EFFECT OF SODIUM BICARBONATE AND ACETIC ACID ON *Hibiscus macrophyllus* SEED-BORNE FUNGAL PATHOGENS, SEED GERMINATION, AND SEEDLING GROWTH

(*Pengaruh Sodium Bikarbonat dan Asam Asetat terhadap Cendawan Terbawa Benih, Perkecambahan Benih dan Pertumbuhan Bibit* Hibiscus macrophyllus**)**

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ABSTRAK

Hibiscus macrophyllus *merupakan salah satu pohon tropis yang memiliki potensi ekonomi tinggi, namun terdapat permasalahan dalam pembibitannya, yaitu dormansi benih dan patogen terbawa benih. Penelitian ini bertujuan untuk mengevaluasi pengaruh sodium bikarbonat dan asam asetat terhadap kolonisasi cendawan terbawa benih, perkecambahan benih dan pertumbuhan bibit* H. macrophyllus. *Rancangan acak lengkap digunakan untuk menguji 6 perlakuan, yaitu: (i) benih tanpa perlakuan, (ii) perendaman dalam air mendidih dan dibiarkan selama 24 jam, (iii) perendaman dalam air mendidih dan biarkan selama 24 jam diikuti perendaman dalam asam asetat 1% (15 menit), (iv) perendaman dalam air mendidih dan biarkan selama 24 jam diikuti perendaman dalam sodium bikarbonat 5% (15 menit), (v) perendaman dalam asam asetat 1% (15 menit) diikuti perendaman dalam air mendidih dan biarkan selama 24 jam, dan (vi) perendaman dalam sodium bikarbonat 5% (15 menit) diikuti perendaman dalam air mendidih dan biarkan selama 24 jam. Perendaman dalam sodium bikarbonat 5% (15 menit) diikuti perendaman dalam air mendidih dan biarkan selama 24 jam secara nyata mampu menekan cendawan terbawa benih. Aplikasi sodium bikarbonat 5% dan asam asetat 1% tidak dapat meningkatkan perkecambahan benih. Perlakuan sodium bikarbonat diikuti perendaman dalam air mendidih memberikan pertumbuhan diameter bibit, panjang daun, lebar daun, panjang akar, jumlah daun terbaik.*

Kata kunci: asam asetat,* Hibiscus macrophyllus*, benih, bibit, sodium bikarbonat

ABSTRACT

Hibiscus macrophyllus, an important tropical tree, have high economic potential, however there are the problems in seedling procurement, i.e. seed dormancy and seed-borne pathogen. The purpose of the research was to evaluate the effect of sodium bicarbonate and acetic acid on the fungal colonization, seed germination, and seedling growth of *H. macrophyllus*. A completely randomized design was used to test the six treatments: (i) untreated seed, (ii) soaking seeds in boiling water and left 24 hours, (iii) soaking in boiling water and left 24 hours followed by soaking in acetic acid 1% (15 minutes), (iv) soaking in boiling water and left 24 hours followed by soaking in sodium bicarbonate 5% (15 minutes), (v) soaking in acetic acid 1% (15 minutes) followed by soaking in boiling water and left 24 hours, and (vi) soaking in sodium bicarbonate 5% (15 minutes) followed by soaking in boiling water and left 24 hours. Soaking in sodium bicarbonate 5% (15 minutes) followed by soaking in boiling water and left 24 hours could significantly decrease the fungal colonization. Sodium bicarbonate 5% and acetic acid 1% treatments could not improve seed germination. The sodium bicarbonate treatment followed by soaking in boiling water increased the seedling diameter, leaf length, leaf wide, root length, and leaf number.

Keywords : acetic acid, *Hibiscus macrophyllus*, seed, seedling, sodium bicarbonate

I. INTRODUCTION

Hibiscus macrophyllus is a potential fast-growing pioneer species for forest plantation program. The plant commonly found in a community forest in Java. This species is a multipurpose plant as building materials,

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plywood, furniture, chopsticks, toothpicks, fire rods, crates, boats, and pulp raw materials (Heyne, 1987; Basri, Prayitno, & Pari, 2012; Nazmurakhman, 2014).

There are two main obstacles in the preparation of seedling stock which include seed dormancy and seed-borne fungal pathogen. *H. macrophyllus* seed has strong dormancy and the germination capacity at untreated seed is between 5.5% ─8.3% (Sudrajat & Bramasto, 2018). Rahmayani (2002) reported that *H. macrophyllus* seed has a hard integument that causes strong dormancy (physical dormancy).

Several methods have been used to break the *physical dormancy*, including mechanical scarification such as boiling water treatment and chemical scarification such as acid and alkali treatments. Soaking seed in boiling water before planting was a general method for breaking seed dormancy. It is widely reported that the positive effect of sulphuric acid to enhance seed germination. In fact, sulphuric acid has a negative effect due to its hazards to the environment and human health.

Seed-borne pathogens especially fungal pathogens can decrease seed viability. An alternative method for controlling seed-borne fungal pathogens and for increasing seed germination and seedling growth is necessary.

Besides being able to break dormancy, acid, and alkali treatment can also inhibit fungal growth (Turkkan, Ozcan, & Erper, 2017; Hassan, El-kadi, & Sand, 2015). Inorganic salts and organic acids are known for antifungal properties. The use of sodium bicarbonate and acetic acid has no negative effect on the environment and human health, easy to apply, cheap and effective alternative methods.

Carbonate and bicarbonate are known to have an inhibitory effect against fungal plant pathogens (Turkkan, *et al*., 2017) as well as capable to break seed dormancy. It has been reported that sodium bicarbonate could inhibit growth numerous fungal species such as *Candida* sp., *Pythium* sp., *Rhizopus stolonifer*, *Aspergillus niger* (Kareem, Afolabi, Shorinmade, & Akinbode, 2018), *Fusarium oxysporum* (Turkkan & Erper, 2014), and *Penicillium digitatum* (Zamani, Sharifi Tehrani, Ahmadzadeh, Hosseininaveh, & Mostofy, 2009). Acetic acid used for inhibiting the growth of fungal, such as *A. flavus*, *P. purpurogenum*, *F. oxysporum*, and *R. nigricans*, is also be able to reduce production fungal toxin such as aflatoxin (Hassan *et al.*, 2015).

Based on the problems of seed germination and seedling growth, then it is important to find methods to inhibit seed-borne fungi contamination and increase seed germination and seedling growth. The purpose of the research was to determine the effect of

sodium bicarbonate, acetic acid on inhibition of fungal pathogen, seed germination and early seedling growth of *H. macrophyllus*.

II. MATERIALS AND METHODS

A. Materials

The materials used in this research were *Hibiscus macrophyllus* seed, acetic acid 1%, sodium bicarbonate 5%, aqua dest, hot plate, petri dish (diameter 15 cm), filter paper, soil sand (1:1 v/v) media, cover glass, microscope slide, microscope.

B. Experimental methods

The six seed treatments were involved in this study which consisted of untreated seed (control), soaking seed in boiling water and let for 24 hours, soaking seed in boiling water for 10 minutes and let for 24 hours followed by soaking in acetic acid 1% for 15 minutes, soaking seed in boiling water for 10 minutes and let for 24 hours followed by soaking in sodium bicarbonate 5% for 15 minutes, soaking seed in acetic acid 1% for 15 minutes followed by soaking in boiling water and let for 24 hours, soaking seed in sodium bicarbonate 5% for 15 minutes followed by soaking in boiling water and let for 24 hours. After soaking, the treated seed and untreated seed were sown on sterilized paper for determining fungal colonization and soil sand media for determining seed germination and seedling growth. Fungal contamination was observed morphologically and the species identification was performed following the method described by Barnet and Hunter (1998). The level of contamination or fungal colonization was monitored weekly during seed germination periods using the paper method.

C. Data Analysis

The experimental design was a completely randomized design (CRD) with 4 replications which 50 seeds for each replication. Results obtained were measured by analysis of variance (One-Way ANOVA) and significant differences between the means were determined by using the Duncan's Test.

III. RESULT AND DISCUSSION

A. Result

1. Fungal contamination

Two fungal species were isolated from the treated seed and untreated seed of *H. macrophyllus*, i.e. *Aspergillus* sp. and *Fusarium* sp. (Table 1). The percentage of fungal on seed treated by soaking in sodium bicarbonate 5% for 15 minutes followed by soaking in boiling water and let for 24 hours (F) was significantly different from any others. The percentage of fungal pathogens on this treatment was the lowest.

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Table (*Tabel*) 1. Effect of sodium bicarbonate and acetic acid on the percentage of the seed-borne fungal pathogen (*Pengaruh sodium bikarbonat dan asam asetat terhadap cendawan terbawa benih*)

Treatment (*Perlakuan*)	The percentage of fungal pathogens (*persentase cendawan patogen*) (%)	The percentage of *Aspergillus* sp. (*persentase* Aspergillus *sp.*) (%)	The percentage of *Fusarium* sp. (*persentase* Fusarium *sp.*) (%)
A	63 a	60.5 a	2.5a
B	47.5 a	47 a	0.5 a
C	42 a	41.5 a	0.5 a
D	40.5 a	10 b	32 a
E	37.5 a	36.5 a	1 a
F	2.5 b	2.5 b	0 a

Remarks (*Keterangan*) : A=untreated seed (control); B=seeds were soaked in boiling water and let for 24 hours; C= seeds were soaked in boiling water for 10 minutes and let for 24 hours followed by soaking in acetic acid 1% for 15 minutes; D= seeds were soaked in boiling water for 10 minutes and let for 24 hours followed by soaking in sodium bicarbonate 5% for 15 minutes; E= seeds were soaked in acetic acid 1% for 15 minutes followed by soaking in boiling water and let for 24 hours; F=seeds were soaked in sodium bicarbonate 5% for 15 minutes followed by soaking in boiling water and let for 24 hours. Values with the same letters on the same column showed not significantly according to Duncan's Test. (*A = benih tanpa perlakuan (kontrol); B = benih direndam dalam air mendidih dan biarkan selama 24 jam; C = benih direndam dalam air mendidih selama 10 menit dan biarkan selama 24 jam diikuti dengan perendaman dalam asam asetat 1% selama 15 menit; D = benih direndam dalam air mendidih selama 10 menit dan biarkan selama 24 jam diikuti dengan perendaman dalam sodium bikarbonat 5% selama 15 menit; E = benih direndam dalam asam asetat 1% selama 15 menit diikuti dengan perendaman dalam air mendidih dan biarkan selama 24 jam; F = benih direndam dalam sodium bikarbonat 5% selama 15 menit diikuti dengan perendaman dalam air mendidih dan biarkan selama 24 jam. Nilai dengan huruf yang sama pada kolom yang sama menunjukkan tidak berbeda nyata menurut Uji Duncan*).

2. Germination capacity and germination rate

The germination capacity was not significantly different from each other on soil media, whereas, on the paper media, those treatment significantly affected on the seed germination capacity (Figure 2). The highest germination capacity was obtained from seeds that were soaked in boiling water and let for 24 hours both on soil media and paper media.

This treatment increased the germination capacity by 11.5% on soil media and 21% on paper media compared with the control.

Application of acetic acid before and after boiling water significant increase in germination rate on soil media (Table 2). Seeds were soaked in boiling water followed by soaking in acetic acid produced the highest germination rate

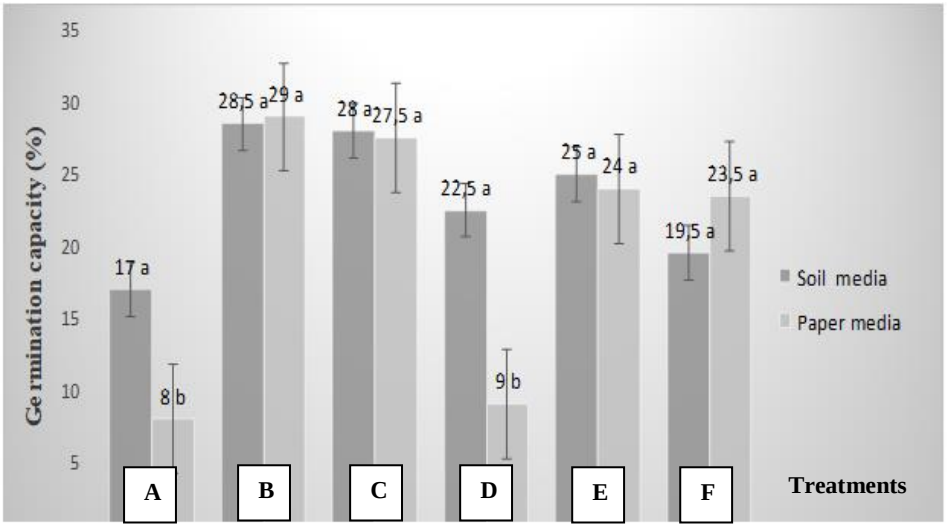

Figure (*Gambar*) 1. Germination capacity of *H. macrophyllus* on the several seed treatments (*Daya berkecambah benih* H. macrophyllus *pada beberapa perlakuan benih*)

Remarks (*Keterangan*): A=untreated seed (control); B=seeds were soaked in boiling water and let for 24 hours; C= seeds were soaked in boiling water for 10 minutes and let for 24 hours followed by soaking in acetic acid 1% for 15 minutes; D= seeds were soaked in boiling water for 10 minutes and let for 24 hours followed by soaking in sodium bicarbonate 5% for 15 minutes; E= seeds were soaked in acetic acid 1% for 15 minutes followed by soaking in boiling water and let for 24 hours; F=seeds were soaked in sodium bicarbonate 5% for 15 minutes followed by soaking in boiling water and let for 24 hours. Values with the same letters on the same column showed not significantly according to Duncan's Test. (*A = benih tanpa perlakuan (kontrol); B = benih direndam dalam air mendidih dan biarkan selama 24 jam; C = benih direndam dalam air mendidih selama 10 menit dan biarkan selama 24 jam diikuti dengan perendaman dalam asam asetat 1% selama 15 menit; D = benih direndam dalam air mendidih selama 10 menit dan biarkan selama 24 jam diikuti dengan perendaman dalam sodium bikarbonat 5% selama 15 menit; E = benih direndam dalam asam asetat 1% selama 15 menit diikuti dengan perendaman dalam air mendidih dan biarkan selama 24 jam; F = benih direndam dalam sodium bikarbonat 5% selama 15 menit diikuti dengan perendaman dalam air mendidih dan biarkan selama 24 jam. Nilai dengan huruf yang sama pada kolom yang sama menunjukkan tidak berbeda nyata menurut Uji Duncan*).

Table (*Tabel*) 2. Effect sodium bicarbonate and acetic acid on germination rate (*Pengaruh sodium bikarbonat dan asam asetat terhadap kecepatan berkecambah*)

Treatment (*Perlakuan*)	Germination rate (%/etmal) (*Kecepatan berkecambah*) (%/etmal)	
	Soil media (*Media tanah*)	Paper media (*Media kertas*)
A	0.61 b	0.13 b
B	0.77 b	0.91 a
C	2.22 a	0.83 a
D	0.81b	0.28 b
E	1.75 a	0.75 a
F	0.86 b	1.09 a

Remarks (*Keterangan*): A=untreated seed (control); B=seeds were soaked in boiling water and let for 24 hours; C= seeds were soaked in boiling water for 10 minutes and let for 24 hours followed by soaking in acetic acid 1% for 15 minutes; D= seeds were soaked in boiling water for 10 minutes and let for 24 hours followed by soaking in sodium bicarbonate 5% for 15 minutes; E= seeds were soaked in acetic acid 1% for 15 minutes followed by soaking in boiling water and let for 24 hours; F=seeds were soaked in sodium bicarbonate 5% for 15 minutes followed by soaking in boiling water and let for 24 hours. Values with the same letters on the same column showed not significantly according to Duncan's Test. (*A = benih tanpa perlakuan (kontrol); B = benih direndam dalam air mendidih dan biarkan selama 24 jam; C = benih direndam dalam air mendidih selama 10 menit dan biarkan selama 24 jam diikuti dengan perendaman dalam asam asetat 1% selama 15 menit; D = benih direndam dalam air mendidih selama 10 menit dan biarkan selama 24 jam diikuti dengan perendaman dalam sodium bikarbonat 5% selama 15 menit; E = benih direndam dalam asam asetat 1% selama 15 menit diikuti dengan perendaman dalam air mendidih dan biarkan selama 24 jam; F = benih direndam dalam sodium bikarbonat 5% selama 15 menit diikuti dengan perendaman dalam air mendidih dan biarkan selama 24 jam. Nilai dengan huruf yang sama pada kolom yang sama menunjukkan tidak berbeda nyata menurut Uji Duncan*).

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3. Seedling growth

Seeds were soaked in sodium bicarbonate followed by on boiling water produced the highest seedling growth although there was not significantly different from control on height, shoot, and root biomass (Table 4). The treatment gave a significant difference in seedling diameter, leaves length, leaves wide, root length compared to control, and just soaked in boiling water.

Table (*Tabel*) 4. Effect sodium bicarbonate and acetic acid on seedling growth (*Pengaruh sodium bikarbonat, asam asetat terhadap pertumbuhan bibit*)

Treatment (*Perlakuan*)	Height (*Tinggi*)	Diameter (*Diameter*)	Leaves length (*Panjang daun*)	Leaves wide (*Lebar daun*)	Root length (*Panjang akar*)	Leaves number (*Jumlah daun*)	Shooth biomass (*Biomassa akar*)	Root Biomass (*Biomassa batang*)
A	2.67 a	0.65b	2.53 b	1.75 b	3.25 d	3.75 bc	0.0263 a	0.0038 ab
B	3.11 a	0.68b	2.68 b	1.85 b	3.78 c	4.12 ab	0.0192 a	0.0036 ab
C	2.89 a	0.64b	2.47 b	1.75 b	4.63 a	3.3 c	0.0183 a	0.0046 a
D	3.33 a	0.65b	2.71 b	1.95 ab	4.63 a	4.45 ab	0.0202 a	0.0033 b
E	2.95 a	0.72 ab	2.83 b	2 ab	4.41 ab	4.4 ab	0.0216a	0.0037 ab
F	3.38 a	0.79 a	3.33 a	2.3a	4.07 abc	4.75 a	0.027 a	0.0047 a

Remarks (*Keterangan*): A=untreated seed (control); B=seeds were soaked in boiling water and let for 24 hours; C= seeds were soaked in boiling water for 10 minutes and let for 24 hours followed by soaking in acetic acid 1% for 15 minutes; D= seeds were soaked in boiling water for 10 minutes and let for 24 hours followed by soaking in sodium bicarbonate 5% for 15 minutes; E= seeds were soaked in acetic acid 1% for 15 minutes followed by soaking in boiling water and let for 24 hours; F=seeds were soaked in sodium bicarbonate 5% for 15 minutes followed by soaking in boiling water and let for 24 hours. Values with the same letters on the same column showed not significantly according to Duncan's Test. (*A = benih tanpa perlakuan (kontrol); B = benih direndam dalam air mendidih dan biarkan selama 24 jam; C = benih direndam dalam air mendidih selama 10 menit dan biarkan selama 24 jam diikuti dengan perendaman dalam asam asetat 1% selama 15 menit; D = benih direndam dalam air mendidih selama 10 menit dan biarkan selama 24 jam diikuti dengan perendaman dalam sodium bikarbonat 5% selama 15 menit; E = benih direndam dalam asam asetat 1% selama 15 menit diikuti dengan perendaman dalam air mendidih dan biarkan selama 24 jam; F = benih direndam dalam sodium bikarbonat 5% selama 15 menit diikuti dengan perendaman dalam air mendidih dan biarkan selama 24 jam. Nilai dengan huruf yang sama pada kolom yang sama menunjukkan tidak berbeda nyata menurut Uji Duncan*).

B. Discussion

1. Fungal contamination

The percentage of fungal on seed treated by soaking in boiling water and using acetic acid or sodium bicarbonate before or after boiling water (B, C, D, E, F) was significantly different from untreated seed (control). It is suggested that seed soaked for 24 hours on water could eliminate fungi on the seed surface especially *Aspergillus* sp. The fungi washed away when the water thrown away. The results exhibited that *Aspergillus* sp. was

dominant fungi especially on untreated seed, the percentage of fungal reached 63%.

Acetic acid and sodium bicarbonate were effective treatments for suppressing fungal growth especially *Aspergillus* sp. Palmer, Kenneth Horst and Langhans (1997) observed that bicarbonate was effective in inhibiting the fungi growth due to its ability to directly interact with the membrane by altering membrane activity or cellular physiology. Sodium bicarbonate were been able to induce defense mechanism by producing increasing the activity of -1,3-glucanase, peroxidase, and phenylalanine ammonia-lyase (PAL) enzymes (Youssef, Sanzani, Ligorio, Ippolito, & Terry, 2014).

Bicarbonate and acetic acid were capable to suppress fungi growth due to pH. (Youssef *et al*., 2014). Poschenrieder, Fernandez, Rubio, Perez, Teres dan Barcelo (2018) reported that bicarbonate plays in controlling cell pH of all organism. PH plays important in cell membrane permeability and enzymes that responsible for degrading the substrate. Turkkan and Erper (2014) reported that the growth of *F. oxysporum* f.sp. *cepae* did not inhibit by various salts in which pH values between 6─9. Sodium bicarbonate 3% has pH value 8.4 (Smilanick, Mansour, Margosan, Mlikota Gabler, & Goodwine, 2005) while acetic acid 5% has pH value 2.56 (Hassan, *et al*, 2015).

Seeds were soaked in sodium bicarbonate 5% for 15 minutes followed by soaking in boiling water and let for 24 hours was the best treatment for reducing the percentage of seed-borne fungal pathogen.

2. Seed germination and germination rate

Application of acetic acid 1% and sodium bicarbonate 5% both before and after boiling water could not increase germination capacity compare to treatment B (seeds were soaked in boiling water and let for 24 hours). Gonzales (2015) studied that acetic acid 0.1% could increase the percentage germination of eggplant seed by 10%, in contrary acetic acid 1% decrease seed germination. It is suggested that there are differences in responses to defend species and types of seed.

Application sodium bicarbonate 5% both before and after boiling water caused the germination capacity was lower than the application of acetic acid 1%. It is suggested that the concentration of sodium bicarbonate was too high so it can be phytotoxic due to the residual chemicals that may be leftover.

3. Seedling growth

In contrary to the germination capacity, the treatment of sodium bicarbonate before boiling water gave a significant difference in seedling diameter, leaves length, leaves wide, root length, leave numbers to compare to control. The results indicated that this treatment could improve seedling growth.

Application of sodium bicarbonate both before and after boiling water could increase seedling height as well as the application of acetic acid before boiling water. The results obtained in agreement with the findings of Helmy (2016) who determined that sodium bicarbonate could enhance chamomile height, a flower of numbers and dry biomass.

The seedling height of control was the lowest. On the other hand, acetic acid (C, E) given the height growth was higher than control but those were lowest than seed were soaked on boiling water and let for 24 hours (B). It suggested that the C and E treatments could remain of acetic acid they cause has a phytotoxic effect. These results agree with those of de Tunes *et al*. (2012) who reported that acetic acid reduces seedling development.

IV. CONCLUSION

Sodium bicarbonate 5% and acetic acid 1% could reduce the percentage of seed-borne fungal pathogen colonization. Application of sodium bicarbonate 5% and acetic acid 1% could not improve seed germination. Acetic acid 1% could faster seed germination. Application of sodium bicarbonate 5% for 15 minutes followed by soaking on boiling water then let for 24 hours could increase seedling growth.

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