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Research Article

Comparing seeds germination of some local plant species on two hydroseeding mulches for post mining revegetation

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Abstract: The aims of this study were to determine seed germination rate of some local plant species in two hydroseeding mulches containing different tackifier concentration, as well as to determine the optimal hydroseeding mulch media composition for germinating seeds. This study used seeds of 13 local plant species: two species of Cyperaceae (*Cyperus brevifolius, C. javanicus*), five species of Leguminosae (*Cajanus cajan, Crotalaria pallida, Sesbania grandiflora, S. sesban, Tephrosia purpurea*), and six species of Poaceae (*Eleusine indica, Paspalum conjugatum, Sorghum timorense, S. bicolor, Sporobolus indicus, Themeda arundinaceae*). Two hydroseeding mulch media with different tackifier composition were mixed with seeds of each species and then sowed in pots. Each treatment was repeated three times. Moistened cotton wool was used as control and comparative media for observing seed viability. Seed germination in mulch media was observed during 13 days. The results showed that only 8 of 13 species could be germinated: *S. indicus, S. timorense, T. arundinaceae, C. cajan, C. pallida, S. grandiflora, S. sesban,* and *T. purpurea.* The highest germination rate was shown by *S. sesban* (67%) in M_2 medium and the lowest one was shown by *T. arundinaceae* (2%) in both media. The fastest germination time was recorded for *C. pallida* and *S. sesban* seeds that germinated in 2 days after sowing (DAS) in both media, while *S. timorense* and *T. arundinaceae* seeds showed the lowest ones in 11 DAS. The fluid M_1 medium was optimal for seeds germination of *S. sesban* (50%) and *S. grandiflora* (35%), while the thicker M_2 medium was optimal for seeds germination of *S. sesban* (67%) and *S. timorense* (50%) in 13 DAS. The maximum germination rate was generally reached in 11 DAS.

Keywords: *seed, hydroseeding, germination rate, mulch*

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Introduction

Indonesia is one of the countries with significant role in producing global mines such as coal, gold, nickel, copper, and tin, and is among the largest thermal coal exporting countries in the world. High mineral and coal deposits placed Indonesia as a country with high mining prospects (Devi and Prayogo, 2013; PwC Indonesia, 2017). However, mining exploitation practiced in Indonesia, especially by open mining techniques, resulted in massive alteration and degradation of physical, chemical and biological aspects of the environment. To restore the function of degraded environments, land reclamation effort needs to include several stages; one of those is revegetation (Kilowasid et al., 2011; Sudarmadji and Hartati, 2016). Revegetation promotes some post mining development, creates aesthetically landscaping, and increases post-mining productivity (Skousen and Zipper, 2010). Selection of appropriate plant species, planting methods, and maintenance of vegetation after program, are necessary in the revegetation stages (Sudarmadji and Hartati, 2016). A used method in revegetation activities on degraded lands is hydroseeding (Albaladejo-

Montoro et al., 2000; Martinez-Ruiz et al., 2007; Skousen and Zipper, 2010). Hydroseeding is a method of land revegetation for stabilizing overburden surfaces and preventing soil erosion through spraying of mixed mulch fibers, seeds, fertilizers, and water on the soil surface. Mulch applications can reduce soil evaporation, maintain soil moisture, suppress weed growth, control temperature and soil structure, and affect soil microorganisms. Such environmental conditions will allow seeds germination (Cereno et al., 2011; Cadre et al., 2017).

The use of local plants as seed sources is highly recommended in post mining land revegetation due to its ability to adapt to local biotic and abiotic conditions (Oliveira et al., 2013). Several previous greenhouse studies have demonstrated the successful germination of local plant seeds from Cyperaceae, Poaceae, and Leguminosae families in some hydroseeding mulch media containing natural gum. The seeds of *C. brevifolius, C. eragrotis, C. odoratus, C. strigosus, E. indica, Eulalia amaura, P. conjugatum, S. indicus, C. cajan, C. pallida, Desmodium triflorum, Indigofera spicata,* and *S. grandiflora* were able to germinate and grow on these media (Yulianingsih and Arisoesilaningsih, 2015; Baiti and Arisoesilaningsih, 2015; Rahma and Arisoesilaningsih, 2015; Azalia et al., 2016). However, the previous mulches were not resistant under heavy rain, the slurry tended to be disintegrated and finally seeds germinated in low rate. Moreover, research in laboratory, greenhouse, and field conditions conducted by Oliveira et al. (2012) indicated that the success of local seed germination in the field has a low frequency. In order to improve the mulch performance, the natural gum could then be replaced by degradable tackifier. So far, the influence of the components of the hydroseeding mulch media on the performance of local plant seeds germination has not been widely reported. The main purpose of this study was to determine seed germination rate of some local plant species in two hydroseeding mulches containing different tackifier concentration. Therefore, we could determine the optimal hydroseeding mulch media composition for the seed germination in the laboratory scale before trial in the field.

Materials and Methods

This research was conducted in the Laboratory of Ecology, Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang. The study used seeds from some local plant species, i.e. two species of Cyperaceae (*C. brevifolius, C. javanicus*), five species of Leguminosae (*C. cajan, C. pallida, S. grandiflora, S. sesban, T. purpurea*), and six species of Poaceae (*E. indica, P. conjugatum, S. bicolor, S. timorense, S. indicus, T. arundinaceae*). Available seeds were soaked in water for 12 hours. The floating seeds were discarded and the submerged seeds were removed to be soaked in GA3 solution for 1 hour. Furthermore, the seeds that have been soaked in GA3 solution were washed. Hydroseeding mulch as seeds germination media was made from tackifier agent, water, compost, and fiber composition. Two variations of hydroseeding mulch media composition were used, the first mulch media with less tackifier agent content (M_1) and the second with twice of tackifier agent content (M_2). For seed viability test, moistened cotton wool was also used as a control medium.

The prepared seeds of each species were sown by mixing them into 30 grams of mulch. The total weight of used seeds of each species was different and depended on the seed size. Seeds of Cyperaceae, Poaceae, *C. pallida, S. sesban,* and *T. purpurea* were respectively used 0.1 grams. While seeds of *C. cajan* and *S. grandiflora* used were 1 gram. Three replications were performed on each species. Seeds germination in M_1 and M_2 media were observed for 13 days under room temperature (20.9 ± 0.5 °C) with $77.5 \pm 1.9\%$ of relative humidity and 23.7 ± 7.5 lux of light intensity conditions. Data were tabulated and analyzed using Ms. Excel and R software.

Results and Discussion

Germination rate among local species seeds

Generally, based on the seed viability test on moistened cotton wool media, it was known that the seeds of *C. brevifolius, C. javanicus, E. indica, P. conjugatum, S. bicolor, S. indicus, T. arundinaceae, C. cajan, S. grandiflora,* and *T. purpurea* had a low viability rate, as indicated by its germination rate of $\leq 25\%$. While *S. sesban* seeds showed 73% germinating seeds and considered had a moderate seed viability, then *S. timorense* and *C. pallida* seeds showed a higher viability. Its germinating seeds reached $\geq 75\%$. Seed germination is a controlled process by a number of mechanisms required for the growth and development of embryos, with the end result is new individuals (Miransari and Smith, 2014). The low rate of seed viability could be resulted by a drastic fluctuation of humidity and temperature after storage condition that influences the seed moisture content (Shaban, 2013). Patil and Krishna (2016) reported that the moisture content of *Calamus thwaitesii* and *Calamus nagabettai* seeds affected the germination rate. The seeds with higher moisture content showed higher germination rate.

Moreover, it was also recorded that the seeds germination at first days in mulch media was longer than in the moistened cotton wool media (Table 1). Seeds germination of each species in mulch showed different germination rate in 13 DAS (Table 1). The seeds of Leguminosae showed a higher germination rate and a faster germination time compared to the seeds of Cyperaceae and Poaceae. This supported a finding of Azalia et al. (2016) that the germination rate of Leguminosae seeds was higher and the germination time was faster than the rate and time of germination of Poaceae and Cyperaceae seeds.

Seeds of *C. brevifolius* and *C. javanicus* of Cyperaceae did not show any germination in both mulch media until 13 DAS. The mean of germination time of Cyperaceae seeds in some media was 19 DAS (Azalia et al., 2016). A factor influencing the time of sedge seed germination is temperature. Cyperaceae grown in open habitats requires a higher temperature than plants from the same family growing in the forest area. The optimum temperature for sedges seed growth ranges from 20ºC to 25ºC (Żukowski et al., 2010).

Table 1. The seeds germination rate of some local plant species in hydroseeding mulch media

No	Species	Germination rate*(%)			First Days of Germination (DAS)		
		M_1	**M_2**	**C**	**M_1**	**M_2**	**C**
1	*C. brevifolius*	0	0	0	-	-	-
2	*C. javanicus*	0	0	0	-	-	-
3	*E. indica*	0	0	0	-	-	-
4	*P. conjugatum*	0	0	0	-	-	-
5	*S. bicolor*	0	0	6	-	-	3
6	*S. indicus*	6	3	16	4	4	2
7	*S. timorense*	6	50	97	11	7	1
8	*T. arundinaceae*	2	2	0	11	11	-
9	*C. cajan*	0	20	13	-	3	2
10	*C. pallida*	10	2	87	2	2	1
11	*S. grandiflora*	35	19	20	4	4	2
12	*S. sesban*	50	67	73	2	2	1
13	*T. purpurea*	13	20	0	4	3	-

Note: M_1 = Mulch 1, M_2 = Mulch 2, C = Control, DAS = days after sowing, * = until 13 DAS

The seeds of Poaceae showed variable germination rates among species and experiments. Sown seeds of *E. indica*, *P. conjugatum*, and *S. bicolor* did not germinate in two mulches. This was different with the results reported by Azalia et al. (2016) that germination rate of *E. indica* reached 13% and *P. conjugatum* reached 4% in 10 DAS. Furthermore, a study conducted by Rahma and Arisoesilaningsih (2015) showed that the fastest germination time of *E. indica* and *P. conjugatum* was in 3 DAS with the highest germination rate of 19% and 44% until 15 DAS. The possible factor influencing these variations was seed dormancy. As reported by Hu et al. (2014), the rate of seedling emergence from fresh seeds, that had non-deep physiological dormancy, was significantly lower than the seeds stored for 1 year. Another study conducted by Gasque and García-Fayos (2003) showed that the seedling emergence of the recently collected seeds (2% ± 2) was lower than those stored for two months (3% ± 4) and four months (32% ± 3). Seeds of *S. indicus, S. timorense,* and *T. arundinaceae* germinated at different times. The seeds of *S. indicus* initiated to germinate in 4 DAS, both in M_1 and M_2 media. However, the highest germination rate until 13 DAS was present in M_1 medium (6%), while seed germination in M_2 medium only reached 3%. The mean of seed germination starting time of *S. indicus* was 3 DAS with the highest germination rate of 58% in 15 DAS (Rahma and Arisoesilaningsih, 2015). The first germination of *S. timorense* seeds occurred in 7 DAS in M_2 medium, with germination rate of 50% in 13 DAS. However, in M_1 medium, seed germination occurred a week later (11 DAS) with a rate of 6% in 13 DAS. *Sorghum* is a sensitive plant to low temperature. A low temperature can decrease the rate of seed germination (Razmi et al., 2013). Meanwhile, first seed germination of *T. arundinaceae* occurred in 11 DAS, in both media, with the same seed germination rate (2%) until 13 DAS. Some environmental factors such as temperature, light, and humidity greatly affect the ability of germination of Cyperaceae and Poaceae seeds. In addition, variations of mass and seed size also affect germination. There is a positive correlation between seed mass and germination index, and between seed mass and germination rate. Seeds with larger size have higher germination rate than seeds with smaller size (Maas, 1989; Wu and Du, 2007; Żukowski et al., 2010; Khan et al., 2014).

C. cajan seeds of Leguminosae just germinated in M_2 medium, in 3 DAS, with 20% of germination rate until 13 DAS. The similar result was reported by Azalia et al. (2016) that *C. cajan* seeds were able to germinate in all mulch media with the fastest germination time in 3 DAS and the mean of seed germination starting time in all media occurred in 7 DAS. Baiti and Arisoesilaningsih (2015) reported that seeds of *C. cajan* sown in mulch media with 0.5 cm depth did not germinate at all. Seed germination of *C. cajan* is hypogeal. Germinating seeds are able to emerge from a depth of up to 5 cm under an optimal germination temperature ranging from 29ºC to 36ºC (Singh and Oswalt, 1992). Seeds of *C. cajan* show a high promising food source or seeds for revegetation program, because of its optimal nutritional content and high tolerance to environmental stress. Furthermore, *C. cajan* has high biomass productivity and moisture contributions to the soil (Odeny, 2007). Species selection and numbers will depend on the seed availability and test plot results.

C. pallida seeds were able to germinate in both media, but the highest germination rate was in M_1 medium (10-16%) at 7-13 DAS (Figure 1). Seed germination rate in M_2 medium only reached 2%. It seemed that M_2 medium containing with more tackifier agent had lower porosity and aeration rate than M_1 medium. According to Purwantoro (2016), media with low porosity and aeration decreased the germination rate. This low germination rate could also be increased by soaking *C. pallida* seeds in hot water with 60ºC temperature for 30 minutes (Kak et al., 2007). The time required for seeds to germinate in both media was the same, in 2 DAS. These results indicated that the germination time of *C. pallida* seeds in M_1 and M_2 media was a day faster than those in previous study conducted by Azalia et al. (2016), as well as Baiti and Arisoesilaningsih (2015).

The seeds of *S. grandiflora* and *S. sesban* germinated in both media. Seeds of *S. grandiflora* initiated to germinate in 4 DAS in both media with the highest germination rate in M_1 medium (35%) until 13 DAS. In M_2 medium, seed germination rate reached 19%. Meanwhile, *S. sesban* seeds in both media were able to germinate in 2 DAS. The highest rate of seed germination was shown by M_2 medium (67%) until 13 DAS, whereas in M_1 medium, the highest seed germination rate (58%) occurred in 5-9 DAS. However, this rate decreased to 50% in 13 DAS (Figure 1). The enhancement of seed germination rate of *Sesbania* could be conducted by soaking the seeds in distilled water for 24 hours or in a solution of GA3 (250 mg/L) for 24 hours (Shreelalitha et al., 2015). Although the light can affect seed germination in general, the treatment of dark and light conditions does not show the difference in seeds germination rate of *S. sesban* as reported by Dan and Brix (2007). Otherwise, the temperature is very influential on seed germination. The optimal temperature for seed germination of *S. sesban* is 30ºC to 37ºC, with seed germination rate reaches 85%. While in 5ºC and 13ºC, *S. sesban* seed did not germinate at all.

Seeds of *T. purpurea* showed the highest germination rate in M_2 medium (20%) in 9-13 DAS with first germination time in 3 DAS, while in M_1 medium, the highest germination rate (16%) was observed in 7-9 DAS and decreased to 13% in 13 DAS. Seeds of *T. purpurea* in M_1 media initiated to germinate in 4 DAS. The germination capacity of *T. purpurea* seeds was significantly greater in the temperature range 30ºC-35ºC (Sy et al., 2001). Seeds germination rate of *T. purpurea* could be increased by soaking seeds in boiling water or in IAA and GA3 solutions. Seeds soaking treatment in boiling water (100ºC) for 90 seconds increased the seeds germination rate compared to treatment in hot water (60ºC) and normal water (Naikawadi, 2016).

Selecting optimal mulch medium

Comparing the germination rate of seeds in M_1 and M_2 media, based on the Mixed Model Analysis, specifically, both media were suitable for germination of *S. sesban* seeds with the highest rate among species. While *S. grandiflora* seeds germinated better in M_1 medium than in M_2 medium. Otherwise, germination of *S. timorense* seeds was better in M_2 medium. Overall, M_2 medium was better than M_1 medium for seed germination (Figure 2). Although M_2 medium had lower porosity and aeration rate than M_1 medium, its more tackifier agent content was expected to decrease the moisture content by absorbing the water.

Matsushima and Nakagami (2013) reported that germination media with moderate moisture content (8%-11%) gave faster seed germination time than those the higher. The medium with moderate water holding capacity was better for seed germination and the most suitable range of medium water content for the process of seedling emergence and growth was about 12%. Either low or excessive media water content would reduce the ability of seedling emergence and growth (Gairola et al., 2011; Tang et al., 2016). The analysis of all seeds germination rate in both media using Cubic Equation (Table 2) showed equation as follow,

$$y = -0.0217x^3 + 0.2869x^2 + 1.6164x - 2.5073$$

from this equation, it was known that the maximum germination rate of all seeds in both media occurred in 11 DAS (Figure 3).

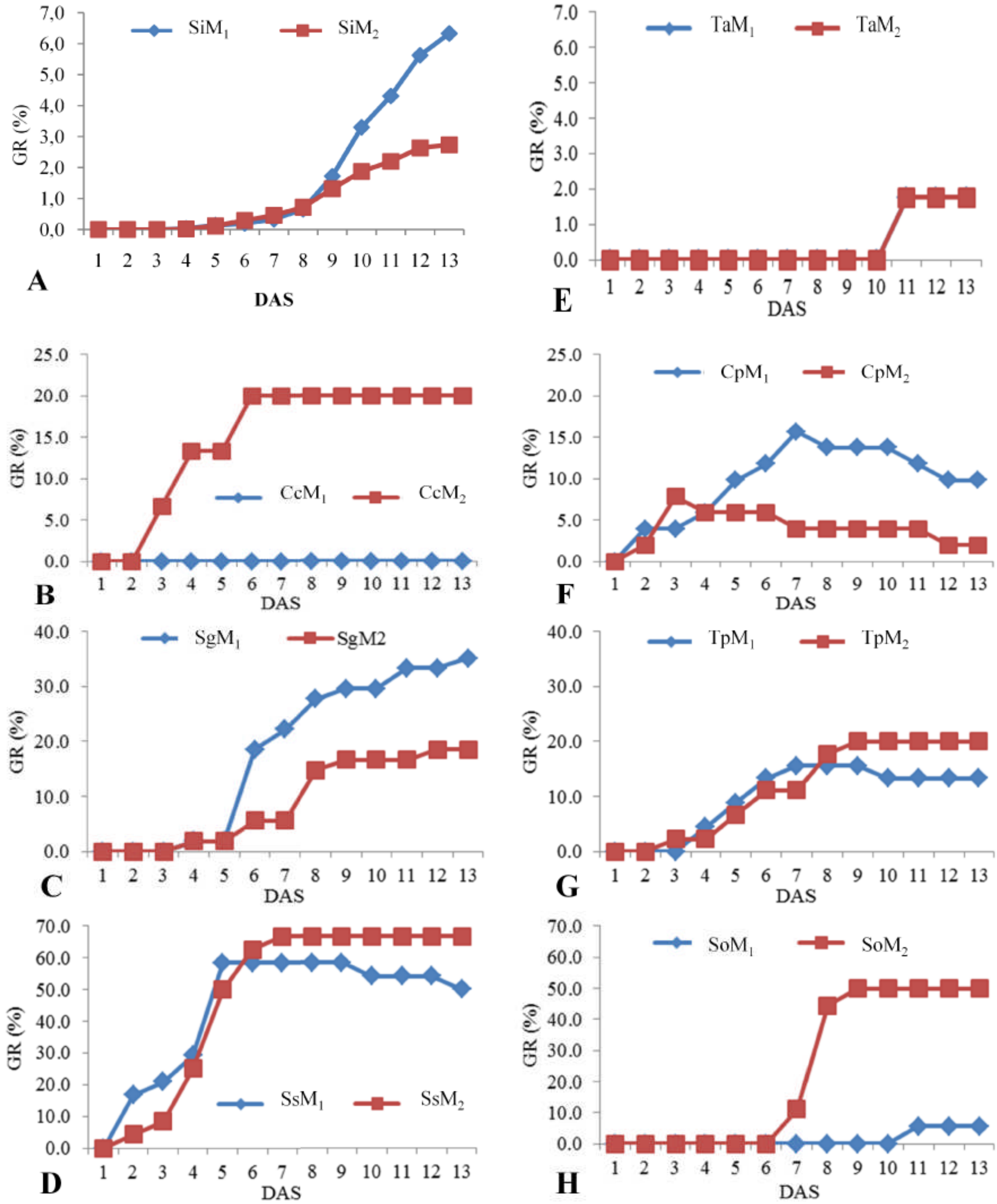

Figure 1. Germination rate of some local plant species until 13 DAS in both media.
Descriptions: A) *S. indicus*, B) *C. cajan*, C) *S. grandiflora*, D) *S. sesban*, E) *T. arundinaceae*, F) *C. pallida*, G) *T. purpurea*, H) *S. timorense*, GR = germination rate, DAS = days after sowing, M_1 = Mulch 1, M_2 = Mulch 2.

Table 2. Model summary and parameter estimates

No	Equation	Model Summary					Parameter Estimates			
		R Square	F	df1	df2	Sig.	Constant	b1	b2	b3
1	Logarithmic	0.921	128.1	1	11	0.000	-4.0	9.8		
2	Inverse	0.677	23.0	1	11	0.001	19.2	-25.1		
3	Quadratic	0.975	197.5	2	10	0.000	-6.1	4.3	-0.2	
4	Cubic	0.988	256.3	3	9	0.000	-2.5	1.6	0.3	0.0
5	Compound	0.397	7.2	1	11	0.021	0.1	1.7		
6	Power	0.685	23.9	1	11	0.000	0.0	3.6		
7	S	0.936	159.7	1	11	0.000	4.6	-12.5		
8	Growth	0.397	7.2	1	11	0.021	-2.2	0.5		
9	Exponential	0.397	7.2	1	11	0.021	0.1	0.5		
10	Logistic	0.397	7.2	1	11	0.021	9.3	0.6		

Note: Dependent variable = germination rate, Independent variable = days after sowing

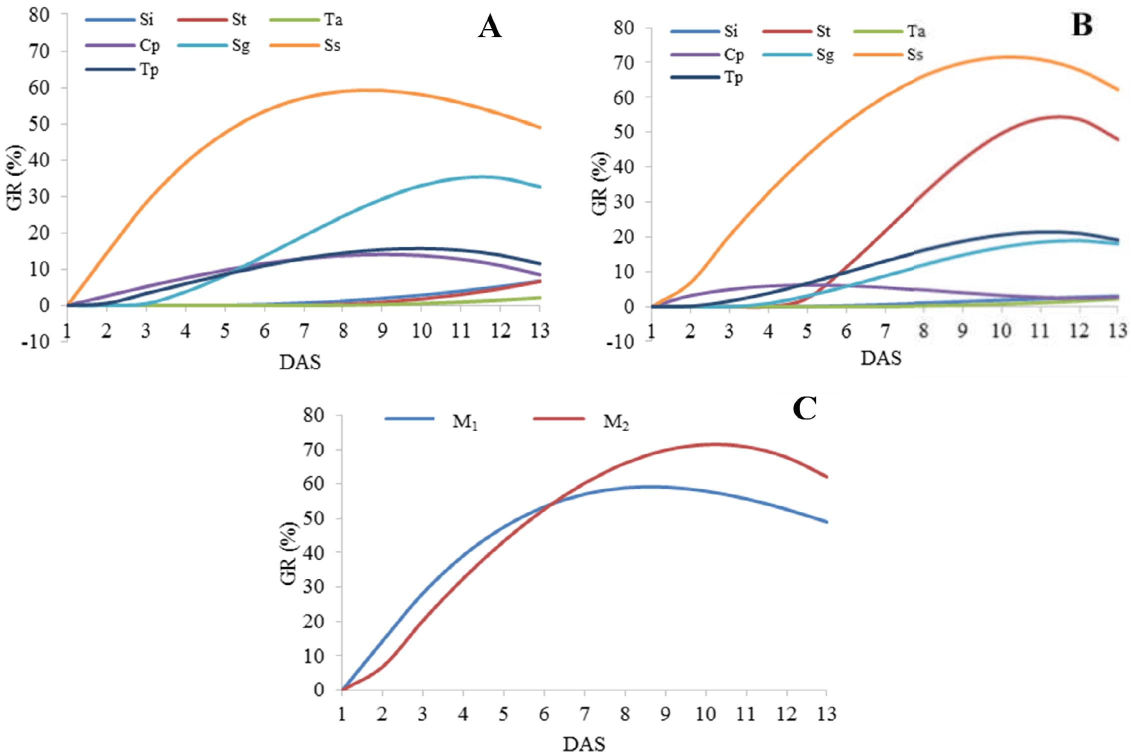

Figure 2. Mixed model of seeds germination.
Descriptions: A. Seeds germination in M_1 medium, B. Seeds germination in M_2 medium, C. Comparison between seeds germination in M_1 and M_2 media, Cp = *C. pallida*, Sg = *S. grandiflora*, Si = *S. indicus*, Ss = *S. sesban*, St = *S. timorense*, Ta = *T. arundinaceae*, Tp = *T. purpurea*, DAS = days after sowing, M_1 = Mulch 1, M_2 = Mulch 2.

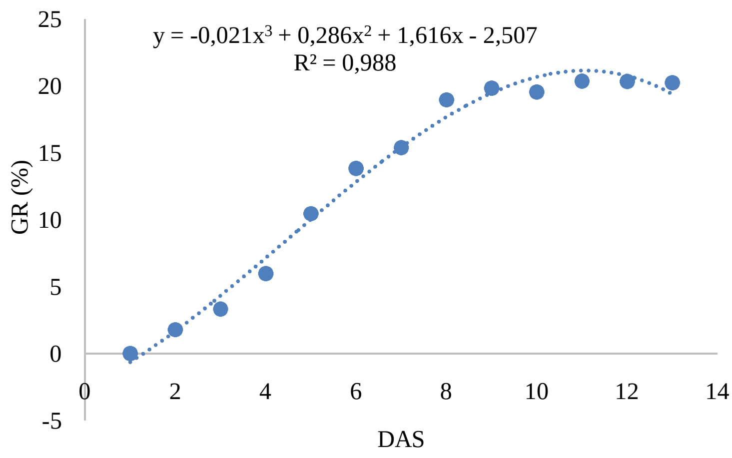

Figure 3. Model of all species seeds germination rate in both mulch media
Descriptions: GR = germination rate, DAS = days after sowing.

Conclusion

Seeds of *S. indicus*, *S. timorense*, *T. arundinaceae*, *C. pallida*, *S. grandiflora*, *S. sesban*, and *T. purpurea* were able to germinate in M_1 and M_2 media, while *C. cajan* only germinated in M_2 medium. Seeds of *C. brevifolius*, *C. javanicus*, *E. indica*, *P. conjugatum*, and *S. bicolor* did not show any germination. The highest seed germination rate was observed on *S. sesban* (67%) in M_2 medium and the lowest seed germination rate was shown by *T. arundinaceae* (2%) in both media. M_1 medium was optimal for seed germination of *S. sesban* and *S. grandiflora*, while M_2 medium was optimal for seed germination of *S. sesban* and *S. timorense*. The maximum germination rate occurred in 11 DAS.

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