Management effort of mottle virus by leaf extract of moringa (Moringa oleifera Lam.) and bougainvillea (Bougainvillea spectabilis Willd.) on Peanut

Fahma Zakiya Zulfi¹, Tri Asmira Damayanti^{1*}, Giyanto¹

¹Departemen Proteksi Tanaman, Fakultas Pertanian, Institut Pertanian Bogor, Jl. Kamper, Kampus IPB Dramaga, Bogor, 16680, Indonesia *Corresponding author. Email: triadys@apps.ipb.ac.id

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ABSTRACT

Peanut mottle virus is one of peanut production constrain worldwide. It is transmitted by aphid vectors and by seeds. One of the efforts to manage viruses is by using plant extracts. This study aims to test the potency of moringa and bougainvillea leaf extract to suppress mottle virus infection in peanuts. The test consisted of four treatments: healthy control, untreated control infected by the virus, moringa, and bougainvillea leaf extracts treatment infected by the virus. The disease assessment and agronomic parameters were observed weekly. The results showed that the spray application of moringa and bougainvillea leaf extract showed lower disease incidence, significantly able to prolong the incubation period, decreased disease severity, and as well as virus titer significantly in comparison to the control. The area under the disease progress curve among leaf extract treatments was significantly different but was significantly lower in comparison to untreated control infected by the virus. The germination percentage, vigor index, and plant height were not significantly different among treated plants and control, except for the leaf numbers. In addition, the growth vigor of treated plants was better than the control. These indicate that both plant leaf extracts have antiviral activity and the ability to improve plant growth.

Keywords: antiviral; bougainvillea; groundnut; moringa; mottle virus.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is on of essential cash crop in Indonesia. The peanut production on 2022 showed tend to decrease approximately 258.968-ton dry pod in compare to production on 2016 (Ditjen Tanaman Pangan 2023). Some factors affect to fluctuation of peanut production such as decreasing of cultivation area, low seed quality, poor drainage as well as plant pest and diseases interference. One of pathogens which is difficult to manage effectively is plant viruses (Saleh and Baliadi 2015).

Peanut mottle virus (PeMoV), Bean common mosaic virus strain Black eye (BCMV-BlC) and BCMV strain Peanut stripe (Pst) is associated as the mottle disease on peanut. Their cause economically yield loss globally wherever peanut was grown. These viruses is member of Potyvirus genus. The particle of Potyvirus are flexuous filaments with length size 740-750 nm and diameter 11-15 nm (King et al. 2012). The mottle disease firstly in Indonesia on 1970. It caused yield loss about 29.6%-70% in Georgia and 40% di India (Hema et al. 2014; Reddy dan Thirumala-Devi 2003). One of management effort to manage mottle virus is using sodium nitroprusside (SNP) and salicylic acid (SA) treatments. Leaf spraying of SNP and SA prior virus inoculation able to induce plant systemic resistance against PeMoV (Kobeasy et al. 2011). Utilizing of plant extractd in plant protection still few in compared to its utilization for humans' health. However, some plant virus effectively able tu suppressed by leaf extract containing antiviral substances (Al-Ani et al. 2011;

Madhusudhan *et al.* 2011). Previously, mechanical transmission of BCMV strain *Black eye cowpea* (BCMV-BlC) able to decreased severity and virus titer significantly by using leaf extract of bougainvillea (*Bougainvillea spectabilis*), four o'clocks (*Mirabilis jalapa*), and *Celosia cristata* (Damayanti dan Panjaitan 2014). Further, those of plant extracts able to manage *Squash mosaic virus* (SqMV) infecting ridged gourd effectively (Putri and Damayanti 2020). However, those plant extracts unable to suppresed the *Cowpea mild mottle virus* (CPMMV) titer on soybean, but interestingtly those of leaf extracts able to increases soybean tolerance even infected by virus and able to maintain the yield as well as healthy plants (Nurhidayah 2019). The ability of plant extract to manage virus disease may depends on virus species and it host.

Moringa leaf (*Moringa oleifera*) is a famous as medicinal herbal for human health, animal and fisheries until present. Moringa A extracted from its seed demonstrated contain virucidal against Influenza virus A (IVA) (Xiong *et al.* 2021). It possesses antiviral activities, antibacterial and antimicrobes due to of its phytochemical subctances such as saponins, triterpenoids, and tannins (Biswas *et al.* 2020; Arifan *et al.* 2021). Moringa leaf also play a role as ecofriendly biopesticide in integrated pest management biological strategy (Goss 2018). However, the effectiveness of moringa leaf extract in controlling plant virus still needs to study. Its potency to manage plant pathogens is still challenging and few study, due to its rich phytochemical substances. Therefore it is necessary to study the potential of



Agrovígor: Jurnal Agroekoteknologi http://journal.trunojoyo.ac.id/agrovigor ISSN: 1979-5777 (*Print*), 2477-0353 (*Online*) moringa and paperflower (*Bougainvillea*) leaf extract to manage one of essensial virus infecting peanut in Indonesia. The expectation of the research output is to obtain the alternative eco-friendly management strategy for mottle virus by using crude leaf extract of those of plants. The research aimed to test the effectiveness of moringa and paperflower leaf extract to control mottle virus infecting peanut.

MATERIAL AND METHODS

Virus Propagation

Peanut mottle virus (PeMoV) isolate is a collection of Laboratory of Plant Virology IPB University. The virus was propagated on healthy peanut plants by mechanical inoculation. Infected leaves was grinded in phosphate buffer pH 7.0 containing 1% β -Merchaptoetanol with rasio leaf and buffer 1:10 (w/v) using a sterile mortal and pestile. The healthy peanut plants (10 days after sowing) were added by wounding agent carborumdum 600 mesh prior mechanical inoculation. The leaf juice (sap) containing virus was spread to leaves and then rinsed by steril water to remove the carborundum residues. After inoculation, plants were maintained in green house until symptoms present as virus inoculum.

Preparation of Plant Extracts and Treatments

Moringa and paperflower leaf extracts were made by grinding the freah leaves in phosphate buffer pH 7.2 with ratio of leaf and buffer 1:10 (w/v). The treatments consist of (1)untreated healthy control, (2) untreated control infected by mottle virus, (3) plants treated by moringa leaf extract, and (4) plants treated by bougainvillea leaf extract. Before planting, peanut seeds var. Kancil were soaked in sterile water for control and in leaf extracts for 1 hr. Each treated seed was planted in a polybag pot containing mixture of soil, hull of rice and manure with ratio 1:1:1 (w/w/w). Each pot consists of three seeds. After plant growth, one plant each pot with the best growth perform was selected as test plant, while the remains plants were discarded. A day before challenge inoculation (9 days after planted), the test plants were sprayed with leaf extracts, while control plants were sprayed by sterile water. The next day, the test plants were mechanically inoculated by mottle virus, except for healthy control. The mechanical inoculation was carried out as previously described above. The leaf extract treatment was repeated at 2 and 4 weeks after inoculation.

Observation Variables

The disease assessment parameters were observed weekly including incubation periods, type of symptoms, disease incidence and severity, Area under Disease Progress Curve (AUDPC) and virus accumulation which is detected serologically by double antibody sandwich ELISA (DAS-ELISA). Incubation period was determined since virus inoculation to first symptom appears. The disease incidence (DI) was measured at 4 weeks post inoculation by using formula;

$$\text{DI}+:\left(\frac{n}{N}\right)x\ 100\%$$

n, is a number of infected plants, and N is a total number of test plants observed.

Disease severity was determined by using severity scoring adopted from mild mottle virus (Andayanie *et al.* 2019; Kasim 2023):

Score 0 = No symptom

Score 1 = 10% mild mottle on leaf surface

Score 2 = 50% mottle on leaf surface, turn yellow

Score 3 = 75-100% mottle on leaf surface, turn yellow, leaf malformed

Score 4 = mottle, turn yellow, leaf malformed, mosaic Score 5 = mottle, turn yellow, leaf malformed, mosaic and stunting

Area under disease progress curve (AUDPC) measured based on weekly development of severity scoring using formula described by Cooke (2006):

AUDPC - DS =
$$\sum_{i=1}^{n} \left(\frac{X_i + X_{(i+1)}}{2} \right) (t_{(i+1)} - t_i)$$

AUDPC = Area Under Disease Progress Curve; Xi = severity score at observation week-i; X(i+1) = severity score at observation week -(i+1); ti = day after planted at observation time-i; t(i+1) = day after planted at observation time-(i+1).

The agronomic variables such as germination, vigour index, plant's height and leaf numbers were observed as supporting data.

Detection of virus serologically

To detect the effect of treatments on virus titer, the test plants were collected at 4 WPI. The virus detected by Direct Antibody Sandwich ELISA (DAS-ELISA) method using antiserum specific for *Peanut mottle virus* (PeMoV). The ELISA was conducted following protocol provided by antiserum producer (DSMZ, Germany). The results were measured quantitatively by using ELISA *reader* (Multiscan, Thermoscientific) at wavelength 405 nm. The positive reaction is determined based on at least twice of negative control (healthy) ELISA absorbance value.

Data Analysis

The experiment is arranged by using Completely Randomized Design which is consist of four treatments with ten plants each treatment as repetition. The obtained data was tabulated by using Microsoft Excel 2019 program, while analysis of variance (ANOVA) was processed by using Mini Tab 2021 program, and further test was analyzed by Tukey test at confidence level α =5%.

RESULTS AND DISCUSSION

Disease Assessment Parameters

The tested plants treated by leaf extracts showed significantly able to prolong incubation period compared to untreated control. The longest incubation period showed by bougainvillea leaf extract; significantly longer than test plants treated by moringa leaf extract and untreated control. It is indicating that leaf extract treatments able to delay the symptoms appearance. The disease incidence of leaf extract treated plants also lower than untreated control;

50

bougainvillea leaf extract treatment showed lowest incidence than other treatments (Table 1).

The severity score of leaf extract treated plants showed significantly lower than untreated control plants. However, the severity score of treated and untreated plants were higher than healthy control plants. Generally, the type of symptoms of leaf extract treated plants were milder mottle to moderate than severe mottle symptoms on untreated control plants (Table 2, Fig 1).

The area under disease curve (AUDPC) based on disease severity scores in parallel with severity score. The AUDPC of leaf extract treated plants significantly lower than untreatead control plants. The lowest AUDPC showed by bougainvillea leaf extract treatment (Table 2).

Treatment ^a	Incubation Period (DPI) ¹	Incidence (%)
Bougainvillea leaf extract	$32.1\pm6.7a^2$	50
Moringa Leaf Extract	$18.7 \pm 4.8b$	90
Untreated control	$12.0 \pm 2.0 \mathrm{c}$	100
Healthy control	-	-

¹-day post inoculation

² number followed by same letter indicates not significantly different based on Tukey test at level α =5%

Table 2 Severity, AUDPC, and Type of symptom					
Treatment	Severity score ^a	AUDPC (Unit) ^b	Symptom		
Bougainvillea leaf extract	$1.0 \pm 0.8b$	$17.1 \pm 2.6c$	Mild mottle to moderate		
Moringa Leaf Extract	$1.1 \pm 0.7b$	$22.7\pm1.8b$	Moderate mottle		
Untreated control	$3.0 \pm 0.0a$	$27.7 \pm 1.4a$	Severe mottle		
Healthy control	$0.0 \pm 0.0c$	$0.0\pm0.0d$	-		

^a number followed by same letter means not significantly different based on Tukey test at level α =5% Area under disease progress curve

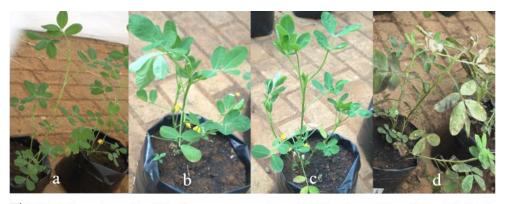


Figure 1 Symptom of mottle disease on peanut . a. Healthy, b. mild mottle, c. Moderate mottle, d. severe mottle

Treatment	ELISA Absorbance Value ^a	notes ^b	RIL (%) ^c
Control (-) ELISA	0.186 ± 0.004	Negative	-
Control (+) ELISA	0.378 ± 0.238	Positive	-
Bougainvillea leaf extract	$0.131 \pm 0.034b$	Negative	82.4
Moringa leaf extract	$0.171 \pm 0.064b$	Negative	77.0
Untreated control	$0.743 \pm 0.278a$	Positive	0.0
Healthy control	$0.195\pm0.025b$	Negative	-

^aNumber followed by same letter means not significantly different based on Tukey test at level α =5% ^bPositive reaction if EAV \geq 0.372

^cRelative inhibition level: EAV untreated control-EAV treatment / EAV untreated control x 100%

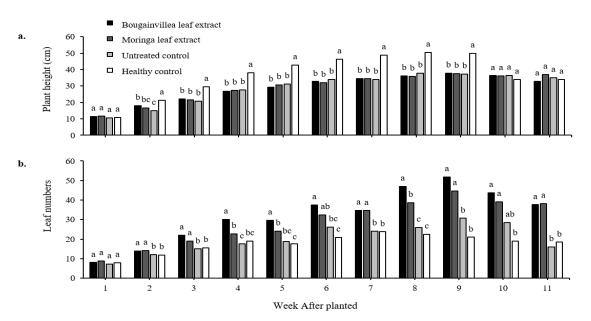


Figure 2. Average of plant height (1) and leaf numbers of test plants (b) at 1-11 weeks post inoculation. The same letter above bar chart indicates not significantly different at α =5%.



Figure 3. Representative of peanut plant growth. A. Healthy control, b. Bougainvillea leaf extract treated plant, c. Moringa leaf extract treated plant, and d. Untreated control. b to d plants infected by mottle virus.

Based on ELISA test showed that virus titer correlates to severity score. The ELISA absorbance value of bougainvillea and moringa leaf extract treatments significantly lower as well as healthy control in compared to untreated control. It is indicating that both leaf extracts have antiviral substances which capable to reduce the virus titer tremendously to detected negatively. The relative inhibition level of virus of bougainvillea and moringa leaf extracts is 82.4% and 77.0%, respectively (Table 3).

Agronomic Parameter

Generally, based on agronomic observation, leaf extract treatment did not significantly affect to germination period, percentage of germination and vigor index of all test plants in compared to untreated control. However, the vigor of leaf extract treated seedling were better growth than untreated control (data not shown). It is indicated that leaf extracts have no adverse effect on peanut seedling. Further, the extract treatments did not significantly affect to plant height in compared to untreated control healthy plants. However, the leaf leaf extract treatment affects significantly to more leaf numbers of test plants even infected by virus in compared to untreated control virus infected and healthy plants (Fig.2a-b). Moreover, peanut plants treated by either moringa or bougainvillea leaf extracts showed better growth vigour and delaying of leaf senescence (Fig. 3).

Based on disease assessment parameters indicates that leaf extract treatments increase plants' tolerances against mottle virus infection. It is showed by lower disease incidence and significantly lower disease severity, AUDPC and virus titer compared to untreated control. Application of leaf extracts before challenged virus inoculation might able to induced plants' systemic resistance. Induced resistance using vary external treatments not cause plant immune or not infected by pathogens at all, but the treatment only increased plants' resistance level and inhibit development of the disease (Suganda *et al.* 2002). It is necessary to determine the antioxidant enzymes which involve in inducing plants' systemic resistance by leaf extract treatments in the future. Interestingly, moringa leaf extract showed ability to reduce virus titer as well as bougainvillea leaf extracts. It is indicating that moringa leaf extract has antiviral activity, as previously described by Biswas *et al.* (2021). Moringa phytochemical possesses antiviral activities, antibacterial and antimicrobes due to of its phytochemical substances such as saponins, triterpenoids, and tannins (Biswas *et al.* 2020).

Moringa leaf extract is well known contains several protein and enzymes which play a role as plant growth hormones to increase the growth of tomato (Culver et al. 2012) and increased yield of tomato and spinach (Hoque et al. 2021). Furthermore, moringa leaf is well known able to promote seed germination, and plant growth. It is containing a wide range of vitamins, essential minerals, amino acid, and phytohormone such as auxins, cytokinins and which may play a role as biostimulant that can be used to improve the growth and productivity various crops (Mashamaite et al. 2022). The phytohormone contain of bougainvillea leaf extract is still unknown, however the growth of plants treated by bougainvillea leaf extract showed better vigour and growth. It is indicating it might possesses phytohormone-like substances which capable to increase plants' growth. The more plant growth well assumed will increase the plants' resistance to combat mottle virus infection. The phytochemical of bougainvillea genus is well known contains flavonoid, phenol, tanins etc which is play a role as antimicrobes and antiviral (Vargas and Petricevich 2018). Bougainvillea spectabilis has proteins belong to a family of ribosome inactivating proteins (RIP). RIP effectively inhibited virus infection such as tospoviruses, tobacco mosaic virus, cowpea aphid borne mosaic virus and cucumber mosaic virus (Balasaraswathi et al.1998; Sadasivam et al.1991), and in this study.

These results supported the previous studies related to bougainvillea leaf extract capability in controlling mechanical infection of BCMV and SqMV (Putri dan Damayanti 2020; Damayanti dan Panjaitan 2014), as well as controlling naturally insect-transmitted viruses on soybean and aphidborne viruses on groundnut in the fields (Cahyati *et. al.* 2023; Kasim 2023). This study extended the effectiveness of bougainvillea leaf extract to control mottle virus. In addition, this the first report of moringa leaf extract capability to control plant virus, especially in Indonesia.

CONCLUSION

Moringa and bougainvillea leaf extracts is effectively able to control mechanical infection of *Peanut mottle virus*. The leaf extract treatments significantly reduced the disease severity, virus titer and AUDPC in compared to untreated control. Both leaf extracts were potentially to utilize as ecofriendly biocontrol agents of plant virus and as biostimulant to increase plants' growth.

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AUTHORS CONTRIBUTIONS

FZZ conducted the experiments in the green house and writing the draft article. TAD and GYT contributed to the research idea, and helping to interprete and analysed the data as well as manuscript correction. All the authors have read and approved the final manuscript.

CONFLICT OF INTEREST

"The authors declare no conflict of interest."

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