



Research Article

Potency of Yeasts to Promote Induced Resistance on Chili Plant (*Capsicum annuum* L.) against Yellow Leaf Curl Disease

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ABSTRACT

Yellow leaf curl is a major disease on chili plants. The use of antagonistic yeasts as the control agents is a good alternative in an environmentally friendly control method. This study was objected to evaluate the potencies of *Rhodotorula minuta* and *Candida tropicalis* to promote induced resistance on chili plants against yellow leaf curl disease. The experiment was arranged in the randomized complete block design with 9 treatments and 3 replications. The treatments were the application of the yeasts at three different times of virus inoculation, as follows: **A.** *R. minuta*, virus inoculation at 3 days after transplanting (dat), **B.** *R. minuta*, virus inoculation at 7 dat, **C.** *R. minuta*, virus inoculation at 10 dat, **D.** *C. tropicalis*, virus inoculation at 3 dat, **E.** *C. tropicalis*, virus inoculation at 7 dat, **F.** *C. tropicalis*, virus inoculation at 10 dat, **G.** control, virus inoculation at 3 dat, **H.** control, virus inoculation at 7 dat, and **I.** control, virus inoculation at 10 dat. No treatment was applied to the control. The yeasts were applied by soaking the chili seeds, and pouring the suspension into the growth media at transplanting. The variables observed were incubation period, disease severity, and disease incidence. Peroxidase activity, phenylalanine ammonia lyase activity, and salicylic acid accumulation were also analyzed. The results showed that the application of *R. minuta* or *C. tropicalis* was able to extend the disease incubation period, but did not reduce the yellow leaf curl disease incidence and severity. The treatment of *R. minuta*, virus inoculation at 7 dat, increased the peroxidase activity from 2590.80 units to 6870.93 units (0.5 minute) and from 577.367 units to 1131.300 units (2.5 minutes), PAL activity from 16.059 to 17.911 A290/mg, and accumulation of salicylic acid from 2.785 to 6.263 ppm. Application of *C. tropicalis*, virus inoculation at 7 dat, increased the peroxidase activity from 2590.80 units to 6033.067 units (0.5 minute) and from 577.367 units to 950.967 units (2.5 minutes), and accumulation of salicylic acid from 2.785 to 6.982 ppm.

Keywords: antagonist; *Candida tropicalis*; Gemini virus; induced resistance; *Rhodotorula minuta*

INTRODUCTION

Gemini virus, the cause of pepper yellow leaf curl virus disease (PYLCV), is a pathogen that cause the low production of chili. The disease was reported to spread in the center of chili production in Indonesia, and caused production loss up to 100% (Hidayat *et al.*, 2006; Adilah *et al.*, 2014).

PYLCV is disseminated by *Bemisia tabaci*, the vector. The rapid population growth of *Bemisia tabaci* makes it becomes a potential virus disseminator (Adilah *et al.*, 2014). The farmers usually control the vector by insecticide application.

Induced resistance on plants may be utilized as an alternative in controlling PYLCV. This method is effective and save to the environment. The induced resistance on plants can be activated by biotic as well as abiotic agents (Kloepper *et al.*, 1992). The plant response of induced resistance will be detected

after the pathogen infection (Olivera *et al.*, 2016), involves elicitors that initiate and increase the biosynthesis of certain compounds (Namdeo, 2007). The elicitors induce the production of secondary metabolites by activation of secondary path in response to stress and pathogen infection. Elicitor in plant defense mechanism is an effective strategy to increase production of secondary metabolites (Sharma *et al.*, 2009). Elicitor can further results in plant defense response, initiated by the formation of Reactive Oxygen Species (ROS) (Habibullah *et al.*, 2018).

Antagonistic microbes are reported to be able to initiate induced resistance in plants. Yeasts can also function as inducer of plant resistance. Some yeasts were reported to induce plant resistance, *Saccharomyces cerevisiae* was one of them. *S. cerevisiae* was reported to induce resistance against downy mildew on wheat by increasing the production

of phytoalexin (Walters *et al.*, 2005). El-Mougy *et al.* (2013) reported that *S. cerevisiae* at 10×10^{10} cfu/ml decreased the disease intensities of downy mildew and powdery mildew on tomatoes, pepper, and cucumber. The decrease was correlated with the increase of peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, β -1,3-glucanase and chitinase activities, both locally and systemically (El-Mougy *et al.*, 2013). Shan *et al.* (2014) reported that yeast XL-1 isolate increased the production of plant resistance enzymes such as polyphenol oxidase, SOD, and β -1, 3- glucanase on melon.

Rhodotorula sp. was also reported to systemically induce potato plant resistance to potato virus Y (PVY) (Al-Ani *et al.*, 2013). As there was no direct contact between PVY and *Rhodotorula* sp., the resistance manifested in the plant against the virus could be attributed to some form of induced systemic resistance. *Pichia membranefaciens* induced the peach resistance to *Penicillium expansum* (Chan *et al.*, 2007), *Metschnikowia fructicola* induced the citrus resistance to *Penicillium digitatum* (Hershkovitz *et al.*, 2012). *Debaryomyces hansenii* decreased the symptoms of *Ulocladium chartarum* infection, the cause of papaya rot, by more than 75% (Sharman *et al.*, 2011). Furthermore, *Candida saitoana* at 10^8 cells/ml controlled *Botrytis cinerea* on apples by 50–70% on day 7 after yeast application (El Ghaout *et al.*, 2003). The systemic resistance to *B. cinerea* was increased with the increase of chitinase and β -1,3-glucanase activity in systemically protected tissue (El Ghaout *et al.*, 2003).

Rhodotorula minuta (isolate of Dmg16BEP) and *Candida tropicalis* (isolate of Lm6BE) are yeasts isolated from chili fruit and they were proven to control anthracnose on chili by direct antagonism mechanism (Hartati, 2016). Those yeasts suppressed anthracnose by hyper parasitism mechanism, formation of volatile compounds and formation of ACC deaminase enzyme. *R. minuta* was also reported to initiate induced resistance on chili plants against anthracnose pathogen by increasing peroxidase enzyme activity, and *C. tropicalis* was able to increase the chili plant growth (Hartati *et al.*, 2019a; 2019b). The ability of those yeasts in inducing the plant resistance to the PYLCV is not known yet. This study was objected to evaluate the potencies of *R. minuta* (Dmg16BEP) and *C. tropicalis* (Lm6BE) as induced resistance agents on chili plants to yellow leaf curl virus.

MATERIALS AND METHODS

The experiment was carried out at the Laboratory of Plant Protection Biotechnology and Green House of the Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran. It was arranged in the randomized complete block design. The treatments were 2 yeast species of *R. minuta* (isolate of Dmg16BEP) and *C. tropicalis* (isolate of Lm6BE) and a control, and 3 different inoculation times at 3, 7, and 10 days after transplanting (dat), as follow: **A.** *R. minuta*, virus inoculation at 3 dat, **B.** *R. minuta*, virus inoculation at 7 dat, **C.** *R. minuta*, virus inoculation at 10 dat, **D.** *C. tropicalis*, virus inoculation at 3 dat, **E.** *C. tropicalis*, virus inoculation at 7 dat, **F.** *C. tropicalis*, virus inoculation at 10 dat, **G.** control, virus inoculation at 3 dat, **H.** control, virus inoculation at 7 dat, **I.** control, virus inoculation at 10 dat. The treatments were replicated 3 times. The number of plants per replication was 10. The yeast isolates were applied two times. First the chili seeds were soaked in the yeast suspensions before planting, second the suspensions were poured into the media at transplanting. The virus was inoculated according to the treatments, which were at 3, 7, or 10 dat. The data were analyzed by Analysis of Variance (ANOVA) using SPSS Version 24.0 for windows. If there were significant differences, the data were then analyzed by Duncan multiple range test at 5%.

Preparation of Yeast Cultures and Suspensions.

R. minuta and *C. tropicalis* were first isolated from chili fruit (Hartati, 2016). Two isolates were chosen for this study, i.e. *R. minuta* isolate of Dmg16BEP and *C. tropicalis* isolate of Lm6BE. The yeasts were sub cultured on PDA and incubated at room temperature. The suspensions were made by adding 10 ml sterile distilled water on the cultures in the Petri dish, and serially diluted to 10^{-8} . The yeasts population densities were counted by using hemocytometer, up to 10^8 cells/ml.

Rearing of B. tabaci and Preparation of Yellow Leaf Curl Virus Inoculum

The insect was reared on chili plant in iron frame cages covered with cloth, in the glasshouse. The virus was obtained from diseased chili plants cultivated in Jatinangor. To multiply the virus inoculum, nymphs of *B. tabaci*, the vector, were used to transmit the virus from the diseased plants onto healthy chili plants.

Preparation of Chili Plant Growth Media

The seed growth media was a mixture of soil and chicken manure 2:1. The chili seeds were planted in pot-tray 54 cm × 28 cm × 4.2 cm. The seedlings were transplanted on the 21st day onto polybags 18 × 16 cm. The transplanting media was the mixture of soil and chicken manure 15,000 kg/ha, and SP-36 150 kg/ha. Urea 200 kg/ha, ZA 450 kg/ha and KCl 150 kg/ha were applied 3 times on 3, 6 and 9 weeks after transplanting (wat).

Yeast Treatments

The yeast treatment was initiated with soaking the chili seeds in the suspensions of *R. minuta* and *C. tropicalis* at 10⁸ cells/ml for 60 minutes, and then the seeds were wrapped in sterile wet tissue paper and incubated for 12 hours. The treated seeds were planted in the pot-tray. On the 21st day, the seedlings were transplanted and the yeast suspensions of *R. minuta* and *C. tropicalis* were reapplied by pouring 10 ml of each yeast suspension per plant onto the media. After the treatments, the chili plants were inoculated with the virus by using the vector, *B. tabaci*, at 3, 7, and 10 dat.

Inoculation of Pepper Yellow Leaf Curl Virus

The PYLCV was inoculated by using *B. tabaci*. Nymphs of *B. tabaci* were placed and fed on diseased chili leaves, the acquisition was set for 24 hours. To inoculate the virus, those nymphs were placed and fed on healthy chili plants at 3, 7, and 10 dat, according to the treatments. The inoculation period was 24 hrs, 5 nymphs per plant.

Incubation Period, Disease Severity, and Disease Incidence

The effect of the yeasts application on virus inoculated chili plants was assessed by incubation period, disease severity, and disease incidence. Incubation period was measured since the day of PYLCV inoculation until the first day the disease symptom was visible. Disease severity and disease incidence was assessed on 14, 21, and 28 dat. The scoring of yellow leaf curl disease on chili was adapted from Trisno *et al.* (2010) (Table 1). Disease severity was calculated by the formula (Cooke, 2006):

$$\text{Disease severity} = \frac{\sum n \times y}{N \times Y} \times 100\%$$

Table 1. Scoring of yellow leaf curl disease on chili (Trisno *et al.*, 2010)

Score	Disease symptom of PYLCV	Area of diseased leaf (%)
0	No symptom	0
1	Yellowing on the leaf edge, started with the younger leaf	>1-20
2	Yellowing on all leaves and curly	>20-40
3	Yellow small leaves, curly and bend upward, and the plants still grow	>40-60
4	Stunted and yellow plants, curly younger leaves and bend upward, no further growth	>60

N = Total sample number

n = Number of sample with the same score

Y = The highest score

y = Examined score

Disease incidence was calculated by the formula (Cooke, 2006):

$$\text{Disease incidence} = \frac{\sum \text{diseased plants}}{\sum \text{total plant sample}} \times 100\%$$

Ability to Cause Induced Resistance

The ability to cause the induced resistance was calculated by using the formulae as follow (Trisno *et al.*, 2010):

a. Effectiveness in reducing disease incidence

$$\text{EiP} = 1 - \frac{\text{Dt}}{\text{Dc}} \times 100\%$$

EiP = Effectiveness in reducing the disease incidence

Dt = Disease incidence on plants treated with the yeasts

Dc = Disease incidence on control plants (control +)

b. Effectiveness in reducing disease severity

$$\text{EiS} = 1 - \frac{\text{Dts}}{\text{Dcs}} \times 100\%$$

EiS = Effectiveness in reducing the disease severity

Dts = Disease severity on plants treated with the yeasts

Dcs = Disease severity on control plants (control +)

Peroxidase, Phenylalanine ammonia lyase (PAL) and Salicylic acid (SA) Analysis

Analysis of peroxidase, PAL and SA were carried out in the Central Laboratory of Universitas Padjadjaran. Peroxidase activity was analyzed by the modified procedure of Simons & Ross (1970),

PAL activity was analyzed by the procedure of Lisker *et al.* (1983), and SA accumulation was analyzed by high performance liquid chromatography (HPLC) modified from the method of Tenhaken & Rubel (1997).

RESULTS AND DISCUSSION

Effect of Yeasts on Incubation Period

The time needed by plants to response to induction is different, and the response will be expressed after the challenge by pathogen infection. The time of the plant response can be estimated by inoculating the pathogen at different times. Induced resistance treatments by *R. minuta* at virus inoculation at 3, 7 and 10 dat were able to delay the incubation period compared to control, but *C. tropicalis* could delay the incubation period only on virus inoculation at 7 dat. *R. minuta* was able to delay the incubation period by 0.67–10 days, but *C. tropicalis* only delayed the incubation by 2 days (Table 2). Rusli *et al.* (1999) reported that the incubation period of yellow leaf curl virus, transmitted by vector *B. tabaci*, without induced resistance treatment was shorter (10-15 days). The induction treatments were able to delay the symptom development of the yellow leaf curl virus caused by Gemini virus. Gunaeni *et al.* (2015) reported that the incubation of yellow leaf curl virus was 21 days after inoculation on various induced resistance treatments.

The symptom of yellow leaf curl virus on chili plants was first detected on younger leaves or buds as yellow spot around the leaf vein. The spots developed, and extended into vein clearing, concave and curly leaf with slight mosaic or yellowing. The symptoms progressed and became yellowing on all

young leaves and buds, some with green mosaic, concave and curly leaves, smaller and thicker leaves. The disease symptoms on yeast treated chili plants were the same as the symptoms on control plants with no yeast treatment.

Effect of Yeasts on Disease Incidence and Severity

Application of *R. minuta* and *C. tropicalis* did not affect the disease incidence (Table 3). However, compared to control, although they were not significantly different, lower disease incidences on treatments were observed at 21 and 28 dat. At 28 dat, the decrease was on treatment with *C. tropicalis* inoculated at 3 and 10 dat, and treatment of *R. minuta* inoculated at 10 dat. The disease incidence on the treatment of *C. tropicalis*, virus inoculation at 3 dat, on the 21st day was 23,33%, and on the 28th was 33,33%. These were lower than control which were 33,33% on the 21st and 60% on the 28th day, although statistically they were not different. There was only disease incidence on treatment of *C. tropicalis*,

Table 2. Incubation period of the yellow leaf curl virus on chili plants with induced resistance treatments by *Rhodotorula minuta* and *Candida tropicalis*

Treatments	Incubation (days)
<i>R. minuta</i> , virus inoculation at 3 dat	17.67
<i>R. minuta</i> , virus inoculation at 7 dat	18.00
<i>R. minuta</i> , virus inoculation at 10 dat	19.00
<i>C. tropicalis</i> , virus inoculation at 3 dat	13.67
<i>C. tropicalis</i> , virus inoculation at 7 dat	15.00
<i>C. tropicalis</i> , virus inoculation at 10 dat	9.00
Control, virus inoculation at 3 dat	17.00
Control, virus inoculation at 7 dat	13.00
Control, virus inoculation at 10 dat	9.00

Note: dat = day after transplanting.

Table 3. Disease incidences of yellow leaf curl disease on chili plants treated with *Rhodotorula minuta* and *Candida tropicalis*

Treatments	Disease incidences (%) on 14 dat	Disease incidences (%) on 21 dat	Disease incidences (%) on 28 dat
<i>R. minuta</i> , virus inoculation at 3 dat	13.33 a	43.33 ab	53.33 abc
<i>R. minuta</i> , virus inoculation at 7 dat	16.67 a	43.33 ab	73.33 bc
<i>R. minuta</i> , virus inoculation at 10 dat	6.67 a	33.33 ab	33.33 a
<i>C. tropicalis</i> , virus inoculation at 3 dat	10.00 a	23.33 a	33.33 a
<i>C. tropicalis</i> , virus inoculation at 7 dat	10.00 a	46.67 ab	63.33 abc
<i>C. tropicalis</i> , virus inoculation at 10 dat	20.00 a	40.00 ab	40.00 ab
Control, virus inoculation at 3 dat	6.67 a	33.33 ab	60.00 abc
Control, virus inoculation at 7 dat	33.33 a	53.33 ab	80.00 c
Control, virus inoculation at 10 dat	16.67 a	63.33 b	63.33 abc

Note: dat = day after transplanting.

Table 4. Disease severities of yellow leaf curl disease on chili plants treated with *Rhodotorula minuta* and *Candida tropicalis*

Treatments	Disease severities(%) on 14 dat	Disease severities (%) on 21 dat	Disease severities (%) on 28 dat
<i>R. minuta</i> , virus inoculation at 3 dat	2.50 a	5.83 a	10.00 a
<i>R. minuta</i> , virus inoculation at 7 dat	2.50 a	11.67 abc	15.83 a
<i>R. minuta</i> , virus inoculation at 10 dat	5.00 a	10.00 abc	10.00 a
<i>C. tropicalis</i> , virus inoculation at 3 dat	5.00 a	10.83 abc	15.00 a
<i>C. tropicalis</i> , virus inoculation at 7 dat	4.17 a	7.50 ab	18.33 a
<i>C. tropicalis</i> , virus inoculation at 10 dat	1.67 a	8.33 abc	8.33 a
Control, virus inoculation at 3 dat	2.50 a	8.33 abc	14.17 a
Control, virus inoculation at 7 dat	8.33 a	14.17 bc	16.67 a
Control, virus inoculation at 10 dat	4.17 a	15.83 c	15.83 a

Note: dat = day after transplanting.

Table 5. Effectiveness of disease incidence suppression

Treatments	Effectiveness of disease incidence suppression at					
	14 dat		21 dat		28 dat	
	EiP (%)	X	EiP (%)	X	EiP (%)	X
<i>R. minuta</i> , virus inoculation at 3 dat	-100	13.33	-30	43.33	11	53.33
<i>R. minuta</i> , virus inoculation at 7 dat	50	16.67	19	43.33	8	73.33
<i>R. minuta</i> , virus inoculation at 10 dat	60	6.67	47	40.00	47	40.00
<i>C. tropicalis</i> , virus inoculation at 3 dat	-50	10.00	30	23.33	44	33.33
<i>C. tropicalis</i> , virus inoculation at 7 dat	70	10.00	13	46.67	21	63.33
<i>C. tropicalis</i> , virus inoculation at 10 dat	-20	20.00	37	33.33	37	33.33
Control, virus inoculation at 3 dat	-	6.67	-	33.33	-	60.00
Control, virus inoculation at 7 dat	-	33.33	-	53.33	-	80.00
Control, virus inoculation at 10 dat	-	16.67	-	63.33	-	63.33

Notes: dat = day after transplanting; EiP = $1 - (DT/DC) \times 100\%$; X = average.

virus inoculation at 3 dat, which was different to control, virus inoculation at 10 dat, on the 21st day (Table 3). The disease incidences on treatments of *R. minuta* and *C. tropicalis* with virus inoculation on the same dat were not different to control.

The effect of treatments on the yellow leaf curl disease severity was only detected at 21 dat. There were differences between treatments of *R. minuta* inoculated at 3 dat and *C. tropicalis* inoculated at 7 dat compared to control (Table 4). The lowest disease severity (5.83 %) was caused by treatment of *R. minuta* inoculated at 3 dat. The best response to the treatments was shown by those plants inoculated at 3 and 7 dat. When an inducer promotes plant resistant to disease, the plant will need some times to activate the resistance genes. The resistance expression was shown when pathogen started to infect (Ouchi, 1983). Different plants with different inducers need certain time to activate the resistance genes. However, in this study, the yellow leaf curl disease severities on the same virus inoculation times were not different

to control, both by treatment of *R. minuta* and *C. tropicalis*.

Effect of Yeasts on the Effectiveness of the Disease Incidence and Disease Severity Suppressions

Application of the yeasts did not affect the yellow leaf curl disease incidence at 14 days after virus inoculation, at all inoculation times. Based on the effectiveness value of disease incidence on chili plants, at 14 days after virus inoculation, there were three treatments that increased the chili plant resistance to Gemini virus, i.e. treatments of *R. minuta* virus inoculation at 7 dat, *R. minuta* virus inoculation at 10 dat, and *C. tropicalis* virus inoculation at 7 dat, with the effectiveness values of 50–70% (Table 5). However, at 21 to 28 dat, the suppressions were less than 50%, which meant the treatments were not effective (Table 5).

Based on the effectiveness value of disease severity on chili plants, treatments of *R. minuta*, virus inoculation at 7 dat and treatment of *C. tropicalis*,

virus inoculation at 7 and 10 dat suppressed the disease severity at 14 dat (Table 6). The highest suppression of disease severity was caused by the treatment of *R. minuta*, virus inoculation at 7 dat (70%). At 21 and 28 dat, the treatments caused less than 50% effectiveness of disease severity suppression. However, in this experiment, the disease incidences and disease severities on yeast treatments and control were not different.

Effect of Yeasts Treatments on Activities of Peroxidase and PAL, and Accumulation of Salicylic Acid

Results of the enzyme analysis showed that treatments of *R. minuta* and *C. tropicalis*, with virus inoculation at 7 dat, increased the peroxidase activity compared to control (Table 7). The peroxidase activity was increased at 0.5 and 2.5 minutes of incubations. Treatment with *R. minuta* resulted in higher peroxidase activity than treatment with *C. tropicalis*. However, the increase of peroxidase activity was not followed by the decrease of disease severity (Table 4). The increase of PAL activity was observed on the treatment with *R. minuta*. PAL activity was in accordance with the concentration of PAL. The higher the PAL concentration, the higher the PAL

activity. *C. tropicalis* did not increase the PAL activity (Table 7). This was in line with the effectiveness value of disease severity suppression and the extended incubation period on treatment of *R. minuta*.

In induced resistance, the enzyme activities in the path of certain metabolites are increased, and some enzymes such as chitinase, β -1,3-glucanase, peroxidase, pathogenesis related (PR) proteins are also increased (Park & Kloepper, 2000). Synthesis of these proteins was regulated at the mRNA (Park & Kloepper, 2000). *R. minuta* and *C. tropicalis* increased the activity of plant resistance enzymes on 7 dat. However, the increase of peroxidase and PAL activities in this experiment only delayed the disease incubation period for 0.67–10 days and 2 days on the treatments of *R. minuta* and *C. tropicalis* respectively (Table 2).

Some studies on the time period of the production of enzymes related to plant resistance showed that at 7 dat the enzymes activities were increased. Some yeasts such as *Candida saitoana*, *Cryptococcus laurentii*, and *S. cerevisiae* were reported to promote systemic induced resistance by increasing the activities of some enzymes related to plant resistance

Table 6. Effectiveness of disease severity suppression

Treatments	Effectiveness of disease severity suppression					
	14 dat		21 dat		28 dat	
	EiS (%)	X	EiS (%)	X	EiS (%)	X
<i>R. minuta</i> , virus inoculation at 3 dat	0	2.50	30	5.83	29	10.00
<i>R. minuta</i> , virus inoculation at 7 dat	70	2.50	18	11.67	5	15.83
<i>R. minuta</i> , virus inoculation at 10 dat	-20	5.00	37	10.00	37	10.00
<i>C. tropicalis</i> , virus inoculation at 3 dat	-100	5.00	-30	10.83	-6	15.00
<i>C. tropicalis</i> , virus inoculation at 7 dat	50	4.17	47	7.50	-10	18.33
<i>C. tropicalis</i> , virus inoculation at 10 dat	60	1.67	47	8.33	47	8.33
Control, virus inoculation at 3 dat	-	2.50	-	8.33	-	14.17
Control, virus inoculation at 7 dat	-	8.33	-	14.17	-	16.67
Control, virus inoculation at 10 dat	-	4.17	-	15.83	-	15.83

Notes: dat = day after transplanting; EiS = $1 - (Dts/Dcs) \times 100\%$; X = average.

Table 7. Peroxidase and PAL activities (virus inoculation at 7 dat)

Treatments	Enzyme activities			
	Peroxidase 0.5 minutes (unit)	Peroxidase 2.5 minutes (unit)	PAL concentration (ppm)	PAL activity (A290/mg)
<i>R. minuta</i> , inoculated at 7 dat	6870.933	1131.300	123.633	17.911
<i>C. tropicalis</i> , inoculated at 7 dat	6033.067	950.967	108.633	15.722
Control, inoculated at 7 dat	2590.800	577.367	110.933	16.059

Note: dat = day after transplanting.

Table 8. Salicylic acid accumulation on chili plants

Treatments	Concentration of SA (ppm)
<i>R. minuta</i> , inoculated at 7 dat	6.263
<i>C. tropicalis</i> , inoculated at 7 dat	6.982
Control, inoculated at 7 dat	2.785

Notes: dat = day after transplanting; SA = salicylic acid.

such as chitinolytic enzymes, β -1,3-glucanase, peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase at 7 dat (El Ghaouth *et al.*, 2003; Yao & Tian, 2005; El-Mougy *et al.*, 2013; Shan *et al.*, 2014).

Analysis of salicylic acid was performed by using HPLC, it revealed that the retention periods between treatments were almost the same with the salicylic acid standard (2.125 minutes). This showed that the plant samples contained salicylic acid. The accumulation of salicylic acid was increased on chili plants treated with *R. minuta* and *C. tropicalis* with virus inoculation at 7 dat. Induction resistance by *R. minuta* and *C. tropicalis* caused high accumulation of salicylic acid content compared to control (Table 8). This showed that the treatment of induced resistance by *R. minuta* and *C. tropicalis*, virus inoculation at 7 dat, increased the accumulation of salicylic acid. The increase of salicylic acid at 7 and 10 dat was also reported to suppress CMV infection (Taufik *et al.*, 2010). In this study, although salicylic acid accumulation was increased, but the disease severity was not decreased.

Salicylic acid is important in the plant defense mechanism against pathogen. It was reported in some studies that salicylic acid had the roles in increasing the plant resistance to virus infection (Taufik *et al.*, 2010; Faizah *et al.*, 2012; Gunaeni *et al.*, 2015). Accumulation of salicylic acid is a fast reaction of plant to defense against virus infection by mobilization of secondary metabolites (Faizah *et al.*, 2012). Indirectly, it inhibits virus movement in the plant vascular tissues, so the disease symptoms were delayed (Naylor *et al.*, 1998). The accumulation of salicylic acid in this study caused the delay on the incubation period of the yellow leaf curl virus disease. Salicylic acid has a role as signal transduction that activate resistance genes against various pathogens systemically, so that the incubation period is extended (Murphy *et al.*, 2001; Gautam & Stein, 2011; Narusaka *et al.*, 2015).

The increase of peroxidase and PAL activities, and salicylic acid accumulation showed the role of elicitor in signal formation and transduction. The elicitor is received by the receptor in plant. In this experiment, the yeast cells and their metabolites act as the elicitors. It is in accordance with Walters *et al.* (2005) that the active recognition process between yeast cells and plant tissues was caused by the yeast that actively secreted elicitor. Oligosaccharide fragments derived from the polysaccharide of yeast cell wall were proven to act as elicitors that actively promote induced resistance in the host plants (Talarczyk *et al.*, 2002; Walters *et al.*, 2005; Sharma *et al.*, 2009).

The increase of peroxidase activity from 2590.80 units to 6870.93 units (0.5 minute) and from 577.367 units to 1131.300 units (2.5 minutes), PAL activity from 16.059 to 17.911 A290/mg, and accumulation of salicylic acid from 2.785 to 6.263 ppm in this experiment did not reduce the disease incidence and severity. The increase of peroxidase and PAL activities, and accumulation of salicylic acid caused by the induction treatment of *R. minuta* and *C. tropicalis* only extend the incubation period. This condition was assumingly caused by the inadequate concentration of the enzymes and the salicylic acid accumulation to support the plant in combatting the virus infection, the yeasts were applied only by soaking the chili seeds and pouring the suspensions into the media on transplanting, and the severe virus infection compared to the ability of the yeasts in increasing the concentration of the enzymes and the salicylic acid accumulation. It was recommended to reapply the yeast suspension on chili plants at certain age, and the observation on the older chili plant.

CONCLUSIONS

The resistance induction on chili plant by *R. minuta* and *C. tropicalis* delayed the incubation period of yellow leaf curl virus disease for 0.67–10 days and 2 days respectively. The treatments of *R. minuta* and

C. tropicalis on chili plant did not decrease the disease incidence and severity of yellow leaf curl virus disease. However, the treatments of *R. minuta* and *C. tropicalis* on chili plant increased the peroxidase activity by 2.3–2.7 times higher than control, at 0.5 minute incubation (2590.80 units to 6870.933 units), and increased the salicylic acid accumulation 2.2–2.5 times higher than control (from 2.785 to 6.982 ppm). Resistance induction treatments of *R. minuta* increased the activity of PAL (the difference was 1.852 A290/mg, i.e. from 16.059 to 17.911 A290/mg).

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