Jurnal Perlindungan Tanaman Indonesia, Vol. 24, No. 2, 2020: 154–160
DOI: 10.22146/jpti.55057
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Potency of Non-Fungicide Chemicals for Maize Inducing Resistance against Downy Mildew

Muhammad Habibullah[1], Christanti Sumardiyono[2], & Ani Widiastuti[2]*

[1]Study Program of Agrotechnology, Faculty of Agriculture, Universitas Tidar
Jln. Kapten Suparman No. 39, Tuguran, Potrobangsan, Magelang Utara, Kota Magelang, Central Java 56116 Indonesia

[2]Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora No. 1, Bulaksumur, Sleman, Yogyakarta 55281 Indonesia

**Corresponding author. E-mail: aniwidiastuti@ugm.ac.id*

Received April 7, 2020; revised July 20, 2020; accepted December 10, 2020

ABSTRACT

Downy mildew disease control is a big challenge in Indonesia due to ability of pathogen adaptation and favorable environmental condition to the disease. Self-resistance induction by activating plant defense mechanism is a valuable control method to be developed in the future due to its environmentally safety. This study aimed to determine potency of non-fungicide chemicals to suppress downy mildew of maize. The tested chemicals were benzoic acid, sodium benzoic acid, salicylic acid, thiamine, saccharin, and aspirin with a concentration of 2000 ppm. Disease incidence, disease severity, disease progress, leaf tissue lignification, plant growth, chlorophyll content, and plant dry weight were observed to find out the effect of the chemicals application in maize against downy mildew. Results showed that seed application of benzoic acid was able to reduce disease incidence and disease severity by 80%. Benzoic acid treated plants were categorized as moderately resistant until 5-week observation, while inoculated plants (positive control) were susceptible. Based on AUDPC values, plants induced by the chemicals had slower disease development rates compared to positive control. Lignification around the stomata occured in all inoculated plants treated by chemicals. Plant growth between chemical treated plants and negative control (non-inoculated plant) mainly showed no significant different, elaborated that those chemicals were applicable. Chlorophyll content in chemical treated plants was also similar to those of negative control plant. Based on these results, benzoic acid was promoted to be further investigated as maize resistance inducer against downy mildew.

Keywords: AUDPC; benzoic acid; maize; non-fungicide chemicals; sodium benzoate

INTRODUCTION

Downy mildew is one of the important diseases in maize cultivation in Indonesia. Wijaya (2010) stated that *Peronosclerospora maydis*, the causal agent of downy mildew, attacked 200 Ha maize planting areas in Tegal Regency, Central Java Province, Indonesia. Until now, seed treatment is still the one option to control as the serious infection occurred in seedling stage. Metalaxyl is an active ingredient commonly used for maize seed treatment in Indonesia. It is an A1 class pesticide due to the fungicide resistance action committee (FRAC), which is assumed to have a high risk in pathogen resistance (FRAC, 2017). Long term use of fungicides caused *P. maydis* resistance (Isakeit & Jaster, 2005; Sumardiyono, 2008). Based on this fact, an environmentally friendly method for controlling downy mildew is required.

Plant induced resistance is a method to activate self resistance in plants by giving inducer materials which trigger the plant defense mechanism. Therefore plants become more resistant against subsequent pathogen infection (Conrath *et al.*, 2002; Agrios, 2005; Faoro *et al.,* 2008). External inducing materials (elicitors) can be biological, chemical and physical agents (Agrios, 2005; Widiastuti *et al.,* 2013). According to Faoro *et al.* (2008), resistance induction offers several important advantages, such as it is not based on a modification of plant genomes, relatively cheap, it has no environmental risks; and effective against various plant pathogens.

Research using non-fungicide chemicals as inducers of plant diseases in Indonesia has not been much conducted (Habibullah *et al.*, 2018), especially on maize. In previous research, Habibullah *et al.* (2018) reported that maize seed dipping treatment by non-fungicide chemicals such as benzoic acid, sodium benzoic, salicylic acid or saccharin induced early response of plant defense by forming reactive oxygen species (ROS) and the presence of mycelial lignification inside the plant tissues. Continuing the research, this study was conducted to observe those non-fungicide chemicals which were potential to suppress downy mildew of maize.

MATERIALS AND METHODS

Preparation and Inoculation

Maize seeds (sweet corn variety) from local seed producers without fungicide application were used in this research. Seeds were soaked in benzoic acid, sodium benzoate, salicylic acid, thiamine, saccharin and aspirin, each with concentrations of 2000 ppm distilled water for 60 minutes. Seeds were planted in pots (5 kg of soil/pot) with 5 replications and 5 seeds/pot. The positive (+) control was seeds immersed in distilled water and inoculated, while the negative control (-) was seeds immersed in distilled water without inoculation. Inoculation was carried out at 10 days after planting following method of Yuniasih and Sumardiyono (2014). Inoculum source was downy mildew diseased leaves collected from Klaten, Central Java. The leaves were cut approximately 2×3 cm and inserted into the middle of the youngest tested leaves. Inoculation was conducted at around 5 p.m. To maintain moisture, the base of the plant was covered by wet cotton and the whole plants was covered with plastic bag over night.

Observation of Disease Incidence, Severity and Development of Downy Mildew of Maize Plants

Observation of disease incidence (%) and severity (%) of downy mildew was conducted every week for 5 weeks after pathogen inoculation. Disease incidence was calculated using the following equation:

$$I = \frac{n}{N} \times 100\%$$

Note:
I : Disease incidence (%)
n : Number of plants infected with the disease
N : Total number of plants observed

Disease severity was calculated using the following equation:

$$IP = \frac{\sum(n \times v)}{N \times V} \times 100\%$$

Note:
IP : Disease Severity
n : Number of plants observed with a certain score
v : Score
N : The total number of plants observed
V : Highest score (4)

Scoring was conducted according to Agustamia *et al*. (2016) with modifications (Table 1). Plant resistance to downy mildew was characterized based on Sumardiyono *et al*. (2015) (Table 2). The disease progression curve or AUDPC which was the total area under the disease development curve was calculated according to the formula (Narayanasamy, 2002):

$$AUDPC = \sum_{i-1}^{k} \left(\frac{Si + Si-1}{2}\right) \times d$$

Note:
k : number of observations
si : disease intensity at the i-th observation
d : time interval between two observations

Observation of Lignification on Maize Leaf Tissue

Observation of leaf tissue lignification was carried out at the 3rd week after inoculation, following the Sass method (1958) with slight modification. Maize leaves were cleaned, cut approximately 1 cm, then clamped in the middle of cassava cork and thinly sliced transversely using a razor. The leaf slices were soaked with 0.05% phloroglucinol for 15 minutes, then soaked in 25% HCl solution for 30 seconds. Leaf slices were placed on a glass slide, covered by a cover glass and observed microscopically. Leaf tissue showed purple-red discoloration indicated tissue lignification (Sass, 1958).

Table 1. Severity scores of downy mildew

Percentage of disease severities (%)	Scale of scores
>0–10	1
>10–30	2
>30–50	3
>50	4

Table 2. Plant resistance categories based on disease severity

Percentage of disease severities (%)	Resistance Category
>0–10	Resistant
>10–30	Moderately resistant
>30–50	Moderate Susceptible
>50	Susceptible

Observation of Maize Plant Growth

Parameter of plant growth measured in this research was plant height, number of leaves, and root length of the plants. Observation of plant height and number of leaves was done every week during 6-week planting. Plant height measurements were carried out by measuring the length from the base of trunk to the longest leaf tip. Observation of root length was done at the 6th week after the planting.

Chlorophyll Content of Maize Leaves

Observation of chlorophyll content was carried out at 5th week after inoculation. Observation was made on each treatment represented with 3 samples as replication. Leaf samples used were 3 top or young leaves of every single plant. The method referred to Arnon (1949). Fresh leaves were weighed as much as 1 g. Acetone 80% as much as 20 ml was added to leaf samples and crushed using a mortar. The crude extract was filtered using filter paper and then put into a test tube. The solution was poured into a spectrophotometer cuvette up to boundary line and measured the absorbance value. Pure acetone solution was used as a standard solution. Observations were made at wavelengths of 645 nm and 663 nm. The absorbance value obtained was used to calculate the chlorophyll content using the formula according to Arnon (1949):

$$\text{Total chlorophyll} = 20{,}2\ A645 + 8{,}02\ A663\ \text{mgg}^{-}$$

Dry Weight

Dry weight was measured at 6th week after planting. Plants were dried in an oven at 60 ºC for 4 days.

Data Analysis

Agronomic observations of maize plants including height, number of leaves, root length and dry weight were analyzed using ANOVA (analysis of variance) at 5% significance level followed by Duncan New Multiple Range Test (DNMRT) at the same level of significance.

RESULTS AND DISCUSSION

Disease Incidence, Severity, and Progression on Maize Downy Mildew After Treatment

The result showed that the tested chemicals reduced the percentage of disease incidence (Figure 1) and disease severity of downy mildew (Figure 2) of maize plants. Observation at 5 weeks after inoculation proved that disease incidence of plants treated by benzoic acid was 20%, sodium benzoate and saccharin were 30%, aspirin was 40%, and salicylic acid and thiamine were 50%, while inoculated plants (positive control) was 100%. Furthermore, the disease severity of plants treated by benzoic acid was 20%, sodium benzoic was 30%, saccharin was 25%, aspirin was 27.5%, salicylic acid was 35% and thiamine was 40%, whereas in plants that were only inoculated (positive control) was 90%.

The result showed that benzoic acid gave the best result in reducing disease incidence and severity. Benzoic acid suppressed disease incidence and disease severity up to 80% compared to the positive control. Categories of plant resistance is performed in Table 3. Data showed that chemicals increased plant response against downy mildew for moderately

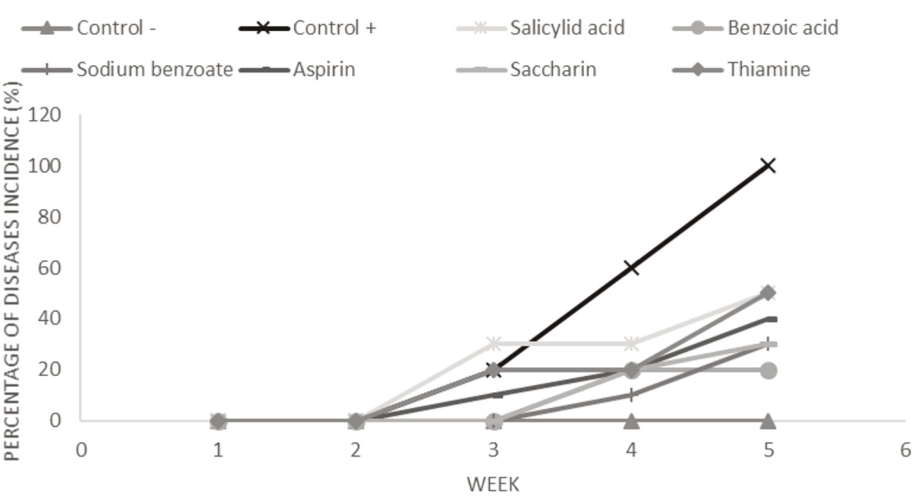

Figure 1. Disease incidence of maize downy mildew after treatments.

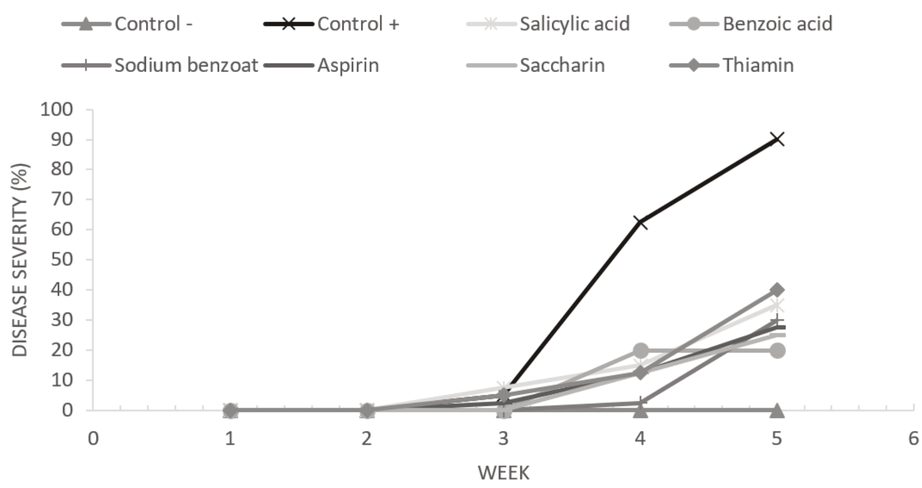

Figure 2. Disease severity of maize downy mildew after treatment

Table 3. Maize resistance category after treatment and inoculation of *Peronosclerospora maydis* at 4 and 5 weeks after inoculation

Treatment	Week 4th	Week 5 th
	Resistance Category	Resistance Category
Control -	- (healthy plants)	- (healthy plants)
Control +	Susceptible	Susceptible
Salicylic acid	Moderately resistant	Moderate susceptible
Benzoic acid	Moderately resistant	Moderately resistant
Sodium benzoate	Resistant	Moderately resistant
Aspirin	Moderately resistant	Moderately resistant
Saccharin	Moderately resistant	Moderately resistant
Thiamine	Moderately resistant	Moderately susceptible

resistant–resistant on week 4, and moderately susceptible–moderately resistant on week 5, while all inoculated plants (positive control) were susceptible (Table 3). Benzoic acid treated plants were consistently categorized as moderately resistant, and sodium benzoate treated plants were categorized as resistant at week 4 and moderately resistant at week 5, while positive control plants were susceptible. Sodium benzoate is a derivative chemical of benzoic acid, therefore they provided relatively similar influence in this research. Based on AUDPC analysis, disease development progressed variably, however benzoic acid, sodium benzoate, and saccharin showed delaying disease incubation period (Table 4).

On data average, all treatments showed slower disease progress compared to positive control. Those data demonstrated that benzoic acid and sodium benzoate treatment possessed potential ability in controlling maize downy mildew. This occurs because of the ability of these chemicals to induce plant resistance so that it is more resistant to pathogen attack. This result is in accordance with Habibullah *et al*. (2018) which showed that chemicals in the form of sodium benzoate and benzoic acid had ability to increase the resistance of maize plants to *P. maydis* attack. In addition, research by Fadel *et al*. (2006) also showed that benzoate compounds could affect the attack of *Ustilazo maydis* on maize.

Lignification of Maize Leaf Tissue

Microscopic qualitative observation (Figure 3) showed that lignification occurred in the tissue around the stomata of maize plants in all inoculated plants treated with chemicals, but it was not found in uninoculated plants. Lignification was indicated by the presence of purplish-red in the leaf tissue dyed with phloroglucinol (Sass, 1958). Lignification surrounding the stomatal area in inoculated plants without any chemical treatments (positive control) showed a general response of maize plants as their

Table 4. Disease development values based on AUDPC analysis on maize after treatment and inoculation of *Peronosclerospora maydis*

Treatment	AUDPC value in every week					
	1	2	3	4	5	Average
Control -	0	0	0	0	0	0
Control +	0	0	2.5	33.75	76.25	22.5
Salicylic acid	0	0	3.75	11.25	25	8
Benzoic acid	0	0	0	10	20	6
Sodium benzoate	0	0	0	1.25	16.25	3.5
Aspirin	0	0	1.25	7.5	20	5.75
Saccharin	0	0	0	6.25	18.75	5
Thiamine	0	0	2.5	8.75	26.25	7.5

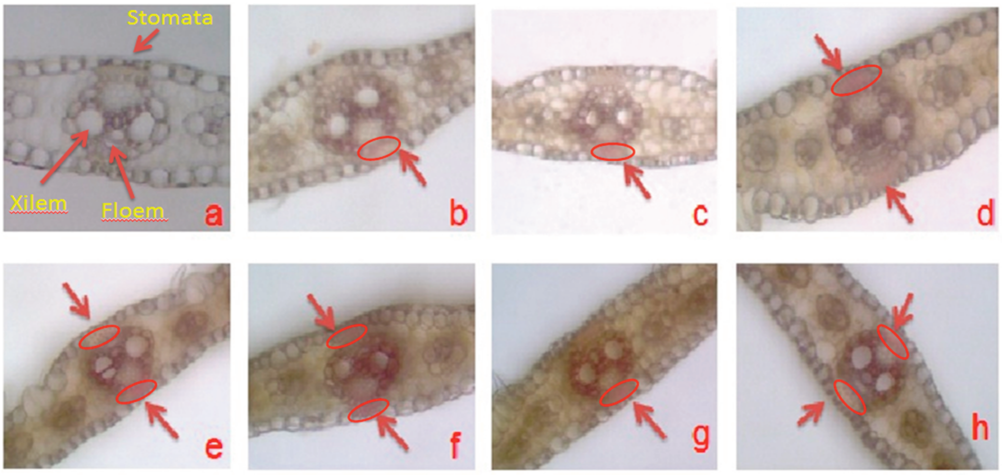

Figure 3. Lignification of maize leaf tissue induced by chemicals after inoculation. negative control (a), positive control (b), benzoic acid (c), sodium benzoate (d), salicylic acid (e), thiamine (f), saccharin (g) and aspirin (h); data was observed on 24 days after planting; the circled part with the arrow is the lignified stomata

defense mechanism agints pathogen infection. He *et al.* (2002) showed that cell wall lignification in pathogen-infected tissue was one of the plant defense systems. This result resembled to Asmawati (2016), which showed lignification in the surrounding of stomata of the maize leaves induced resistance with *Trichoderma* spp. The presence of lignification in the tissues around leaf stomata showed that plants activated their defense mechanism after treated with chemical inducer or pathogen inoculation. Vance *et al.* (1980) stated that increasing level of lignin inhibited physical penetration and invasion of pathogens, inhibited toxins spreading and enzymes released by pathogens, as well as inhibited nutrients supply needed by pathogens.

Previously, Habibullah *et al.* (2018) reported that the same chemical treatment produced reactive oxygen species (ROS) in leaf tissue as plants early response and mycelial lignification of pathogen in leaf tissues. Stomata lignification in this study showed that the chemical treatments possibly induced maize resistance and then suppressed the incidence and severity of the disease. Some previous studies also showed the effect of chemicals on suppressing disease in plants. Fadel *et al.* (2006) reported that treatment of salicylic acid, sodium benzoate, and potassium monophosphate could suppress swollen rot disease of maize plants. In that research, severity of fruit swelling in plants treated with sodium salicylic acid was 10.12%, benzoate was 6.34%, and potassium monophosphate was 5.83%.

Growth of Maize Plants

Result showed that chemical treated plants had better growth compared to positive control and were not significantly different from negative control (Table 5). Taken together with previous data that

Table 5. Maize growth and chlorophyll content after treatment on 6 weeks after planting

Treatment	Plant height (cm)	Number of leaves	Root length (cm)	Plant dry weight (g)	Chlorophyll value (mg/g)
Salicylic acid	107.80 a	5.90 ab	42.50 a	2.60 abc	0.56377
Benzoic acid	96.20 ab	5.80 ab	31. 75 a	3.00 ab	0.50091
Sodium benzoate	96.40 ab	5.70 ab	31.15 a	2.67 ab	0.60493
Aspirin	96.30 ab	5.90 ab	36.75 a	2.53 bc	0.50977
Saccharin	95.40 ab	6.10 a	39.50 a	2.63 ab	0.51381
Thiamin	97.30 ab	5.50 ab	38.05 a	2.13 c	0.54766
Control +	87.40 b	5.20 b	34.10 a	1.57 d	0.35367
Control -	99.20 ab	5.70 ab	38.75 a	3.06 a	0.85099

Note: The numbers in the same column followed by the same letters indicate no significant difference based on the DNMRT test with a 95% confidence level.

chemical treatment reduced disease incidence and severity, it was assumed that plants had better endurance in their growth stage, therefore plant height and the number of leaves were higher than the positive control. On root length, there were no significant difference among treatments, but salicylic acid treated plant showed the longest root (Table 5). Some reports showed that certain chemical treatment influenced plant physiology, such as Hayat *et al.* (2005) which informed that salicylic acid regulated the balance of auxin, cytokinin, and ABA in plant tissues, induced the proliferation and expansion of plant cells, increased the absorption and transportation of nutrients, increased photosynthesis, thereby increased plant growth.

Chlorophyll Content and Dry Weights of Maize Plants

Observations of chlorophyll content (Table 5) showed that the highest value was gained in negative control at 0.85 mg/g, followed by plants treated with sodium benzoate, and other chemicals. The lowest chlorophyll content was shown in the positive control at 0.35 mg/g. Those data related to previous observation that disease incidence and severity was lower after chemical treatments, indicated that plants might compensate the pathogen infection process. Therefore their physiological process was not much disrupted including the clorophyl production. As the consequence, chemical treated plants would grow better. Plant resistance to *P. maydis* caused lower leaf chlorosis or the chlorophyll content in the leaf was well maintained, in accordance with the research of Agustamia (2016) and Asmawati (2016) which showed that plants that were more resistant to *P. maydis* had a higher chlorophyll content than susceptible plants.

The highest dry weight was obtained by negative control at 3.06 g, followed by plant treated with 3 g of benzoic acid, 2.67 g of sodium benzoate, 2.63 g of saccharin, and 2.6 g of salicylic acid. All of these treatments were not significantly different from negative control. The lowest dry weight was found in positive control and significantly different from all treatments. The low dry weight of positive control was probably caused by the lack of chlorophyll content which resulted in inhibition of plant growth rate due to decreasing photosynthate. This is in line with the determination of Habibullah & Suhendra (2020) which showed that maize plants that were induced resistance showed higher dry weight than positive control.

CONCLUSION

The benzoic acid treatment of 2000 ppm gave the lowest disease incidence, intensity and progression compared to other treatments and positive control. Plants resistance category was maintained in moderately resistant up to 5th week after planting. Plant growth, dry weight, and chlorophyll values were not significantly different from negative control. Therefore benzoic acid had the highest potential as a resistance inducer chemical of maize to downy mildew for further research.

ACKNOWLEDGEMENT

This research article is part of a Postgraduate thesis entitled, “Induction of Maize Plants Resistance to Downy Mildew Using Chemicals,” in the name of Muhammad Habibullah, Universitas Gadjah Mada, 2017, under supervision of Christanti Sumardiyono and Ani Widiastuti. Authors declared there is no conflict of interests.

LITERATURE CITED

Agrios. G.N. 2005. *Plant Pathology*. 5th edition. Academic Press, San Diego. 952 p.

Agustamia, C., A. Widiastuti & C. Sumardiyono. 2016. Pengaruh Stomata dan Klorofil pada Ketahanan Beberapa Varietas Jagung terhadap Penyakit Bulai. *Jurnal Perlindungan Tanaman Indonesia* 20: 89–94.

Arnon, D.I. 1949. Copper Enzymes in Isolated Chloroplast, Poliphenol Oxidase in *Beta vulgaris*. *Plant Physiology* 24: 1–15.

Asmawati, L. 2016. *Induksi Ketahanan Jagung terhadap Penyakit Bulai dengan Jamur Trichoderma spp*. Tesis. Fakultas Pertanian Universitas Gadjah Mada, Yogyakarta. 62 p.

Conrath, U., C.M.J. Pieterse, & B. Mauch-Mani. 2002. Priming in Plant–Pathogen Interactions. *Trends in Plant Science* 7: 210–216.

Fadel, F., E.N. Magdy, T. Sobhi, & F. Gamal. 2006. Induction of Disease Resistance by Salicylic Acid, Sodium Benzoate and Potassium Monophosphate against *Ustilago maydis* in Maize Plants, p. 240–250. *In* G. J. Kövics & I. Dávid (eds.), *Recent Developments of IPM. Proceedings of 4th International Plant Protection Symposium*. Debrecen University, Debrecen, Hungary, October 18–19, 2006.

Faoro, F., D. Maffi, D. Cantu, & M. Iriti. 2008. Chemical-induced Resistance against Powdery Mildew in Barley: The Effects of Chitosan and Benzothiadiazole. *BioControl* 53: 387–401.

FRAC (Fungicide Resistance Action Committee). 2017. *FRAC Code List ©*2017: Fungicides sorted by mode of action (including FRAC Code numbering)*. Fungicide Resistance Action Committee (FRAC). https://www.google.co.id/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwii3taH1er-WAhXEq5QKHcOGAhMQFgglMAA&url=http%3A%2F%2Fwww.frac.info%2Fdocs%2Fdefault-source%2Fpublications%2Ffrac-code-list%2Ffrac-code-list-2017-final.pdf%3Fs-fvrsn%3D2&usg=AOvVaw1V4tBNqesJfaD4wLQ2qMeb, modified 01/08/2019.

Habibullah, M. & D. Suhendra. 2020. Use of Bamboo Vinegar as an Alternative to Control Downy Mildew in Maize. *AGRINULA: Jurnal Agroteknologi dan Perkebunan* 3: 17–24.

Habibullah, M., A. Widiastuti, & C. Sumardiyono. 2018. Respons Awal Ketahanan Jagung terhadap *Peronosclerospora maydis* dan Induksi Bahan Kimia. *Jurnal Perlindungan Tanaman Indonesia* 22: 27–32.

Hayat, S., Q. Fariduddin, B. Ali, & A. Ahmad. 2005. Effect of Salicylic Acid on Growth and Enzyme Activities of Wheat Seedlings. *Acta Agronomica Hungarica* 53: 433-437.

He, C.Y., T. Hsiang, & D.J. Wolyn. 2002. Induction of Systemic Disease Resistance and Pathogen Defence Responsses in *Asparagus officinalis* Inoculated with Nonpathogenic Strains of *Fusarium oxysporum*. *Plant Pathology* 51: 225–230.

Isakeit, T. & Jaster, J. 2005. Texas Has a New Pathotype of *Peronosclerospora sorghi*, the Cause of Sorghum Downy Mildew. *Plant Disease* 89: 529.

Narayanasamy, P. 2002. *Microbial Plant Pathogens and Crop Disease Management*. Tamil Nadu Agricultural University, Science Publishers Inc., Coimbatore, India, 553 p.

Sass, J. 1958. *Botanical Microtechnique*. 3rd Edition. The Iowa State College Press, Ames, Iowa. 228 p.

Sumardiyono, C. 2008. Ketahanan Jamur terhadap Pestisida di Indonesia. *Jurnal Perlindungan Tanaman Indonesia* 14: 1–5.

Sumardiyono, C., A. Wibowo, & A. Widiastuti. 2015. *Uji Ketahanan Beberapa Varietas Jagung terhadap Penyakit Bulai (Peronoslerospora maydis) di Klaten*. Laporan Akhir Penelitian: Hibah Penelitian Fakultas Pertanian Universitas Gadjah Mada, Yogyakarta. 10 p.

Vance, C. P., T.K. Kirk, & R.T. Sherwood. 1980. Lignification as a Mechanism of Disease Resistance. *Annual Review of Phytopathology* 18: 259–88.

Widiastuti, A., M. Yoshino, H. Saito, K. Maejima, S. Zhou, H. Odani, K. Narisawa, M. Hasegawa, Y. Nitta, & T. Sato. 2013. Heat Shock-induced Resistance in Strawberry against Crown Rot Fungus *Colletotrichum gloeosporioides*. *Physiological and Molecular Plant Pathology* 84: 86–91.

Wijaya, R. 2010. *220 Hektare Lahan Jagung Diserang Penyakit Bulai*. Suaramerdeka.com. http://suaramerdeka. com, modified 09/02/2017.

Yuniasih, D.A. & C. Sumardiyono. 2014. *Ketahanan Peronosclerospora maydis terhadap Fungisida*. Tesis. Fakultas Pertanian Universitas Gadjah Mada, Yogyakarta. 64 p.