Journal of Natural Resources and Environmental Management
13(3): 398–407. http://dx.doi.org/10.29244/jpsl.13.3.398–407
E-ISSN: 2460-5824
http://journal.ipb.ac.id/index.php/jpsl

Effect of chitosan-PMAA-nutrients slow-release fertilizer on germination of *Falcataria moluccana* (Miq.) Barneby & J. W. Grimes

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Article Info:
Received: 28 - 12 - 2022
Accepted: 03 - 02 - 2023

Keywords:
High concentration, MAA, nutrient release, seeds germination

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***Abstract**. Slow-release fertilizer (SRF) with chitosan (CS) coating as a supply of plant nutrients is an alternative to the efficient use of conventional chemical fertilizers and reduces environmental pollution. However, its potential in woody plants is limited. This study aimed to analyze the effect of SRF on* Falcataria moluccana *germination and SRF phosphorus release rate. This study used SRF from polymerizing CS with methacrylic acid (MAA) to trap nutrients (CS-PMAA-nutrients SRF). The seeds of* F. moluccana *were germinated at six concentrations SRF: without fertilizer (F0), SRF CS dose 0.5% weight 0.03 g (F1), SRF CS 0.5% 0.01 g (F2), SRF CS 0.7% 0.03 g (F3), SRF CS 0.7% 0.01 g (F4), and conventional fertilizer (F5) for 21 days. SRF can increase the germination of* F. moluccana. *F4 increased normal sprout (10%) and germination rate (16%) of* F. moluccana. *Furthermore, SRF with a weight of 0.01 g produced a higher germination value than a weight of 0.03 g. The release rate of phosphorus in SRF is slower (91.80%) than conventional fertilizers. SRF application can reduce nutrients lost and increase the efficiency of nutrient absorption. Hence, it can improve* F. moluccana *growth.*

How to cite (CSE Style 8th Edition):
Madani HN, Wijayanto N, Hartoyo ADP. 2023. Effect of chitosan-PMAA-nutrients slow-release fertilizer on germination of Falcataria moluccana (Miq.) Barneby & J. W. Grimes. JPSL **13**(3): 398–407. http://dx.doi.org/10.29244/jpsl.13.3.398–407.

INTRODUCTION

Chemical fertilizers have been overused all over the world. The use of global chemical fertilizers is known to reach 190 million tons, where Indonesia ranks 5th after China, India, US, and Brazil in 2018 (Ludemann et al. 2022). Given the importance of chemical fertilizers for plants in Indonesia, the amount of fertilizers utilized grew from 1.2 million tons in 1980 to 6.9 million tons in 2020 (IFA 2022). Applying chemical fertilizers significantly increases plants' growth (Amanullah et al. 2016; Purbajanti et al. 2019; Anisuzzaman et al. 2021). However, excessive and continuous fertilizer application decreases fertilizer efficiency and the soil's chemical, physical, and biological capabilities (Krasilnikov et al. 2022). The unbalanced use of chemical fertilizers also triggers changes in soil pH and soil acidification, resulting in a decrease in organic matter and soil organisms, stunting of plant growth and yield, and greenhouse gas emissions (Chandini et al. 2019; Pahalvi et al. 2021). The nutrients lost from applying conventional chemical fertilizers to agricultural systems are still relatively high. Some studies reported that 55% nitrogen, 70% phosphorus, and 70% potassium from fertilizers are lost due to leaching and runoff until they collect in water bodies (Mo'allim et al. 2018; Alfaro et al. 2008; Islam et al. 2014). This causes environmental problems such as eutrophication to human health problems (Jiao et al. 2017; Chandini et al. 2019).

Falcataria moluccana (Miq.) is a pioneer tree species of the family Fabaceae and belongs to the type of fast-growing plant (Baskorowati 2014). *F. moluccana* is widely grown in Indonesia forest plantations due to: (1) rapid growth that can be harvested within 4–7 years and can improve environmental quality; (2) good adaptation to various types of soil, including dry, moist, and even acidic soils provided sufficient drainage, as well as; (3) the quality of its wood that is acceptable to the panel and carpentry wood industry (Yunanto et al. 2019; Julian et al. 2019; Prematuri et al. 2020; Nuroniah et al. 2021). With short-cutting rotations and high demand, environmental factors, especially the availability of nutrients in the soil, can limit the productivity of *F. moluccana*. One solution that can be done to restore the availability of nutrients is fertilization. However, fertilization can be expensive if the application rate exceeds plant nutrient uptake and does not increase tree productivity (Henderson and Jose 2012). Phosphorus (P) is one of the macronutrients that many plants need for growth and is an essential factor in forming nucleic acids (DNA and RNA) and phospholipids. Phosphorus is also the main component of adenosine triphosphate (ATP), which is a source of plant metabolic energy (Haleem et al. 2020; Bhardwaj et al. 2022). Unfortunately, in soil, phosphorus is very quickly unavailable to plants due to the binding of phosphorus by aluminum and iron at low pH (acid) and calcium at high pH (alkaline) (Miller 2016). This is why the need for fertilizers that release phosphorus slowly so plants can absorb it optimally to reduce the loss of nutrients to the environment.

In the last decade, slow-release fertilizers (SRF) has become a new alternative to reduce chemical fertilizers while reducing environmental pollution (Lubkowski 2016). SRF is a fertilizer that has the property of slowly releasing nutrients through a coating mechanism with semi-permeable materials (Cole et al. 2016). One of the potential coating materials for SRF is chitosan. Chitosan (CS) is the most abundant natural fiber after cellulose which is non-toxic and biodegradable (Sharif et al. 2018). CS has relative hydrophobic properties, especially insoluble in water and organic solvents. This character makes CS have great potential as an SRF coating (Nguyen and Wang 2017). In their research, Corradini et al. 2010 and Hasaneen et al. 2016 succeeded in synthesizing and characterizing NPK fertilizer with CS nanoparticle coatings through oxidative decarboxylation reactions using potassium persulphate ($K_2S_2O_8$). Several studies on CS-PMAA-NPK fertilizer have only been carried out on the growth of agricultural crops, such as wheat (Abdel-Aziz et al. 2016), peas (Khalifa and Hasaneen 2018), and potatoes (Elshamy et al. 2019). Application of CS-PMAA-NPK fertilizer with a lower amount resulted in higher plant growth compared to conventional NPK with the same amount. Research on CS-PMAA-NPK SRF on forestry plants, especially *F. mollucana* has never been done. Based on this background, research related to SRF on *F. moluccana* germination needs to be carried out. Therefore, this study aimed to analyze the effect of SRF on *Falcataria moluccana* germination and SRF phosphorus release rate.

METHOD

Location and Period

The research was conducted in the Silvicultural laboratory, Department of Silviculture, Faculty of Forestry and Environment, IPB University, Bogor, Indonesia. SRF nutrient analysis was carried out at the integrated laboratory of IPB University. The research was conducted in June–July 2022.

Tools and Materials

The tools and materials used were *F. moluccana* seeds, chitosan, methacrylic acid (MAA) (Himedia Lab. USA), potassium persulphate ($K_2S_2O_8$) (Himedia Lab. USA), urea, superphosphate, KCl, filter paper, distilled water, petri dishes, germinator, magnetic stirrer, oven, and analytical balance.

CS-PMAA-Nutrients SRF Production

The CS-PMAA-Nutrients SRF synthesis process is divided into two stages: 1) CS-PMAA (poly (methacrylic acid)) production, and 2) the incorporation of NPK into CS-PMAA. CS-PMAA was obtained by combining MAA with chitosan (CS) (de Moura et al. 2008). CS was dissolved in a 0.5% MAA solution (V/V) for 12 h using magnetic stirring. The CS doses used were 0.5 and 0.7% (W/V). A total of 0.2 mmol $K_2S_2O_8$ was added to the CS-PMAA solution using magnetic stirring at 70 °C for 1 hour and cooled in an ice bath. Sources of nitrogen, phosphorus, and potassium in SRF are obtained from urea, superphosphate, and potassium chloride. The amount of NPK dissolved in CS-PMAA was 0.36 g N, 0.14 P, and 0.09 K. Incorporation of NPK into CS-PMAA was carried out by dissolving it into 100 mL of CS-PMAA solution and accompanied by magnetic stirring for 6 hours at 25 °C (Corradini et al. 2010). The solution was then dried in the oven at 70 °C for 24 hours.

Germination Test of *F. moluccana*

The germination test used *F. moluccana* seeds whose dormancy was broken by soaking the seeds in hot water for 1 minute and continued in water for 24 hours. The germination test was carried out by placing six seeds on filter paper in a petri dish. SRF is applied in the middle of the seed pool. The petri dish was kept in the germinator for 21 days. Each petri dish was watered daily using 5 ml of distilled water.

Variable Measurement

Germination test parameters observed in the seeds of *F. moluccana* include normal sprouts (NS), germination rate (GR), maximum growth potential (MGP), mean germination time (MGT), sprout height (SH), percent sprout life (PSL). NS, GR, MGP, MGT, and PSL were calculated by the following formula:

$$\text{NS (\%)} = \frac{\Sigma \text{ normal sprouts on days 7 and 14}}{\Sigma \text{ seeds tested}} \text{ x } 100\% \quad \text{(Halindra et al. 2017)}$$

$$\text{GR (\%)} = \frac{\Sigma \text{ normal sprouts on 5 and 7 days}}{\Sigma \text{ seeds tested}} \text{ x } 100\% \quad \text{(ISTA 2014)}$$

$$\text{MGP (\%)} = \frac{\Sigma \text{ germinated seeds (normal \& abnormal) on day 21}}{\Sigma \text{ seeds tested}} \text{ x } 100\% \quad \text{(Al-Ansari and Ksiksi 2016)}$$

$$\text{MGT (days)} = \frac{\text{N1T1} + \text{N2T2} + \ldots + \text{NxTx}}{\Sigma \text{ germinated seeds}} \quad \text{(Ali et al. 2020)}$$

Notes:
N = The number of seeds that germinated per unit time
T = Amount of time between initial testing to final testing at intervals (days 1, 2, 3, …, 7)

$$\text{PSL (\%)} = \frac{\Sigma \text{ seeds live on day 21}}{\Sigma \text{ seeds tested}} \text{ x } 100\%$$

Analysis of Nutrient Release of Phosphorus of SRF

The nutrient release test was carried out to see the pattern of phosphorus nutrient release from the fertilizer. The test was carried out using the shaking method. The fertilizer was put into distilled water and shaken for 15, 30, 45, and 60 minutes (Handayani et al. 2015). Element P was analyzed using a spectrophotometer with a reduction of molybdophosphoric acid by stannous chloride to become molybdenum blue. Elemental P is measured at a wavelength of 625 nm (APHA 23rd (2017): 4500-P.D).

Data Analysis

The germination test used a complete randomized design (CRD) with 1 factor and four repetitions. Factor in this study was fertilizer types with six levels, namely; i) without fertilizer (control/F0), ii) SRF with a CS dose of 0.5% and a weight of 0.03 g SRF (F1), iii) SRF CS 0.5% weight 0.01 g (F2), iv) SRF CS 0.7% weight 0.03 g (F3), v) SRF CS 0.7% weight 0.01 g (F4), and vi) conventional NPK fertilizer (F5). Analysis using CRD with the following models (Mattjik and Sumertajaya 2013):

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Description:
i = Fertilizer types
j = Number of repetitions
Y_{ij} = The results of observations of plant germination at the i^{th} fertilizer types factor and the j^{th} replication
μ = General average
α_i = Effect of i^{th} fertilizer types
ε_{ij} = Random effect of the i^{th} fertilizer types and the j^{th} repetition

The germination data obtained were analyzed using a variance analysis (ANOVA) with a confidence interval of 95%. If the germination parameters are significantly affected by fertilizer, then the test is continued with the Duncan Multiple Range Test (DMRT) to see differences in treatment levels.

RESULTS AND DISCUSSION

The effect of fertilizer on the germination of seeds of *F. moluccana* was carried out by evaluating NS, GR, MGP, MGT, SH, and PSL. Based on Table 1, the application of CS-Nutrients SRF on sprout tests exert a different effect on *F. moluccana*. The observations for 21 days showed that SRF had a significant effect on all parameters of *F. moluccana* seed germination except MGP and MGT.

Table 1 The results of the variance analysis and DMRT test of the effect of fertilizer on the germination test of *F. moluccana*

Parameters[a]	F Test[b]	Types of fertilizers[c]					
		F0	F1	F2	F3	F4	F5
NS	*	54.17[a]	25.00[b]	33.33[b]	31.25[b]	60.41[a]	0[c]
GR	*	64.58[ab]	47.91[bc]	56.25[ab]	54.16[b]	77.08[a]	27.08[c]
MGP	ns	83.33	75.00	83.33	70.83	87.50	54.18
MGT	ns	1.57	1.34	1.34	1.50	1.13	1.06
SH	*	3.33[a]	1.67[b]	1.65[b]	1.40[b]	2.50[ab]	0.23[c]
PSL	*	70.83[a]	37.50[bc]	37.50[bc]	33.33[c]	62.50[ab]	0[d]

[a] NS: normal sprouts, GR: germination rate, MGP: maximum growth potential, MGT: mean germination time, SH: sprouts height, PSL: percent sprout life; [b] (ns): no significant effect, (*): significant effect on the test level of 5%; [c] numbers followed by the same letter on one line indicate values that do not differ significantly (DMRT)

Normal Sprouts (NS)

A normal sprout has all its essential structures (root system and buds) with the potential to continue its development and produce normal plants (de Medeiros et al. 2020). The results showed fertilizer significantly affected NS *F. moluccana* (Table 1). Based on Table 1, the fertilizers that have the most significant effect on the NS of *F. Moluccana* sequentially were: F4 > F0 > F2 > F3 > F1 > F5. The F4 treatment produced better NS than the control on *F. moluccana* seeds. The above results show that SRF fertilizer treatment with lower concentrations (F2 & F4) results in higher NS values than with high concentrations (F1 & F3). Khalifa and

Hasaneen (2018) revealed the results of nanoCS-NPK fertilizer at higher concentrations, decreasing root length and inhibiting secondary root growth in *Pisum sativum* seeds.

Germination Rate (GR)

Germination rate indicates the ability of seeds to become a benchmark for seeds to grow normally under optimum environmental conditions. Fertilizer treatment significantly influenced the change of GR *F. moluccana* (Table 1). Further tests using DMRT for the GR of *F. moluccana* resulted in a sequence of treatments: F4 > F0 > F2 > F3 > F1 > F5. This result is similar to the effect of fertilizer on the NS of *F. moluccana*. Bhardwaj et al. (2022) revealed that the interaction and response of SRF fertilizer formulations produce a different pattern and vary depending on plant species, type of formulation, and plant growth stage at the time of application. CS-NPK fertilizer SRF at a dose of 100% produces low growth in potato crops, and on the contrary, it provides high growth in wheat crops (Elshamy et al. 2019; Abdel-Aziz et al. 2016).

Maximum Growth Potential (MGP)

The results showed that fertilization had no significant effect on the MGP parameter of *F. moluccana* (Table 1). The DMRT assay on MGP *F. moluccana* yielded a sequence of treatments: F4 > F2 = F0 > F1 > F3 > F5. F4 produced the highest MGP value (87.50%), followed by F2, which had the same value as F0. The exciting thing was that the F5 treatment produced the lowest germination value on MGP, and the two previous parameters, NS and GP. *F. moluccana* seed germination response can be seen in Figure 1. Conventional NPK fertilizer (F5) produced the lowest MGP value and differed from other treatments, worth 54.18%. NPK fertilizers applied to seeds directly without media (soil) are known to have the potential to release ammonia (NH_3) and ammonium (NH_4^+) which are harmful to seeds through ammonia toxicity and osmotic damage to seeds. Seed osmotic damage will limit water absorption and lead to physiological dryness of the seed (Pereira et al. 2012). Codognoto et al. (2019) revealed that seeds exposed to NPK directly over an extended time decreased seed germination, bud, and root length and increased seed mortality.

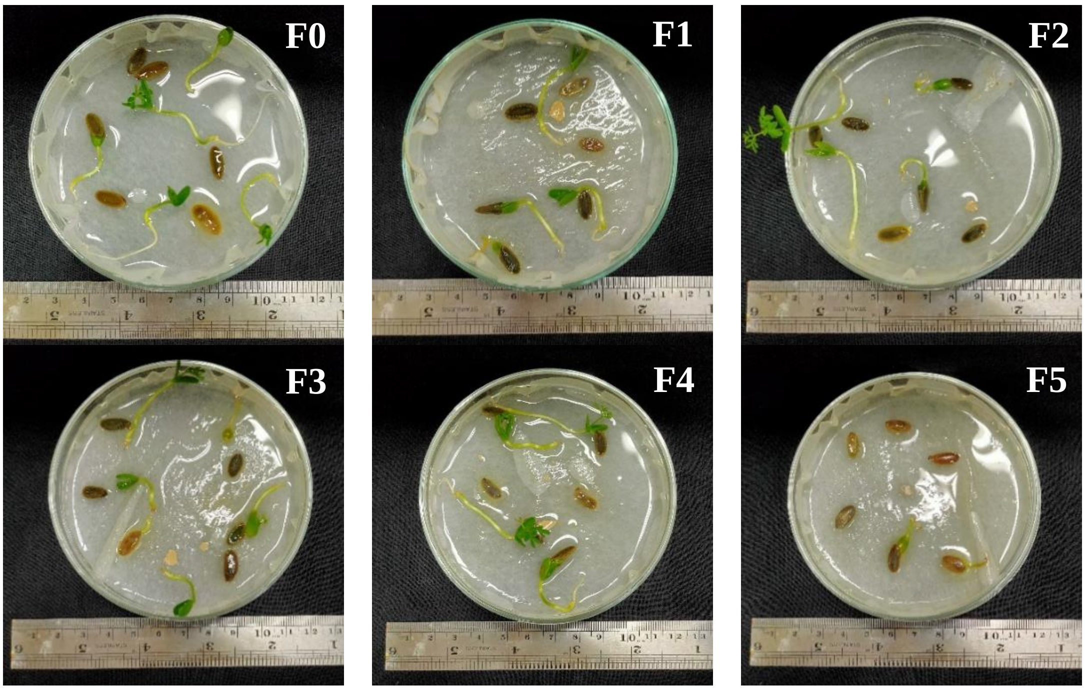

Figure 1 7-day-old *F. moluccana* seed germination response

Mean Germination Time (MGT)

The application of CS-Nutrients SRF had no significant effect on the MGT parameter of *F. moluccana* (Table 1). The MGT value indicates the time (days) needed for seed germination. A low MGT value indicates that the seed germinates faster than a high MGT value. The DMRT test on MGT *F. moluccana* yielded a sequence of treatments: F5 > F4 > F1 = F2 > F3 > F0. The MGT response showed that the F5 treatment produced the lowest MGT value of 1.06 days, followed by F4, F1, and F2, indicating the time it took for *F. moluccana* seeds to germinate the shortest compared to other fertilizer treatments but not significantly different. In their research, Sharma et al. (2020) found the results of increasing the value of the vigor index of corn plant seeds given CS-nano fertilizer. The vigor value of seeds increased by 68% compared to water control. Furthermore, higher concentrations of CS-nano fertilizers showed no inhibitory effect on the germination of corn crops. The above results differ from those found in this study, with higher concentrations (F1 & F3) resulting in slower germination rates than low concentrations (F2 & F4). This is in line with the results of Saharan et al. (2016), who found that seed vigor values decreased with increasing concentrations of Cu-CS-nano fertilizer in corn seeds. Growth inhibition by high concentrations of CS-NPK SRF has been reported to increase plant DNA damage, thereby inhibiting seed germination (Khalifa and Hasaneen 2018).

Sprout Height (SH)

The results showed that fertilizer treatment significantly affected the SH of *F. moluccana* (Table 1). The highest 21-day-old sprouts of *F. moluccana* were found in the F0 treatment and followed by the F4 treatment (Figure 2).

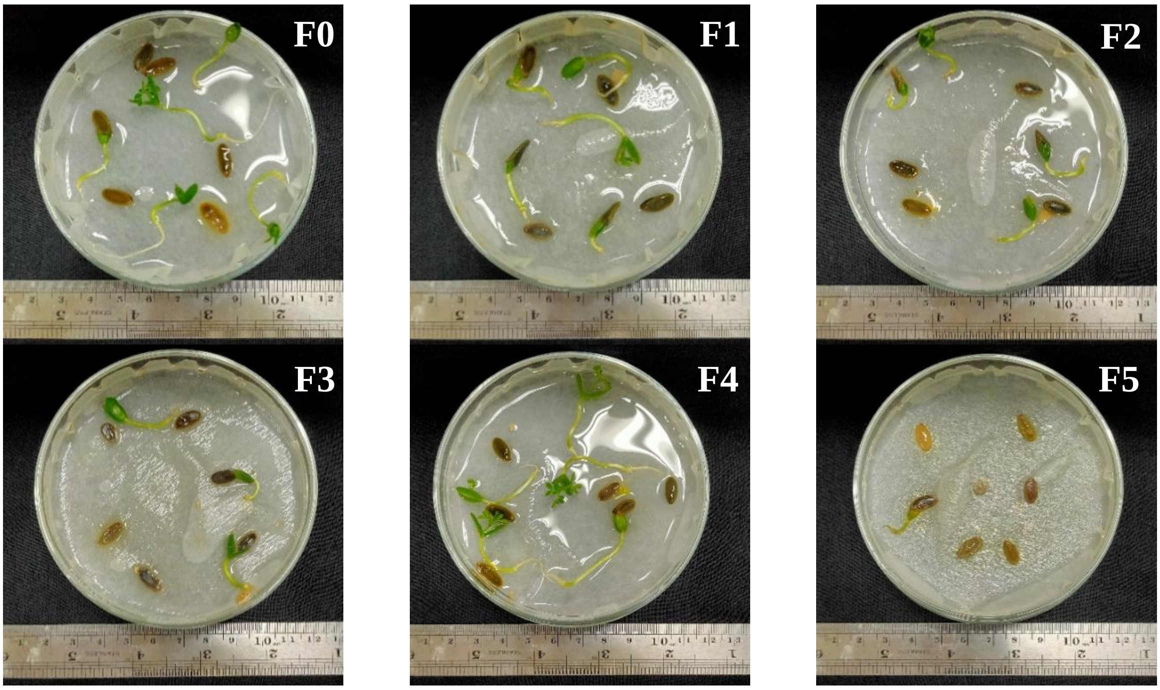

Figure 2 7-day-old *F. moluccana* seed germination response

The above results show that the SRF treatment has not been able to increase the height of the sprouts of *F. moluccana*. The study's results were similar to those of Khalifa and Hasaneen (2018), where there was a decrease in the length of the roots of *Pisum sativum* sprouts with CS-NPK SRF treatment compared to water control. Leonardi et al. (2021) also found that the application of CS-CuO-nano fertilizer has yet to increase the height of *Fortunella margarita* sprouts. Sathiyabama and Parthasaranthy (2016) obtained different research results, who obtained an increase in chickpea sprout height with CS-nano fertilizer application within ten days of observation. Saharan et al. (2015) also obtained positive growth results in *Solanum lycopersicum* sprouts applied with CS-Cu-nano fertilizer compared to water control. The interesting thing that was found from the results of this study and some of the studies above is related to fertilizer concentration, where higher fertilizer concentrations result in lower plant growth and can even reduce plant growth (Saharan et al. 2015; Khalifa and Hasaneen 2018; Leonardi et al. 2021).

Percent Sprout Life (PSL)

The results showed that fertilizer treatment significantly affected the PSL of *F. moluccana* in the germinator (Table 1). The F0 treatment resulted in the highest PSL during the 21-day observation. The F4 treatment produced the highest PSL after F0 in *F. moluccana* seeds. These results indicate that CS-Nutrients SRF has not been able to increase the survival rate of *F. moluccana* sprouts compared to the water control. This study's results differ from the research of Sharma et al. (2020), which used CS-Cu-SA-nano fertilizer. CS-Cu-SA-nano fertilizer increased the vigor index value of corn sprouts compared to the control treatment. SRF based on chitosan coating can increase plant vigor and resistance to pathogens (Sathiyabama and Parthasarathy 2016; Choudhary et al. 2019). In addition, chitosan coating positively influences seed germination due to the induction of amylase and protease activity (Saharan et al. 2016). The results found are inversely proportional compared to some of the above results. Higher CS-Nutrients SRF (F1 & F3) inhibit many germination modifiers compared to low concentrations (F2 & F4). The inhibitory effect of germination by high concentrations of CS-NPK SRF in plants is possible due to excessive accumulation of N, P, and K in plant cells toxic to cellular systems (Choudhary et al. 2019).

Phosphorus Release of SRF

The amount of phosphorus released by SRF was obtained by analyzing the amount of phosphorus dissolved in distilled water under shaking conditions. The SRF shaking process in distilled water was carried out at 15, 30, 45, and 60 minutes, and the results are shown in Figure 3. Dissolved phosphorus results show that conventional fertilizers release phosphorus higher than CS-PMAA-Nutrients SRF. P elements released by fertilizer are 4–8.7 mg/L (F1), 2.33–2.44 mg/L (F2), 5–6.14 mg/L (F3), 2.44–3.15 mg/L (F4), 33.63–73.9 mg/L (F5), respectively. These results indicate that CS-PMAA-Nutrients SRF releases phosphorus more slowly (± 91.80%) than conventional NPK fertilizer for 60 minutes. Similar results were found by Plofino et al. (2019), which used CS-PMAA-K where the nutrient release was lower than conventional fertilizer at 60 minutes of observation in water media. Essawy et al. (2016) also obtained lower nutrient release results by superabsorbent hydrogels from CS-cellulose than conventional fertilizers at 240 hours of observation in liquid media. Similar results were discovered by Gumelar et al. (2020), Sharma et al. (2020), and Leonardi et al. (2021).

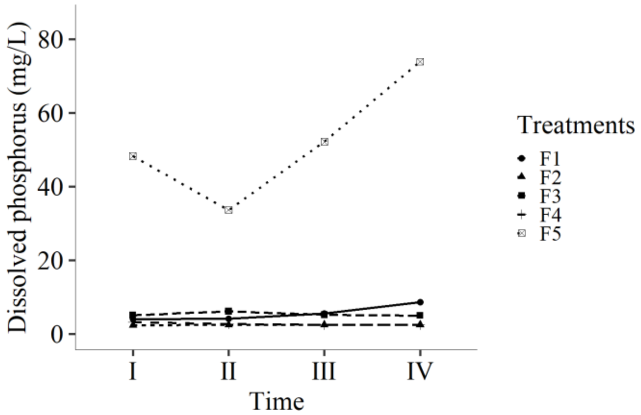

Figure 3 The amount of dissolved phosphorus at 4 observation times in the aquades solvent (15 min (I), 30 min (II), 45 min (III), 60 min (IV))

CONCLUSION

CS-PMAA-Nutrients SRF significantly influenced the germination tests of *F. moluccana*. SRF CS dose of 0.7% weight 0.01 g (F4) results in better germination of *F. moluccana*. The higher SRF weight (F1 & F3) decreased the germination values compared to the lower weight (F2 & F4). Lower germination values are possible due to excessive accumulation of N, P, and K elements in plant cells by high concentrations of CS-PMAA-Nutrients SRF. The phosphorus release rate in SRF is slower (91.80%) than in conventional fertilizers. CS-PMAA-Nutrients SRF is an alternative way to accelerate the germination growth of *F. moluccana* with the efficient use of fertilizers and without producing environmental pollution.

ACKNOWLEDGMENT

We thank the Directorate General of Higher Education, Research, and Technology, and the Ministry of Education, Culture, Research, and Technology, through the Higher Education Leading Basic Research Scheme (PDUPT) 2022, for funding this research. We also thank Dr. Achmad Solikhin and all who have assisted in this study.

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