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Evaluation on the Application of *Pseudommonas fluorescens* and Chitosan Against Soybean Mosaic Virus

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ABSTRACT. *Soybean mosaic virus* (SMV) causes a decrease in soybean [*Glycine max*(*Leguminosae*) Merril] production. Activity of the *Pseudomonas fluorescens* (*Pseudomonadaceae*) and chitosan can increase soybean plant resistance to SMV and stimulate plant growth. Therefore, The study aimed to evaluate the effect of increasing salicylic acid and total phenol contents on the biological control activity of *P. fluorescens* and chitosan against SMV. This study used two soybean varieties (Wilis and Anjasmoro) and threelines (GK/Mlg 3288-7-11, W/PI 200.485-7-14, and GK/PI200.485-7-2). Treatment using seed encapsulation with *P. fluorescens* combined with chitosan on the Wilis variety was the highestpercentage of the total phenolic and salicylic acid content. In addition, Applying *P. fluorescens* with chitosan suppresses disease incidence on the Wilis variety and GK/Mlg 3288-7-11 line. Moreover, the ELISA absorbance value was low (0.182−0.224) and reacted negatively with no symptoms. Therefore, applying *P. fluorescens* combined with chitosan on the GK/Mlg 3288-7-11 line could be developed as the candidate variety for SMV control in the endemic soybean plant area.

Keywords: Disease incidence, ELISA, induce systemic resistance, secondary metabolites, soybean.

INTRODUCTION

Soybean is an important source of vegetable protein. The *Soybean mosaic virus* (SMV) often attacks soybean plants, causing a decrease in soybean production. The virus symptoms showed mild mosaic, cupped down, stunting, and curling. The disease incidence will increase as the vector increases if there is a source of inoculum. Control using resistant varieties is an environmentally friendly way. However, only a few wide varieties are resistant to SMV. Moreover, applying pesticides was ineffective in controlling aphid vectors, *Aphis glycines*(*Aphididae*) Mats. (Andayanie et al., 2017; Andayanie, & Ermawati, 2019). Another approach is to use organic products with inoculation of biocontrol agents or resistance inducers. For example, in recent years, plant growth-promoting rhizobacteria (PGPR), chitosan, and plant extract as induced systemic resistance have the potential to control *Tomato leaf curl virus, Squash mosaic virus*, and SMV, respectively (Andayanie et al., 2019[a]; Andayanie et al., 2019[b]).

Phenolic compounds and salicylic acid can form a biochemical defense response of plants against pathogens. Phenolic compounds are used for pigmentation and reproduction. The combination of chitosan and rhizobacteria decreases the disease severity of the *Squash mosaic virus* on cucumber plants (Mishra et al., 2014; Firmansyah et al., 2017). *Pseudomonas fluorescens* has the nature of PGPR. Several *P. fluorescens* produce 2,4- Diacetyl Phloroglucinol (DAPG) antibiotics and siderophores. In addition, *P. fluorescens* can colonize plant roots and have induced systemic resistance (ISR). Activated defense genes encode chitinase, phenylalanine ammonium lyase (PAL), and other enzymes. The ISR mechanism results in changes in plant physiology, which stimulates the formation of chemical compounds such as jasmonic acid (AJ), ethylene compounds (ET), and hydrogen cyanide (HCN) to defend against pathogen attack (Bakker et al., 2007). *Pseudomonas fluorescens* is one of the antagonist bacteria that produce secondary metabolites, i.e., a phenolic compound. They were stimulated to defend themselves against pathogens. Application of *P. fluorescens* P60 increases the content of phenolic compounds (saponin, and tannin) in plant tissues. Moreover, accumulating phenolic compounds can increase PAL and synthesize chitinase. It functions for plant resistance to wounds and pathogen attacks (Ohri, & Pannu, 2010; Kapoor, & Handa, 2018; He et al., 2021). *Pseudomonas fluorescens* can also produce salicylic acid as one of the signal transduction to activate plant resistance genes through ISR mechanisms (Haggag, & El Soud, 2012). Salicylic acid and peroxidase enzyme activity increased in plants after PGPR inoculation and virus-infected. The function of peroxidase as antioxidant endogenous convert H2O2 to H2O and O2 that play a major role in secondary cell wall biosynthesis and resistance to viral infection. The application of *P. fluorescens*

indirectly affects ISR by accumulating salicylic acid and pathogenesis related-protein. The accumulation of PR-protein played a role in plant defense mechanisms (Andayanie et al., 2021). The genus Pseudomonas mediated induction of systemic resistance elicitation by the Pyoverdines synthesis mediated competition. As a result, there was to suppress pathogen activity by colonizing the roots to produce salicylic acid and phytoalexin (Chen et al., 2000; Ran et al., 2005).

The secondary metabolites of *Piper auritum* (*Piperaceae*) extracts produce chitosan. In addition, chitosan can also make from all parts of the shrimp shells. Chitosan in low doses can improve plant resistance to pathogens through the immune system in plants and increase seed germination. The immune system in plants cannot directly inactivate viruses, but has obvious inhibitory effects on virus replication and long-distance movement in plants. There was a stimulating concentration of total phenol and salicylic acid to defend against pathogen attack. Chitosan can induce systemic resistance against fungi and viruses (El Hadrami et al., 2010; Faisal et al.,2018; Fernández et al., 2021). However, plant species, molecular structure, and molecular weight of chitosan affect antiviral activity. A smaller molecular weight will make it easier to enter host cells to interfere with cellular factors and/or viral proteins that help virus replication. They interfere with viral polymerase activity (in RNA viruses) and prevent the development of new virions. The application of chitosan at a concentration of 0.9 % effectively suppresses the *Bean common mosaic virus* and reduces the population of the *Aphis craccivora* (*Aphididae*) Koch vector on yardlongbeans [*Vigna unguiculata* (*Fabaceae*)] (Kulikov et al., 2006; Megasari et al., 2014). Furthermore, the application of chitosan increases salicylic acid accumulation in grapevine plants. There was an increased salicylic acid accumulation during systemic induction reactions to anthracnose (Prakongkha et al., 2013). Chitosan also induces resistance to the *Tobacco mosaic virus* in Arabidopsis by activating the salicylic acid signaling pathway (Jia et al., 2016). Salicylic acid can respond quickly to pathogen attacks in plant tissues. When plants are infected by pathogens, salicylic acid biosynthesis increases, and the salicylic acid transduction pathway is activated, thereby increasing resistance in plants.

In the present study, we evaluated the effect of increasing salicylic acid and total phenol contents on the biological control activity of *P. fluorescens* and chitosan against the SMV. We also evaluated the disease incidence and detection of SMV.

EXPERIMENTAL SECTION

Materials

This study used two soybean varieties (Wilis and Anjasmoro) and three lines (GK/Mlg 3288-7-11, W/PI 200.485-7-14, and GK/PI200.485-7-2) of F7. Three soybean lines were susceptible to SMV isolate T and collected by Agricultural Faculty of Merdeka Madiun University, Indonesia. The Wilis and Anjasmoro varieties were resistant to SMV isolate T and collected by The Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD).

Microorganism

The SMV-T isolate inoculum was preserved from Laboratory and propagated on soybeanplant in a whitefly-proof screen house, at the Merdeka Madiun University. *P. fluorescen* isolate was obtained by taking rhizosphere from soybean and collected in plant disease laboratory, Faculty of Agriculture, Merdeka Madiun University.

Soybean mosaic virus Inoculation

Inoculum from infected leaves (1 g/50 mL buffer solution) and carborundum 400 mesh 0.5 % (0.5 g/100 mL) were sprayed with compressed air technique (1.9 to1.5 kg/cm^2). Spraying was carried out with a spray time of 1 sec/plant at a distance of 15 to 20 cm from the leaf surface the seven days after planting (DAP).

Soybean Seeds Encapsulation

The encapsulation material used a mixture of compost (50 g), inorganic zeolite carrier (25 g), and clay (25 g) as carrier material and filtered using a 15 µm. Next, the material was added glucose (1 g) and then sterilized using an autoclave to avoid contamination. Finally, soybean seeds and encapsulation material are put into the encapsulator.

Chitosan and *Pseudomonas fluorescens* Solution for soybean Seeds Encapsulation

Inocula of *P. fluorescens* are sprayed in soybean seeds encapsulation with a concentration of 1x10^{10} mL^{-1} colony forming units (CFU). The commercial chitosan from shrimp shells was purchased from Sigma-Aldrich (St. Louis, MI, USA). The chitosan solution was done by Firmansyah et al. (2017) with slight modification: 1. The chitosan solution was dissolved in 0.1 M acetic acid with a concentration of 2 % (by volume) to make a stock chitosan solution; 2. Soybean seeds encapsulation with a concentration of 0.9 % to make chitosan and *Pseudomonas fluorescens* solution. There were sprayed using a hand sprayer while the encapsulator was running. These are so that chitosan and *P. fluorescens* (*Pseudomonadaceae*) are attached to the seeds. Finally, the seed encapsulation is carried out until the coating solutions cover the surface. The seeds are then air-dried for 1 h.

Quantification of Phytochemicals

Estimation of total Phenolic content

A slightly modified method of Emelike et al. (2017) was employed to measure the leaf sample's total phenol content. In brief, the extract of leaf sample (1 mg) was dissolved in methanol solution (1 mL). Methanol solution can dissolve polar and non-polar compounds so it is very good at extracting secondary metabolite compounds contained in the samples used.

Then 250 µL extract solution was mixed with 2.5 mLof 10 % Folin-Ciocalteu's reagent by manual shaking for 30 s. The solution mixture added as much as 2.0mL of 7 % Na_2CO_3. The reaction mixture was vortexed for 10 min until homogenous and incubated in the dark at room temperature for 60 min (Andayanie et al., 2019). The absorbance was measured against a blank at 750 nm with a UV visible spectrophotometer (Merck Spectroquant pharo 300). Blank consists of all reagents except the extract. A standard solution of gallic acid was prepared (0.053125 µg mL^{-1}). The concentration of total phenolics was calculated as mg of gallic acid equivalents per gram (mg GAE. g^{-1}) of extract weight. Three times replicates to the estimation of total phenolic compound.

Percentage increase in total phenolic content at was calculated 15 days after planting (DAP) and 30 DAP by the following formula:

$$\text{Percentage increase in total phenolic content} = \frac{a-b}{b} \times 100\%$$

Where,

a = total phenolic content at 30 DAP

b = total phenolic content at 15 DAP

Estimation of total Salicylic acid content

Estimation of total salicylic acid ($C_7H_6O_3$) in the leaves using the method of Téllez et al. (2014) with a slight modification (Andayanie et al., 2019). The stock solution of salicylic acid was prepared at a concentration of 0.1 mg/mL and used to make a standard solution of 0.001% salicylic acid. The leaf sample (0.50 g) was ground with chloroform, then 100 mL of distilled water was added and centrifuged for 15 min. The absorbance was measured against a blank at 278 nm, and the flow rate was 1.0 mL/min with high performance liquid chromatography (HPLC) to measure the content of salicylic acid. Three times replicates to the estimation of salicylic content. Percentage increase in total salicylic acid content at wascalculated 15 days after planting (DAP) and 30 DAP by the following formula:

$$\text{Percentage increase in total salicylic acid content} = \frac{c-d}{b} \times 100\%$$

Where,

c = total salicylic acid content at 30 DAP

d = total salicylic acid content at 15 DAP

Detection of *Soybean mosaic virus*

The Indirect ELISA was conducted following method described by Koenig (1981) with a slight modification. Leaf samples were ground in an extraction buffer (0.05 M sodium carbonate buffer pH 9.6) until pulverized. The extract was filtered to obtain sap as antigen with ratio of (w/v= 1:10). SMV polyclonal antibodies were diluted 1000 times from an initial titer of 1000 to 10000. Each sample was placed in each well of a polystyrene microtiter plate (Nunc-immuno-Plate, InterMed), then the following materials were added: 1) antigen (10 μL) incubated for four h at 37 °C; 2), BSA (Bovine serum albumin) 0.05 % (150 μL) incubate one h at 37 °C; 3) polyclonal antibody SMV(100 μL) incubated for 18 h at 40 °C; 4) general conjugate (100 μL) of a 1:3,000 dilution, followed by incubation for two h at 37 °C; 5) the substrate in the form of 1 mg/ml p-nitrophenyl phosphate in 10 % diethanolamine pH 9.8 (200μL was incubated for 1 to1.5 h at room temperature. At the end of each stage, washing is carried out three times each 3 min using 0.02 M PBS, which contains 0.05 % Tween 20 (PBS-T). The ELISA results were analyzed quantitatively with an ELISA reader (Allsheng Model Flex A-200, Zhejiang, China) at 405 nm. The sample was declared positive if the ELISA absorbance value (NAE) of the test sample was two times greater than the NAE of the ELISA negative control (healthy plants).

Disease Incidence

The Indirect ELISA was conducted disease incidence showed the presence or absence of disease since 15 DAP and 30 DAP. Percentage of infected leaves on soybean plant was calculate by using the formula:

$$\text{Disease incidence (DI)} = \frac{a}{N} x\ 100\ \%$$

Where,

DI = Disease incidence (%)

a = The number of positive plants detected by the virus

N = Total number of plants assessed

Statistical Analysis

The experiment was arranged using a Randomized Complete Block Design (RCBD) with three replicates. The treatment are (varieties/lines, chitosan, *P. fluorescens*) and arranged factorially. Each treatment consisted of thirty plants with ten plant samples. All data were subjected toanalysis of variance (ANOVA). Statistical analyses were accomplished with SPSS program version 16.0 (*Statistical Package for Social Sciences,* USA) and separated by Duncan Multiple Range Test (DMRT) at $p \leq 0.05$ confidence level.

RESULTS AND DISCUSSION

The use of *P. fluorescens*, chitosan, and *P. fluorescens*, in combination with chitosan showed an increase in total phenol content in two soybean varieties (Anjasmoro, Wilis) and three lines (GK/Mlg 3288-7-11, W/PI 200.485-7-14, and GK/PI200.485-7-2) from 15 DAP to 30 DAP. In addition, there were higher increases in total phenolic content than for the untreated controls. Applying seed encapsulation *P. fluorescens* combined with chitosan on the Wilis variety (A2B4) was the highest percentage of the increase in total phenolic content. In this study, we showed that A2B4 was insignificantly different ($P < 0.05$) with *P. fluorescens* combined with chitosan on the GK/Mlg 3288-7-11 line (A3B4). The application of *P. fluorescens* on the Wilis variety showed significantly different results compared to the combination *P. fluorescens* with chitosan (**Table 1**).

Chitosan can induce systemic resistance against fungi and viruses (El Hadrami et al., 2010; Faisal et al.,2018; Fernández et al., 2021). However, plant species, molecular structure, and molecular weight of chitosan affect antiviral activity. Increasing the content of phenolic compounds can increase nitrogen availability in soybean plants and stimulate the activity of microorganisms in the soil. Moreover, the phenolic compound will be mineralized nitrogen immobilized in the cell structure of soil microorganisms to increase soybean plant growth (Li et al., 2008). Therefore, if nitrogen availability in low conditions, the content of phenolic compounds is also standard for decreasing nodulation and nitrogen fixation. In the present study, the difference in phenolic compounds in each treatment is suspected that each plant has a different ability to produce phenolic compounds. Results show that using *P. fluorescens* (A5B2) is a better potential for increased total phenolic content than the application combined with chitosan (A5B4) on the GK/PI200 line. On the other hand, using *P. fluorescens* without chitosan showed a lower bacterial population. Furthermore, *P. fluorescens* was able to increase plant phenol content. The accumulation of phenolic compounds in plants can stimulate PAL activity and synthesize chitinase to improve plant resistance to wounds and pathogen attacks (Ohri and Pannu, 2010; Kapoor & Handa, 2018; He et al., 2021; Andayanie et al., 2022; Shahzad et al., 2022).

Further information showed that salicylic acid activity increased in all treatments from 15 DAP to 30 DAP. Our results show that *P. fluorescens* application was a higher percentage of increasing salicylic content than the application of chitosan alone. However, using *P. fluorescens* combined with chitosan is the best potential to increase the total salicylic content percentage of the Wilis variety (A2B4). There was insignificantly different ($P < 0.05$) in salicylic acid content with the GK/Mlg 3288-7-11 line at 30 DAP (A3B4) (**Table 2**).

Applying chitosan increased of total salicylic acid content in the GK/Mlg 3288-7-11 (A3B3) line. There was showed no significance with applying *P. fluorescens* in combination with chitosan (A5B4 and A3B3). It was suspected that chitosan with a low molecular weight accumulation will influence the increase in salicylic acid and phenylalanine triggers. In contrast, studies carried out by Prakongkha et al. (2013) have shown that using chitosan alone causes an increase in the accumulation of salicylic acid and triggers phenylalanine ammonia-lyase (PAL) production. There was reduced phenolic production to produce systemic acquired resistance (SAR).Furthermore, Misra et al. (2014) have shown that chitosan can increase the *P. fluorescens*population. Moreover, the application

Table 1. The increase of total phenolic content at 15 DAP and 30 DAP in the leave of soybean plants

Treatments	Total phenol content (mg GAE g^{-1} of extract)		The increase of total phenolic content (%)
	15 DAP	30 DAP	
A1B1	0.086 e	0.132 d	34.843 e
A1B2	0.360 b	1.238 b	61.632 cd
A1B3	0.261 c	0.515 cd	49.320 de
A1B4	0.475 a	1.126 b	68.028 c
A2B1	0.091 de	0.144 d	36.806 e
A2B2	0.224 cd	0.547 cd	78.976 b
A2B3	0.115 d	0.718 c	63.649 cd
A2B4	0.378 b	1.976 a	84.919 a
A3B1	0.064 e	0.119 d	46.218 de
A3B2	0.492 a	1.743 a	71.773 bc
A3B3	0.263 c	0.566 cd	53.534 d
A3B4	0.280 c	1.940 c	82.685 a
A4B1	0.089 de	0.129 d	31.756 e
A4B2	0.112 d	0.142 d	35.972 e
A4B3	0.245 c	0.568 cd	56.049 d
A4B4	0.360 b	1.122 b	70.420 bc
A5B1	0.084 e	0.137 d	38.686 e
A5B2	0.356 b	1.225 b	70.939 bc
A5B3	0.250 c	0.580 cd	56.897 d
A5B4	0.292 c	0.843 c	65.362 cd
CV	3.97	4.12	

Means followed by the same letter in the same bars, are not significantly different ($P < 0.05$).
A1:Anjasmoro variety; A2: Wilis variety; A3: GK/Mlg 3288-7-11 line; A4: W/PI 200.485-7-14 line; A5: GK/PI200 line; Abbreviations: A1.485-7-2 line; B1: control; B2: *P. fluorescens* ; B3: chitosan; B4: *P. fluorescens* in combined with chitosan.

Table 2. The increse of total salicylic acid content at 15 DAP and 30 DAP in the leave of soybean plants

Treatments	Total phenol content (mg GAE g^{-1} of extract)		The increase of total salicylic content (%)
	15 DAP	30 DAP	
A1B1	0.323 d	0.565 d	42.832 e
A1B2	0.419 c	1.378 b	69.593 ab
A1B3	0.331 d	0.617 c	46.353 d
A1B4	0.608 a	1.321 b	53.974 c
A2B1	0.329 d	0.598 d	44.983 d
A2B2	0.418 c	0.933 b	55.198 bc
A2B3	0.366 cd	0.850 b	56.941 bc
A2B4	0.452 c	1.844 a	75.488 a
A3B1	0.315 d	0.626 c	49.681 cd
A3B2	0.545 b	1.625 ab	52.402 c
A3B3	0.426 c	0.895 b	66.461 b
A3B4	0.616 a	1.817 a	61.098 b
A4B1	0.268 e	0.572 d	44.019 de
A4B2	0.381 cd	0.749 c	46.523 d
A4B3	0.323 d	0.546 d	43.608 de
A4B4	0.317 d	0.982 ab	54.715 bc
A5B1	0.298 d	0.513 d	41.910 e
A5B2	0.372 cd	0.855 b	56.491 bc
A5B3	0.325 d	0.579 d	43.869 de
A5B4	0.421 c	1.204 b	65.033 b
CV	2.71	0.54	

Means followed by the same letter in the same bars, are not significantly different ($P < 0.05$).: A1: Anjasmoro variety; A2: Wilis variety; A3: GK/Mlg 3288-7-11 line; A4: W/PI 200.485-7-14 line; A5: GK/PI200 line; Abbreviations: A1.485-7-2 line; B1: control; B2: *P. fluorescens* ; B3: chitosan; B4: *P. fluorescens* in combined with chitosan.

Table 3. The average disease incidence and increase of total phenolic and salicylic contentbased on serological reaction

Treatments	The increase of total		Disease Incidence (%)	Incubation period (DAP)
	Phenolic	Salicylic acid		
A1B1	34.848 e	42.832 e	38.961	7.298
A1B2	61.632 cd	69.593 ab	16.392	9.650
A1B3	49.320 de	46.353 d	19.086	10.264
A1B4	68.028 c	53.974 c	14.538	12.812
A2B1	36.806 e	44.983 d	31.245	8.253
A2B2	78.976 b	55.198 bc	9.830	14.512
A2B3	63.649 cd	56.941 bc	14.875	10.495
A2B4	84.919 a	75.488 a	4.452	24.561
A3B1	46.218 de	49.681 cd	26.718	8.780
A3B2	71.773 bc	66.461 b	9.467	15.394
A3B3	53.534 d	52.402 c	17.875	10.276
A3B4	82.685 a	61.098 b	6.178	21.478
A4B1	31.756 e	44.019 de	39.263	7.245
A4B2	35.972 e	46.523 d	37.168	8.120
A4B3	56.049 d	43.608 de	16.891	10.295
A4B4	70.420 bc	54.715 bc	9.862	15.223
A5B1	38.686 e	41.910 e	36.430	7.526
A5B2	70.939 bc	56.491 bc	9.825	14.471
A5B3	56.897 d	43.869 d	16.309	10.298
A5B4	65.362 cd	65.073 b	15.750	13.642

Means followed by the same letter in the same bars, are not significantly different ($P < 0.05$): A1: Anjasmoro variety; A2: Wilis variety; A3: GK/Mlg 3288-7-11 line; A4: W/PI 200.485-7-14 line; A5: GK/PI200 line; Abbreviations: A1.485-7-2 line; B1: control; B2: *P. fluorescens* ; B3: chitosan; B4: *P. fluorescens* in combined with chitosan.

of chitosan at 120 mg L^{-1} increased the chili plant's totalphenolic, flavonoid, and salicylic acid in the chili plant (Amkha and Rungcharoenthong, 2021). Applying *P. fluorescens* in combination with chitosan decreased symptom development. The Wilis variety indicated the lowest disease incidence whereas control treatment have tended fast symptom development for incubation period (8.253 DAP). Decreasing the disease incidence can be stimulated by phenol and salicylic content, respectively.

Applying seed encapsulation of *P. fluorescens* more suppresses disease incidence on the GK/PI200 line (A5) than a combination of Pseudomonas with chitosan. There was effectively delayed symptom development and the potential for inducing resistance against SMV. Metabolic changes in plants lead to the induction of resistance in plants by increasing salicylic acid and phenolic compounds. Increased activity of phenolic and salicylic acid content was affected by SMV attack in the field. Increasing phenol and salicylic acid content indicate that soybean plants have controlled the disease incidence by systemic resistance induction. There were related to the incubation periods of SMV and absorbance value at 405 nm.The present study showed salicylic acid functions as a plant hormone and phenolic compound to regulate a significant signaling network under environmental stress and plant pathogens. Flavonoids are phenolic compounds and secondary metabolites. This compound has antioxidant properties to overcome damage caused by biotic and abiotic stress. Other studies showed that an increase in the total phenol content of 0.8 % could affect disease suppression up to 100 %, while the 1 % salicylic acid content has a 10.2 % chance of disease incidence in the chili plant (Tian et al., 2007; Widnyana et al., 2013).

Various symptoms of SMV showed blistered leaf surface, thickening of the leaf veins, leaf malformations, leaf margins curling upwards (cupping), and yellowing. We applied chitosan alone to the seed encapsulation, which did not effectively reduce the ELISA absorbance value. We found that chitosan, combined with *Pseudomonas* in the seed encapsulation effectively reduced the ELISA absorbance value under field conditions. The ELISA absorbance value reacted negatively with no symptoms in the A2B4 (*P. fluorescens* in combined with chitosan on the Wilis variety) and A3B4 (*P. fluorescens* combined with chitosan on GK/Mlg 3288-7-11 line) treatments. There was most inhibit SMV infection. Moreover, A2B2 (*P. fluorescens* on the Wilis variety) and A4B4 (*P. fluorescens* in combined with chitosan on W/PI 200.485-7-14 line) responded negatively, and the plant showed little chlorosis. Similarly, A3B2 (*P. fluorescens* on the Wilis variety) and A5B2 (*P. fluorescens* on GK/PI200 line) showed little chlorosis and mild mosaic with light green. However, there were negative reactions and no significant different ($P < 0.05$) in salicylic acid content (**Table 4**).

Table 4. Effect of increasing salicylic acid and total phenol contents on ELISA absorbancevalues

Treatments	The increase of total		ELISA absorbance values/Reaction	Type of symptoms
	Phenolic	Salicylic acid		
A1B1	34.848 e	42.832 e	1.819/+	R, SM, S
A1B2	61.632 cd	69.593 ab	0.715/+	MMD, BL, ML
A1B3	49.320 de	46.353 d	1.781/+	BL, CD, R,
A1B4	68.028 c	53.974 c	0.663/+	MML, MMD, BL
A2B1	36.806 e	44.983 d	2.723/+	CD, R, C, SM
A2B2	78.976 b	55.193 bc	0.197/−	NS, Cl
A2B3	63.649 cd	56.941 bc	0.715/+	MMD,BL, MdM
A2B4	84.919 a	75.488 a	0.182/−	NS
A3B1	46.218 de	49.681 cd	1.920/+	BL, CD,R, S
A3B2	71.773 bc	66.461 b	0.304/−	NS, MML
A3B3	53.534 d	52.402 c	1.346/+	ML, MMD, CD,
A3B4	82.685 a	66.098 b	0.213/−	NS
A4B1	31.756 e	44.019 de	1.839/+	C, SM,S
A4B2	35.972 e	46.523 d	1.576/+	MMD, C, R
A4B3	56.049 d	43.608 de	1.281/+	ML, C; SM
A4B4	70.420 bc	54.715 bc	0.224/−	NS, Cl
A5B1	38.686 e	41.910 e	1.365/+	BL, CD, SM
A5B2	70.939 bc	56.491 bc	0.298/−	NS, MML, MMD
A5B3	56.897 d	43.869 d	1.124/+	MdM,S
A5B4	65.362 cd	65.073 b	0.687/+	MMD,MdM, C

Means followed by the same letter in the same bars, are not significantly different ($p < 0.05$). Abbreviations: NS: no symptom; CL:chlorosis; MM; mild mosaic; MML: mild mosaic with light green; MMD: mild mosaic with dark green; BL: dark mosaic with blanching of the leaf bones; MdM: moderate mosaic; ML: malformation of leaves; C: curling; CD: cupped down; R: rugose; SM: severe mosaic; S: stunting. The test is declared positive if the EAV (ELISA absorbance values) of the sample is twice the EAV of the negative ELISA control (positive if EAV > 0.327).

The pattern of *Pseudomonas* combined with chitosan showed to inhibit SMV infection by inactivating SMV replication, multiplication, and cell to cell movement in the host. In addition, there were antiviral effects against SMV and triggers of induced systemic resistance (ISR) response in the soybean plant. ISR activates defense genes mediated by jasmonic acid, ethylene, and salicylic acid to protect against SMV. Different results were reported by Prakongkha et al. (2013), that developed chitosan to induce resistance in grapevine against anthracnose disease.

CONCLUSIONS

Chitosan can stimulate total phenol and salicylic acid content in *P. fluorecens* (*Pseudomonadaceae*). Applying *P. fluorescens* combined with chitosan can induce resistance against the SMV. In addition, chitosan, combined with Pseudomonas in the seed encapsulation, effectively reduces the ELISA absorbance value under SMV-infected field conditions. Applying *P. fluorescens* combined with chitosan on GK/Mlg 3288-7-11 line (A3) could be developed as the candidate variety for SMV control in the endemic soybean plant area with ELISA absorbance values < 0.327.

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