



Research Article

Inhibition of *Rehmannia mosaic virus* Infection by *Ganoderma* sp. Extract

Mustika Ajeng Kartini Putri Pertiwi¹⁾, Suryanti^{1)*}, & Susanto Somowiyarjo¹⁾

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

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

Received September 14, 2019; revised August 12, 2020; accepted December 8, 2020

ABSTRACT

In fruiting bodies and mycelia of several fungi belonging to a Basidiomycetes group, among others, *Ganoderma* contains active polysaccharides and has potential as an antiviral substances. This study aimed to determine the effect of mycelium and fruiting body extract of *Ganoderma* sp. against *Rehmannia mosaic virus* (ReMV) infections on *Chenopodium amaranticolor* with variations of dilution and application times. The results showed that mycelium and fruiting body extract of *Ganoderma* sp. could inhibit ReMV infections. Antiviral extract which were applied 4 hours before, and at the same time with virus inoculation resulted in a higher viral inhibition rates than when applied 4 hours after virus inoculation. Fruiting body extract at 10^{-1} and 10^{-2} dilutions delayed symptom appearance caused by ReMV infection when applied 4 hours before virus inoculation. Mycelium extract at dilution of 10^{-2} and 10^{-3} applied 4 hours before virus inoculation resulted in the highest inhibition rate (100%) which was indicated as the number of local lesions on *Chenopodium amaranticolor* leaves. Meanwhile the fruiting body extract dilution of 10^{-1} resulted in highest inhibition (99.55%) when applied 4 hours before virus inoculation.

Keywords: antiviral substances; *Ganoderma* sp.; *Rehmannia mosaic virus*

INTRODUCTION

Rehmannia mosaic virus (ReMV) was first reported in Henan, China as a virus of *Rehmannia glutinosa* Libosch. Symptoms caused by this virus is a systemic mosaic similar to symptoms caused by *Tobacco mosaic virus*. ReMV can be transmitted mechanically on *Nicotiana glutinosa* and result in necrotic lesions. In addition, this virus also causes the same symptoms on *Nicotiana tabacum* var. *Xanthi*, *N. rustica*, *Datura stramonium* and *Chenopodium amaranticolor* (Zhang *et al.*, 2008). This virus was first reported in Japan on chili pepper variety Fushimi-amanaga. Symptoms from this infection are mosaic symptoms on leaves which continuously develop and cause leaves to fall, necrotic lesions on stems, and malformed fruit (Kubota *et al.*, 2012).

According to Endarsih *et al.* (2017), *Rehmannia mosaic virus* are known as the cause of systemic mosaics on tobacco. Symptoms caused by this infection are leaf malformations and systemic mosaics. This virus causes variety of symptoms on several plants. Chlorotic systemic lesions were shown on cucumber plants. Symptoms on *C. amaranticolor* are chlorotic and local necrotic lesions, while on chili pepper,

tobacco, and eggplants, symptoms found was systemic mosaics (Nurviani, 2018). Therefore, this virus has a wide host range and can easily be transmitted; thus, there is a need for an antiviral compound to manage this virus. Basidiomycota is a phylum recognized to contain many natural antibiotic compounds. Some species have been reported to possess antifungal and antiviral compounds, inhibit phytopathogenic nematodes and myeloma cell growth, as well recognized as antioxidants (Somowiyarjo, 1999; Sokovic *et al.*, 2013; Sivanandhan *et al.*, 2017).

Indonesia has high biodiversity of fungal species, such as the genera *Ganoderma*, which are still unknown of their practical use (Tampubolon *et al.*, 2013). Some species are known as plant pathogens of several important plantation crops (Semangun, 2000; Purnamasari *et al.*, 2012). However, research by Kovalenko *et al.* (2008) reported that metabolites produced by *G. lucidum* and *G. applanatum* were able to inhibit Tobacco mosaic virus infection by 65–70% at the concentration of 1000 $\mu\text{g/mL}$. This goal of this study was to determine the antiviral potential of mycelium and fruiting body of *Ganoderma* sp. against *Rehmannia mosaic virus* infection.

MATERIALS AND METHODS

Test Plant Preparation

Test plants used in this study were approximately 2-months-old *C. amaranticolor* that were grown in dark conditions 24 hours prior to inoculation.

Ganoderma sp. Isolation and Propagation

Ganoderma sp. used in this study was obtained from a river tamarind (*Leucaena leucocephala*) tree located on Kalimantan Street, Sinduadi, Sleman, Special Region of Yogyakarta. Fungus was isolated and the growing mycelia were then cultured on a new PDA medium. This later was stored as a pure culture for subsequent tests.

Ganoderma sp. Extract Preparation

Ganoderma sp. extract was prepared from mycelia and fruiting body. Mycelia that were dried in an oven, weighted, macerated using a porcelain mortar, and sterile water was added to obtain an extract concentration of 1 g/mL. *Ganoderma* sp. extract from fruiting body was obtained by drying macerated fruiting body, weighting and adding sterile water to obtain extract concentration of 1 g/mL.

Virus Inoculation and Propagation

Virus isolate used in this study was obtained from tobacco plants with mosaic symptoms located at Kedu, Temanggung, Central Java. Virus were then propagated on tobacco plants and molecularly identified using TobRT-up1 and TobRT-do2 primer pairs. Sequence results confirmed that the virus isolate had 97% homology with *Rehmannia mosaic virus* (ReMV) of *Capsicum annuum* Japan isolate (Endarsih *et al.*, 2017).

Virus inoculation was conducted using a sap substance made by adding 10 mL of phosphate buffer 0,01M pH 7 to 1 g of macerated infected tobacco leaves. Virus sap substance was diluted to 10^{-4} and was inoculated by damaging plant leaves with rubbing 600 mesh of carborundum and followed by rubbing virus sap substance. Leaves were cleaned using sterile water after inoculated with virus sap substance.

Ganoderma sp. Extract as an Antiviral Agent

Both *Ganoderma* sp. extract types were diluted to 10, 100, and 1000 times using sterile water. Each dilution series were applied 4 hours before, 4 hours after, and same time as virus inoculation on test plants with a ratio of 1:1 between virus sap substance

and *Ganoderma* sp. extract. This study was designed with a complete randomized design with 5 replications for each treatment combination. Application was conducted by rubbing *Ganoderma* extract on leaf surface of *C. amaranticolor* with the following treatments:

- P1 : Inoculated with virus and without *Ganoderma* sp. extract application
- P2 : *Ganoderma* sp. extract application 4 hours before virus inoculation
- P3 : *Ganoderma* sp. extract application same time as virus inoculation
- P4 : *Ganoderma* sp. extracts application 4 hours after virus inoculation

Observation in this study included (1) symptoms and virus incubation period done every day for 10 days after *C. amaranticolor* leaf inoculation; (2) number of local lesions on each treatment, which were done every day for 10 days after *C. amaranticolor* leaf inoculation; (3) inhibition percentage of ReMV infection on test plants that were calculated using the following formula (Lu *et al.*, 2013):

$$P = [(a - b) / a] \times 100\%$$

- P : Inhibition percentage
- a : Number of local lesions on leaves inoculated with virus without *Ganoderma* sp. application
- b : Number of local lesions on leaves inoculated with virus with *Ganoderma* sp. application

Average local lesions and inhibition rates were analyzed using ANOVA. If significant differences were found, a Duncan post hoc test (DMRT) was conducted. All tests were conducted at $\alpha=0.05$.

RESULTS AND DISCUSSION

Disease Symptoms and Incubation Period

Local lesions appeared on *C. amaranticolor* leaf inoculated with ReMV (Figure 1). The symptoms started with yellow lesions (Figure 1.b), which enlarged causing several small lesions forming a larger one and turned brownish 7 days after inoculation (Figure 1.c). Leaf areas around the lesions turned yellow followed by death of the leaf causing leaf to fall (Figure 1.d).

Incubation period of the virus is shown in Figure 2. ReMV had an incubation period of 4 days, which was implied by observations on leaves inoculated with virus without antiviral treatment (positive

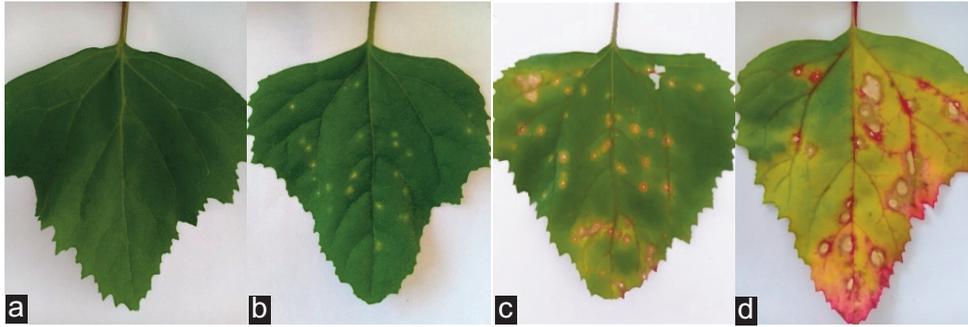


Figure 1. *Rehmannia mosaic virus* (ReMV) infection development on *Chenopodium amaranticolor*: (a) untreated leaf; (b) symptoms on leaf inoculated with virus 4 days after inoculation (DAI), 7 DAI (c), and 15 DAI (d)

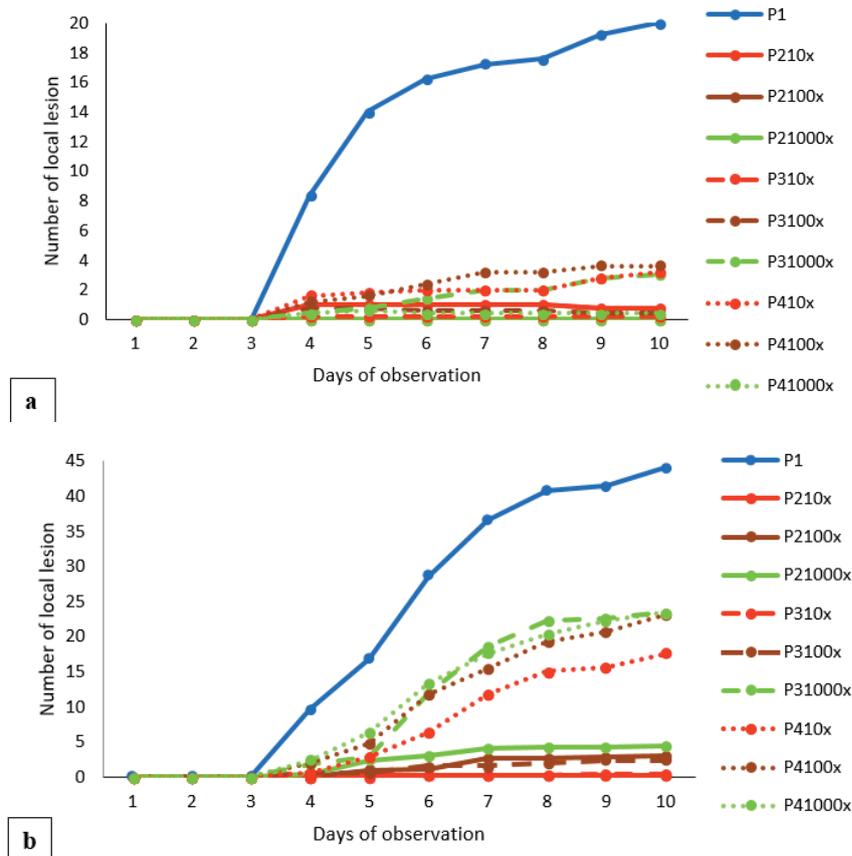


Figure 2. Development of local lesions numbers on *Chenopodium amaranticolor* leaves treated with (a) mycelium extract (b) *Ganoderma* sp. fruit body extract for 10 days after inoculation; control (P1), antiviral application 4 hours before virus inoculation at 10× dilution (P210×), antiviral application 4 hours before virus inoculation at 100× dilution (P2100×), antiviral application 4 hours before virus inoculation at 1000× dilution (P21000×), antiviral application together with virus inoculation at 10× dilution (P310×), antiviral application together with virus inoculation at 100× dilution (P3100×), antiviral application together with virus inoculation at 1000× dilution (P31000×), antiviral application 4 hours after virus inoculation at 10× dilution (P410×), antiviral application 4 hours after virus inoculation at 100× dilution (P4100×), antiviral application 4 hours after virus inoculation at 1000× dilution (P41000×)

control/P1). Leaves treated with almost all antiviral substances at various dilution series and application times combinations showed similar incubation periods with the positive control (Figure 2). However,

several antiviral treatments showed longer incubation periods. Antiviral treatment using extract of *Ganoderma* sp. fruiting body and applied 4 hours before inoculation at 10× and 100× dilution had 5 days incubation period.

Antiviral application together with virus inoculation at 10x dilution showed similar incubation period (Figure 2.b). Antiviral treatment using mycelia extract 4 hours before inoculation with dilution of 100x and 1000x did not show symptoms until observation were finished (10 days after inoculation) (Figure 2.a).

***Ganoderma sp.* Extract as an Antiviral Substance against ReMV**

The effects of mycelial extract against ReMV infection is shown in Table 1. Positive control showed the greatest number of local lesions compared to leaves treated with antiviral substance. Antiviral substance applied 4 hours before inoculation resulted in the least number of lesions compared to other application time treatments, whereas antiviral applied 4 hours after inoculation showed the highest number of lesions. The effect *Ganoderma sp.* fruiting body extract against ReMV (Table 1) showed that the highest number of lesions was the positive control (44 lesions). Antiviral substance applied 4 hours before and same time as virus inoculations showed relatively fewer lesions compared to antiviral substance applied 4 hours after inoculation. Dilution rates corresponded positively with the number of lesions.

ReMV Inhibition Rate of Ganoderma sp. Extracts

Mycelium extract was able to inhibit up to 100% with an average number of local lesions of 0, while fruiting body extract showed the highest inhibition rate of 99.55% with an average 0.2 lesions (Figure 3). The both highest inhibition rates were obtained from application times of 4 hours before inoculation.

Antiviral substance application before virus inoculation tend to show higher inhibition rates compared to other application times. This was shown by the fewer numbers of lesions. According to Awasthi *et al.* (2016), there are 3 mechanism that may occur when virus infections are inhibit, such as (1) direct effects on virus by non-activating or alternating virus characteristics; (2) effects on infectious process of viruses, especially ones that are highly related to ribosomal inactivating protein (RIPs), by hindering virus replications and protein synthesis of host ribosomal cells; and (3) affecting host susceptibility by changing cell metabolism and producing antiviral substance. The later mechanism in an indirect mechanism, which frequently occurs as induced local and systemic resistance mechanisms.

Table 1. Number of local lesions on *Chenopodium amaranticolor* leaves infected by *Rehmannia mosaic virus* and treated with *Ganoderma sp.* extracts

Treatment	Σ Local Lesion**)	
	Mycelium Extract	Fruit Body Extract
P1*)	20 a***)	44 a
P210x	0.8 b	0.2 c
P2100x	0 b	3 c
P21000x	0 b	4.4 c
P310x	0.2 b	0.4 c
P3100x	0.4 b	2.4 c
P31000x	3 b	23.4 b
P410x	3.2 b	17.6 b
P4100x	3.6 b	23.2 b
P41000x	0.4 b	23.4 b

- *)P1 : Control
 P210x : Antiviral application 4 hours before virus inoculation, at 10x dilution
 P2100x : Antiviral application 4 hours before virus inoculation, at 100x dilution
 P21000x : Antiviral application 4 hours before virus inoculation, at 1000x dilution
 P310x : Antiviral application together with virus inoculation, at 10x dilution
 P3100x : Antiviral application together with virus inoculation, at 100x
 P31000x : Antiviral application together with virus inoculation, at 1000x
 P410x : Antiviral application 4 hours after virus inoculation, at 10x
 P4100x : Antiviral application 4 hours after virus inoculation, at 100x
 P41000x : Antiviral application 4 hours after virus inoculation, at 1000x

***) Average number of local lesions on *C. amaranticolor* leaves from 5 replication.

****) Numbers followed by different letter of the same column are significantly different based on a DMRT post-hoc test at $\alpha = 0.05$. For analytical purposes, data were transformed using $\sqrt{(x+0.5)}$.

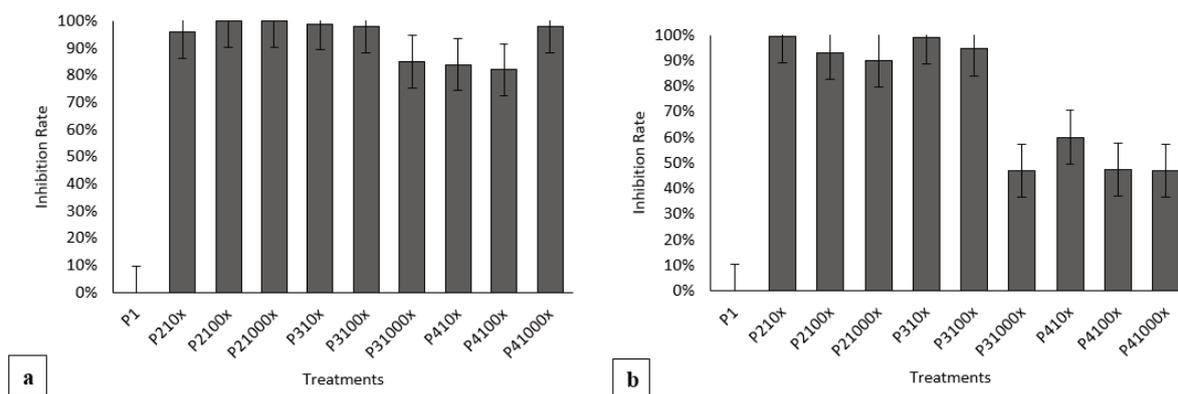


Figure 3. Infection inhibition of *Rehmannia mosaic virus* infection by (a) mycelia; (b) fruiting body extract of *Ganoderma* sp.; control (P1), antiviral application 4 hours before virus inoculation at 10× dilution (P210×), antiviral application 4 hours before virus inoculation at 100× dilution (P2100×), antiviral application 4 hours before virus inoculation at 1000× dilution (P21000×), antiviral application together with virus inoculation at 10× dilution (P310×), antiviral application together with virus inoculation at 100× dilution (P3100×), antiviral application together with virus inoculation at 1000× dilution (P31000×), antiviral application 4 hours after virus inoculation at 10× dilution (P410×), antiviral application 4 hours after virus inoculation at 100× dilution (P4100×), antiviral application 4 hours after virus inoculation at 1000× dilution (P41000×)

Antiviral substance application together with virus inoculation showed decent results. Infection inhibition rates were positively correlated to dilution rates of antiviral substance. According to Rini (2004), higher dilution rates caused lower effectivity in suppressing local lesions resulted from this infection.

Application time of antiviral substance after virus inoculation resulted in lower inhibition rates. According to Narwal *et al.* (2001), antiviral application after virus inoculation were more effective when applications were done immediately after virus inoculation. Inhibition rates will decrease over time as time between virus inoculation and antiviral substance application increases. The same research showed that antiviral substance application 4 hours after inoculations were only able to inhibit up to 46%. Furthermore, antiviral substance application after inoculation was not able to eliminate virus. This may imply that this antiviral may not have chemotherapeutic mechanism as shown by the use of ribavirin in the research by Roostika *et al.* (2016) which proven to be able to eliminate Sugarcane streak mosaic virus (SCSMV) on sugarcane variety PS862-Crb.

Bioactive compounds contained by *Ganoderma* include terpenoids, steroids, adenosine dan guanosine, protein, polysaccharides, ganoderic acid, germanium, and lectin (Mizuno *et al.*, 1995). Polysaccharides, terpenoids, ganoderic acids, and protein contained in *Ganoderma* have also been reported to contain antiviral

mechanisms (Kovalenko *et al.*, 2008; Zhu *et al.*, 2015), while according to Kobayashi *et al.* (1987), antiviral substance of Basidiomycetes that have inhibition activity against virus infection was polysaccharides and glycoproteins.

In *G. lucidum*, polysaccharides were the main components and more than 200 polysaccharides have been isolated from this species where most of them were β -glucan. Several researches have shown that β -glucan has antiviral activities by hindering virus penetration and replications (Minari *et al.*, 2011; Rouhier *et al.*, 1995). Therefore, β -glucan might be a component with antiviral substance in *Ganoderma* that may inhibit ReMV infection.

CONCLUSION

Mycelia and fruiting body extract of *Ganoderma* sp. possessed antiviral properties against ReMV infection. Mycelial extract at 10^{-2} and 10^{-3} applied 4 hours before virus inoculation resulted in the highest inhibition of 100%, while fruiting body extract at dilution rate of 10^{-1} resulted in the highest inhibition of 99.55% when applied 4 hours after inoculated with virus sap substance.

ACKNOWLEDGEMENT

This article was written by partly using data from the first author's bachelor thesis that was advised

by the second and third writer. We would like to thank the Faculty of Agriculture, Universitas Gadjah Mada for The Lecturer and Student Collaboration Grant 2017 3172/PN/PG/2017 used to fund this study.

LITERATURE CITED

- Awasthi, L.P., H.N. Verma, & S. Kluge. 2016. A Possible Mechanism of Action for the Inhibition of Plant Viruses by an Antiviral Glycoprotein Isolated from *Boerhaavia diffusa* Roots. *Journal of Virology & Antiviral Research* 5: 1–8.
- Endarsih, W., S. Hartono, & S. Sulandari. 2017. Perbaikan Metode Ekstraksi dsRNA Virus secara Sederhana untuk RT-PCR Tiga Virus Tumbuhan. *Jurnal Perlindungan Tanaman Indonesia* 21: 106–113.
- Kobayashi, N., A. Hiramatsu, & T. Akatsuka. 1987. Purification and Chemical Properties of an Inhibitor of Plant Virus Infection from Fruiting Bodies of *Lentinus edodes*. *Agricultural and Biological Chemistry* 51: 883–890.
- Kovalenko, O.G., O.M. Polishchuk, T.A. Krupodorova, N.A. Bisko, & A.S. Buchalo. 2008. Screening of Metabolites Produced by Strains of *Ganoderma lucidum* (Curt.:Fr.) P. Karst and *Ganoderma applanatum* (Pirs.:Waller) Pat. for their Activity against *Tobacco mosaic virus*. *Visnyk Kiyvskogo Nacionalnogo Univresitry* 51: 32–34.
- Kubota, K., T. Usugi, Y. Tomitaka, Y. Matsushita, M. Higashiyama, Y. Kosaka, & S. Tsuda. 2012. Characterization of *Rehmannia mosaic virus* Isolated from Chili Pepper (*Capsicum annuum*) in Japan. *Journal of General Plant Pathology* 78: 43–48.
- Lu, M., Z. Han, Y. Xu, & L. Yao. 2013. *In Vitro* and *in Vivo* Anti-*Tobacco mosaic virus* Activities of Essential Oils and Individual Compounds. *Journal of Microbiology and Biotechnology* 23: 771–778.
- Minari, M.C., V.P. Rincão, S. Soares, & N.M.P.S. Ricardo. 2011. Antiviral Properties of Polysaccharides from *Agaricus brasiliensis* in the Replication of *Bovine herpesvirus 1*. *Acta Virologica* 3: 255–259.
- Mizuno, T., G. Wang, J. Zhang, H. Kawagishi, T. Nishitoba, & J. Li. 1995. *Reishi*, *Ganoderma lucidum* and *Ganoderma tsugae*: Bioactive Substances and Medicinal Effects. *Food Reviews International* 11: 151–166.
- Narwal, S., A. Nalasubrahmanyam, P. Sadhna, H.C. Kapoor, & M.L. Lodha. 2001. A Systemic Resistance Inducing Antiviral Protein with N-glycosidase Activity from *Bougainvillea xbuttiana* Leaves. *Indian Journal of Experimental Biology* 39: 600–603.
- Nurviani. 2018. *Karakterisasi Penyebab Mosaik Tembakau dan Penghambatannya dengan Ekstrak Jantung Pisang*. Tesis. Program Pasca Sarjana Fakultas Pertanian Universitas Gadjah Mada, Yogyakarta. 44 p.
- Purnamasari, M.I., C. Prihatna, A.W. Gunawan, & A. Suwanto. 2012. Isolasi dan Identifikasi secara Molekuler *Ganoderma* spp. yang Berasosiasi dengan Penyakit Busuk Pangkal Batang di Kelapa Sawit. *Jurnal Fitopatologi Indonesia* 8: 9–15.
- Rini, K.I. 2004. *Pengaruh Ekstrak Shiitake terhadap Infeksi Cucumber mosaic virus*. Skripsi. Fakultas Pertanian. Universitas Gadjah Mada, Yogyakarta. 47 p.
- Roostika, I., S. Hartono, & D. Efendi. 2016. Kombinasi Termoterapi dan Khemoterapi dengan Kultur Apeks dan Meristem untuk Eliminasi Virus Mosaik pada Tebu. *Jurnal Litri* 1: 19–28.
- Rouhier, P., M. Kopp, V. Begot, M. Bruneteau, & B. Fritig. 1995. Structural Features of Fungal Beta-d-glucans for the Efficient Inhibition of the Initiation of Virus Infection on *Nicotiana tabacum*. *Phytochemistry* 1: 57–62.
- Semangun, H. 2000. *Penyakit-Penyakit Tanaman Perkebunan di Indonesia*. Gadjah Mada University Press, Yogyakarta. 835 p.
- Sivanandhan, S., A. Khusro, M. G. Paulraj, S. Ignacimuthu, & N.A. al-Dhabi. 2017. Biocontrol Properties of Basidiomycetes: An Overview. *Journal of Fungi* 1: 1–14.
- Soković, M.D., J.M. Glamočlija, & A.D. Ćirić. 2013. Natural Products from Plants and Fungi as Fungicides, p. 185–232. In M. Nita (ed.), *Fungicides: Showcases of Integrated Plant Disease Management from Around the World*, IntechOpen Ltd., London, UK.
- Somowiyarjo, S. 1999. Potensi Dua Jenis Jamur Patogen Tumbuhan, *Ganoderma* spp. dan *Phytophthora* spp., Sebagai Penghasil Senyawa Bioaktif Penghambat Sel Tumor. *Mediagama* 1: 47–51.

- Tampubolon, S.D.B.M., B. Utomo, & Yunasfi. 2013. *Keanekaragaman Jamur Makroskopis di Hutan Pendidikan Universitas Sumatera Utara, Desa Tongkoh Kabupaten Karo Sumatera Utara*. <http://download.portalgaruda.org/article.php?article=110106&val=4112>, modified 11/2/2017.
- Zhang, Z.C., C.Y. Lei, L.F. Zhang, X.X. Yang, R. Chen, & D.S. Zhang. 2008. The Complete Nucleotide Sequence of a Novel *Tobamovirus*, *Rehmannia mosaic virus*. *Archives of Virology* 153: 595–599.
- Zhu, Q., T.H. Bang, K. Ohnuki, T. Sawai, K. Sawai, & K. Shimizu. 2015. Inhibition of Neuraminidase by Ganoderma Triterpenoids and Implications for Neuraminidase Inhibitor Design. *Scientific Report* 5: 1–9.