (2001), consisted of several stages, starting with imbibition through the testa of the seed, hydration of the aleurone of the seed up to the endosperm and embryo. The next step was the activation of enzymes in seeds such as amylase, lipase and protease enzymes in seeds. The enzyme in the next stage breaks down the food reserves in the seeds in the form of proteins, fats, carbohydrates and others into soluble forms. The soluble energy reserves were translocated to the growing point of the embryo. The hormones auxin, gibberellins and cytokinins worked to support the allocation of available energy to the area of cell growth. The radicle and plumule grew through the seed coat, as a result of cell growth, the process of cell enlargement, and the development of the sprout's growing point.

Imbibition mechanism in seeds given invigoration treatment matriconditioning different from the process of seed germination in general without treatment. Controlled imbibition in the matriconditioning treatment extended the time for water absorption which allowed for the improvement

of the metabolic activity of Jatropha seeds. The imbibition mechanism provided controlled hydration to seeds which was controlled by the physical strength of the moist solid media used (fired husks), had a low matrix potential and negligible osmotic potential (Khan, 1992). The media used according to Taylor et al. (1988), must had conditions that had water holding capacity, be able to balance and release water according to the moisture content of the seeds, not be toxic to the seeds and could be cleaned from the seeds.

Seeds that experienced deterioration experienced damage to the permeability and integrity of the cell membrane as indicated by cell leakage on the results of the electrical conductivity test. Free radicals caused damage to cell membranes that interfere with electron transport, scatter free electrons which join with free radicals resulting in damage to the integrity of germ cell membranes (Copeland and Mcdonald, 2001). Direct and uncontrolled hydration could cause additional damage to cell membranes, because seeds with low water content were prone to imbibition injury. A controlled imbibition process would improve germination performance so that seed viability and vigor could be improved, especially in seeds that experience deterioration (Finch-Savage et al., 2004).Orthodox seeds that were sensitive to cold conditions and high in fat, such as nuts, were more susceptible to imbibition injury (Taylor et al., 1988).

Invigoration of matriconditioning showed different results depending on the type of plant seed, the permeability of each type of plant seed was different depending on the physical testa of the seed (thick, hard, thin, soft), and the content in the seed (fat, protein, starch) would affect the rate of imbibition (Finch-Savage et al., 2004). Orthodox seeds with thick skin and high fat content, such as Jatropha curcas, were thought to experience a slower imbibition process than other types of seeds. Hard testa and high fat content in seeds that had low permeability properties slow down the imbibition process, in some seeds it could be increased by matriconditioning treatments (Taylor et al., 1988). Invigoration of matriconditioning in some seeds found an improvement in the integrity of the cell membrane, judging by the decrease in the resulting electrical conductivity (EC).

The matriconditioning invigoration mechanism after imbibition helped the activation of hormones and enzymes in Jatropha seeds last longer, so that the internal potential of the seeds could be maximized. Enzymes that work in the overhaul of seed food reserved during matriconditioning were amylase, lipase and protease enzymes. The endogenous enzyme amylase in the seeds helped break down food reserves in the form of starch into sugar (sucrose) as energy. The lipase enzyme changed the lipids contained in Jatropha seeds into fatty acids and glycerol and is broken down more simply into glucose and ends up as energy. The protease enzymes in the seeds changed the protein preparations in the seeds into amino acids and amides in forming new proteins for germination (McDonald and Copeland, 1995). It happened during the time of matriconditioning invigoration.

The performance of hormones in seeds when active matriconditioning affected seed germination in the form of gibberellin (GA3), auxin (IAA), and cytokinin hormones found in seeds. Cytokinins affected the action of phytochromes, could change the permeability of cell membranes, thereby allowing the release of gibberellin from the scutellum to the aleurone during seed germination (Copeland et al., 2001). Cytokinins studied in many studies affected cell division in germination and helped break seed dormancy (Bradford and Nonogaki, 2007). Gibberellins influence seed germination in mobilizing energy in seeds (Taiz and Zeiger, 2020), and in some circumstances could replace light and temperature in germination (Copeland et al., 2001). The hormone of auxin influenced embryo development and initiated the development of the basal axis in seeds, namely the formation of plumule and radicle into apical shoots and roots (Taiz and Zeiger, 2020). Auxin interacts with light to affect seed germination, at high concentrations, it could inhibit germination (Copeland et al., 2001).

Influence of matriconditioning had been proven to improve the vigor and viability of seeds studied from the metabolic activity of the seeds, when the matriconditioning treatment supported the germination process. Pathogen control as a factor influencing the success of matriconditioning could be integrated with the use of beneficial biological agents for increasing seed viability and vigor. The combination of matriconditioning with beneficial microbes was a research that needed to be further

developed, the use of *Pseudomonas fluorescens* had been used with excess production of enzymes and the resulting siderophores (Khan, 1992).

Administration of biological agents of *Pseudomonas fluorescens* influenced on the viability and vigor of Jatropha seeds. The given effect had positive and negative impacts depending on the concentration and dose of the biological agent population. A positive effect was shown by an increase in the value of viability (germination) and vigor (speed of growth, uniformity of growth, vigor index) of Jatropha seeds at the dose of the biological agent of *Pseudomonas fluorescens* 100 ml l^{-1}. The increase in the viability of Jatropha seeds was thought to have occurred due to the presence of hormones from the secondary metabolites of the biological agent *Pseudomonas fluorescens*. This statement was in accordance with the explanation from Soesanto (2017), which explains that these biological agents produced secondary metabolites in the form of hormones, antibiotics, siderophores, enzymes and fats.

Biological agents *Pseudomonas fluorescens* strain producing high cytokinins which was propagated at 28ºC with a long shaker time of 72 hours, using NB (Nutrient Broth) media capable of producing cytokinin hormones reaching 280 ppm (Kapoor and Kaur, 2016). Cytokinin hormones played a role in the process of seed germination, research by Un et al. (2018), explained that the hormone gibberellins had an effect on increasing the viability of sandalwood seeds by 84%, breaking dormancy and increasing vigor reaching 2.4%/etmal. Cytokinin hormones in this study could penetrate Jatropha seeds with thick skin types. The mechanism of cytokinin hormones in the germination process was to encourage cell division and enlargement, stimulate the embryo and cotyledons to give rise to the seed plumule,

Biological agents of *Pseudomonas fluorescens* produced secondary metabolites, in the form of the hormone auxin indole acetic acid (IAA) (Istiqomah et al., 2017). the bacteria could synthesize IAA up to 68.9 ppm (Tefa, 2018). The auxin hormone produced depends on the nutrients used and the incubation growing environment in the multiplication of these biological agents. The IAA hormone had a positive effect on increasing mung bean germination up to 24% and spurring mung bean root elongation (Janani et al., 2017). This hormone was thought to cause an increase in Jatropha seed vigor in the form of an increase in the value of growth speed, vigor index and the value of growing simultaneity in sprouts. The IAA hormone produced by biological agents played a role in influencing the physiology of seeds, stimulating the growth of the radicle and plumule of the seed during the germination process (Herlina et al., 2017). The auxin mechanism in seed germination started with the hormone penetrating the testa of the seed, entering the embryo and cotyledons,

Hormones were produced by the biological agent of *Pseudomonas fluorescens* apart from the auxin and cytokinin types was also capable of producing hormones of gibberellins reach 69.2 ppm (Tefa, 2015), 25-60 ppm (Kapoor et al., 2016). Gibberellin hormone in the study of Un et al. (2018), explained that it had an effect on increasing the viability of sandalwood seeds by 84%, breaking dormancy and increasing vigor reaching 2.4%/etmal. Hormones produced by the biological agent *Pseudomonas fluorescens* are thought to influence seedling growth and affect germination speed, growth synchrony and the number of Jatropha sprouts. The hormone gibberellin played a role in influencing seed germination in mobilizing the available food reserves in the seed, so that it begins to be broken down into energy, weakening the seed coat that caused dormancy (Taiz and Zeiger, 2020).

Food reserves stored in jatropha seeds in the form of protein, fat, starch, phosphate were used by the seeds as energy and carbon sources after being hydrolyzed by hydrolytic enzymes in the seeds or from exogenous products produced by biological agents. The enzymes produced by the biological agent *Pseudomonas fluorescens* were protease enzymes, chitinase enzymes, cellulases, lipases and amylase enzymes (Soesanto, 2017). Each enzyme produced by these biological agents had a different use, and was thought to influence the process of seed germination. Jatropha seeds that had been imbibed penetrate the seed testa with a soluble amylase enzyme, which functions to catalyze starch in the seed which was converted into soluble sugar in the form of maltose. The dissolved sugar was used as energy for the seed embryo to germinate.

The protease enzyme produced by the biological agent *Pseudomonas fluorescens* catalyzes the protein contained in Jatropha seeds. Proteins that had undergone hydrolysis into amino acids become proteins in a form available to seed embryos in the germination process (Joshi, 2018). Kawi

Researchnski et al. (2021), explained that the lipase enzyme in seeds was shown to change the amount of hydrolyzed fat into free fatty acids and glycerol during the germination process of jojoba seeds. According to Sugiharni (2010), the lipase enzyme produced by *Pseudomonas fluorescens* had been shown to have a function as an enzyme that could hydrolyze fats produced by plant seeds into soluble fatty acids and glycerol which seeds use for germination.

The biological agent of *Pseudomonas fluorescens* in seed treatment was useful as a bioprotectant presumably due to compounds produced from secondary metabolites in the form of antibiotics and siderophores. Antibiotics produced in this study could harm and inhibit the development of other microbes. The antibiotics produced by these biological agents were salicylic acid, pseudomonic acid, cyanhydrate, 2,4-diacetylfluorolucinol (Soesanto, 2017). Antibiotic compounds produced by biological agents could inhibit the growth of pathogens that attack seeds and plants. Permatasari (2016) showed that the results of the *Pseudomonas fluorescens* antagonist test could inhibit the growth of *C. acutatum*, followed by the same results in field conditions on chili plants. Apart from antibiotics, *Pseudomonas fluorescens* also produced siderophore compounds which were useful as inhibitors of pathogen growth. Siderophores were special compounds produced by *Pseudomonas fluorescens* which were useful for binding iron in the growth environment, so that they could suppress the growth of pathogens (Soesanto, 2017).

Pathogenic attack still occured in some sprouts. Sprouts attacked by pathogens had characteristic rot on the stem but did not attack the roots. The pathogen from the sprouts was grown on agar media and it was seen that the disease originated from a fungus with characteristic white hyphae which turned brown after one week of incubation. Even though it was not observed microscopically, it was found that the cause of the stem rot of the sprouts came from a fungus. These pathogens could come from Jatropha seeds (seed-borne diseases) or from the growing environment. Seeds that were damaged by the testa, were opened and inside there were brown hyphae. Washing with 5% sodium hypochlorite during 5 minutes and rinsed with distilled water, to reduce the incidence of diseases that may be caused by seed-borne pathogens (Sutariati et al., 2021).

CONCLUSION

Based on the results and discussion above it could be concluded that; (1) the interaction effect of matriconditioning time and dose of *Pseudomonas fluorescens* was significantly different on the observed variables of growth speed, vigor index and T50. Combination of 24 hours matriconditioning treatment and dose of *Pseudomonas fluorescens* 100 ml l^{-1} produced the best treatment on variables representing seed vigor (growth rate and vigor index), and (2) the effect of a single factor on matriconditioning time was significantly different and the best was the 24-hour treatment period seed viability (germination) and seed vigor (growing rate, growth synchrony and vigor index).The effect of a single factor dose of *Pseudomonas fluorescens* was significantly different and the best at the dose 100 ml l^{-1} to seed viability (germination), seed vigor (growth rate, growth simultaneity, vigor index) and maximum growth potential.

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