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Research Article

Transmission Effectivity of Rice Yellow Stunt Disease by Imidacloprid-Resistant and Susceptible Brown Plant Hopper

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ABSTRACT

The brown plant hopper (BPH) is a major pest of rice and as a vector of *Rice ragged stunt virus* (RRSV) and *Rice grassy stunt virus* (RGSV). Curently, numerous rice yellow stunt disease symptoms are found in the field that caused by the single and simultaneous infection of these two viruses. Brown plant hopper population correlate with the incidence and severity of the disease. Misuse of insecticides, would cause of BPH resistances to imidacloprid. This study aimed to investigate the ability of BPH imidacloprid-resistant and susceptible to transmit of rice yellow stunt disease on rice plants. The variables tested were the acquisition period, inoculation period, number of infesting BPH, and lifespans of the viruliferous BPH that used in this research. Experiments were set as separated Completely Randomized Design with 10 replications for each treatment within an experiment. The results showed that both resistant and susceptible BPH to imidacloprid was able to transmit the virus to healthy plants. The acquisition and inoculation period test showed the BPH could transmit the virus with the shortest acquisition time for 30 minutes followed 24 hours of inoculation, as well as the acquisition time of 10 days with the shortest inoculation time for 30 minutes. Based on the incubation time, symptoms variation, and disease severity, susceptible BPH were more effective in transmitting rice yellow stunt disease than imidacloprid-resistant BPH. Single imidacloprid-resistant or susceptible BPH was proven able to transmit rice yellow stunt disease to healthy plants during its lifespan. Lifespans BPH viruliferous of imidacloprid-resistant were shorter than susceptible, which was 16 days for resistant BPH and 21 days for susceptible BPH.

Keywords: brown plant hopper; imidacloprid resistant; virus transmission; yellow stunt disease

INTRODUCTION

Brown plant hopper (*Nilaparvata lugens* Stall) (BPH) is a major pest in several rice production centers around Indonesia and is able to threat national self-sufficiency of rice (Untung & Trisyono 2009). This is a devastating pest due to its ability to cause *puso* (harvest failure). Besides being pest on rice, BPH are also able to transmit *rice grassy stunt virus* (RGSV) and *rice ragged stunt virus* (RRSV) (Cabautan *et al.,* 2009). Both viruses are persistently transmitted by BPH (Ling, 1977; Hibino, 1996). The occurrence of RRSV and RGSV in Indonesia has been reported since 1977. In 1977, RRSV was found with high incidence in Indonesia and Vietnam (Du *et al.*, 2007). Currently, RRSV has been reported in several rice areas in Java and Bali (Kusuma *et al.*, 2018). RGSV was first reported in Philippines at 1963 (Rivera *et al.,* 1966). This disease later spreaded to South Asia, China, Japan, and Taiwan (Hibino, 1996). In Indonesia during 1970–1977, high incidence of RGSV was occured (Hibino, 1989). In the last ten years, especially in Java and Bali, outbreaks of BPH have been followed by yellow stunt disease with various symptoms. The most found symptoms was yellow stunt with the main symptoms are for plants to be stunted, posses leaf malformation, and turning yellow (Dini *et al.*, 2015; Kusuma *et al.*, 2018; Helina *et al.*, 2018; 2019).

In Indonesia, continuous monoculture of rice is always found in areas where established irrigation system exists. An example of these areas is the District of Bantul, Yogyakarta. Based on observation in 2013, high incidence of yellow stunt symptoms were found in areas where BPH outbreaks and harvest failure occurred in the previous seasons. *Direktorat Perlindungan Tanaman Pangan* (2011) reported that the area of BPH attacks was almost double the area attacked in the previous year of 173,890 ha with damage of 22,613 ha.

This disease is caused by the infections of RGSV and RRSV (Helina *et al*., 2018; 2019). In these areas, farmers heavily rely on continuous use of the same active ingredient, one of these active ingredients was imidacloprid. This practice increases the risk of BPH populations to grow resistances to currently available insecticides. BPH are known to be resistant to 29 active ingredients around the world (Sparks & Nauen, 2015). China, India, Indonesia, and Thailand have reported BPH resistances against insecticides (Catindig *et al*., 2009). Cox (2001) reported that imidacloprid was first used in several areas around Indonesia at 1994. In Banyumas, population outbreaks of resistan BPH due to continuous use of imidacloprid have been reported (Londingkene *et al*., 2016).

Continuous use of imidacloprid can increase resistant ratio (RR) of the next generation and has already been reported in Indonesia. Increases have been reported in Karawang by 108.1 folds, 12.7 folds in Subang (Surahman *et al*., 2016), and 7.0 folds in Klaten (Baehaki, 2011). Resistance ratio against imidacloprid have been reported to increase to the next generation. Diptaningsari *et al.* (2019) reported RR increase of 5 generations from Banyumas by 46.2 folds to 150.39 by the fifth generation. The same results occurred in BPH from Karawang where RR increase by 13.5 folds in the 6th generation (Iswanto *et al*., 2019). Resistance inheritances is autosomal, epigenetic not completely dominant, and not stabil (Diptaningsari *et al*., 2019; 2020). Resistance monitoring is an essential effort to understand population susceptibility to several active ingredients as a management strategy to prevent insecticide resistances within pest populations (Zhang *et al*., 2016).

Resistance mechanisms of insects against insecticides involves overexpression or mutations of enzymes for detoxification genes and amino acid of targeted genes (Sparks & Nauen, 2015). Baehaki *et al.* (2016) reported that BPH from several areas in Java had different levels of resistance to the insecticides imidacloprid, etiprol, tiametoxam, fipronil, BPMC, MIPC, buprofezin, sipermethrin and sihalothrin. Based on previous findings, it is necessary to determine the differences of yellow stunt transmission between susceptible and imidacloprid-resistant BPH populations. This study aimed to investigate the ability BPH imidacloprid-resistant and susceptible to transmit yellow stunt disease on rice plants. The reseach was done on January–March 2018 at Laboratory and a green house at sub Laboratory of Plant Virology, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada

MATERIALS AND METHODS

BPH and Inoculum Source

Rice plants affected by double infections in Bantul were taken as inoculum sources. Double infection was showed with the symptom of stunted plant, turned yellow in the tip of the leaf, there was none of panicles, grow upright, ragged, gall and twisted tip leaf especially in the younger part (Helina *et al*., 2019). Experiments were conducted at the Virology Laboratory, Faculty of Agriculture, Universitas Gadjah Mada. Susceptible and imidacloprid-resistant BPH populations were obtained from the Laboratory of Pest Management, Toxicology Division, Faculty of Agriculture, Universitas Gadjah Mada.

Brown Plant Hopper Rearing

BPH taken from the field were reared on rice plants var. Ciherang aged 2 weeks in plastic containers with tops closed with mesh cloth after reaching 3rd instars. BPH were placed on inoculum sources for virus acquisition. Infectious BPH were then moved to healthy plant using aspirator. Inoculated plants were then transplanted to plastic trays containing growing media. Observations were done every 2 days.

Rice Yellow Stunt Disease Transmission Using Imidacloprid-Resistant and Susceptible Brown Planthoppers

For experiment the acquisition period treatments were conducted using imidacloprid-resistant and susceptible BPH to ingest the virus from diseased plants for 3, 6, 12, 24 hours, and 3, 5, 7, 10 days. The viruliferous BPH were than inoculated on healthy plants for 24 hours. For experiment the inoculation period treatment were conducted using BPH for ingest the virus for 10 days on diseased plants. Viruliferous BPH and then transfered to the healthy plants for 30 minutes, 60 minutes, 3, 6, 12 and 24 hours to inoculate the virus. For experiment the number of BPH were done after acquisition on diseased plant for 10 days, the viruliferous of BPH was transferred to healthy plants with the number of 1, 2, 3, and 5 respectively for each treatment and allowed to inoculate the healthy plant for 24 hours. The virus retention experiment used susceptible and imidacloprid-resistant BPH populations were let to feed on diseased plant for 10 days to acquisition of the virus. One viruliferous BPH then was transfered on to a healthy plant for 24 hours to inoculate the virus. After that, the same of BPH was moved to a new healthy plant and let to transmit the virus again for the next 24 hours. This was repeated until the BPH was died. Inoculated plants were transplanted to growing media on trays and incubated in a greenhouse. Experiments were set as separated Completely Randomized Design with 10 replications for each treatment within an experiment.

Observations

Incubation time, symptoms variations, incidence, and disease severity were observed in this experiment. Observations were done weekly for 4 weeks after treatments. Severity and incidences were calculated using the formula from Zadoks and Schein (1979), with slight modifications. Disease severity were scored for each plant (Table 1).
Based on the scores were processed to calculated disease severity using the following formula:

$$\text{Disease severity} = \frac{m \times v}{N \times Z} \times 100\ \%$$

m : number of infected plants
v : score of infected plants
N : number of plants observed
Z : highest score

Data Analysis

Data were analyzed using ANOVA in R. Treatments that showed significant differences based on their P-value were tested using DMRT post-hoc test at α=5%.

RESULTS AND DISCUSSION

Effect Acquisition Feeding Periode of Susceptible and Imidacloprid-Resistant BPH to Transmission of Rice Yellow Stunt Disease

Experiment of the acquisition period of resistant and susceptible BPH, showed the incubation period were not difference, but based on the incidence and intensity of disease, susceptible BPH was more efficient to transmit the virus (Table 2) Susceptible BPH could more ingest the virus on inoculum source than the resistant. In resistant BPH maybe occuring physiological change caused by imidacloprid compound.

Acquisition feeding periode of imidacloprid-resistant and susceptible BPH on infected plants for 3 hours and inoculation for 24 hours were able to cause symptoms (Table 2). The same data was reported by Morinaka *et al.* (1983), but another reseacher also reported the shorter acquisition time with 2 hours and 1 hours inoculation time could transmit the disease (Chen & Chiu, 1981). The longer of vector acquisition periods in plants, the virus concentration in the vector becomes higher

Table 1. Stunt symptom scoring for rice (Kusumaningrum, 2018)

Score	Symptoms
0	Healthy plants
1	Leaves were yellow or pale green
2	Leaves were yellow or pale green; small, ragged, twisted tips, and gall appear
3	Leaves were yellow or pale green, small, ragged, twisted tips, gall appear, excessive tillering
4	Leaves were yellow or pale green, small, ragged, twisted tips, gall appear, excessive tillering, and stunted

Table 2. Effect of acquisition period on yellow stunt symptoms after 4 weeks since innoculation

Resistant Status	Acquisition Period	Incubation time (days)	Symptom Variations
Imidacloprid-resistant	3 hours	12.25	Yellow and shrinked leaves
	6 hours	12	Yellow and shrinked leaves
	12 hours	11.7	Yellow and shrinked leaves
	24 hours	11.6	Leaves are yellow, shrinked, and ragged on their perimeters
	3 days	11.1	Leaves are yellow, shrinked, and ragged on their perimeters
	5 days	10.9	Leaves are yellow, shrinked, and ragged on their perimeters
	7 days	10.7	Leaves are yellow and shrinked, tillers are fanning, and young leaves are ragged
	10 days	10.2	Leaves are yellow and shrinked, tillers are fanning, gall appear at leaf bases, and young leaves are ragged
Susceptible	3 hours	12.12	Yellow and shrinked leaves
	6 hours	11.1	Yellow and shrinked leaves
	12 hours	11.1	Yellow and shrinked leaves
	24 hours	10.9	Leaves are yellow, shrinked, and ragged on their perimeters
	3 days	10.8	Leaves are yellow, shrinked, and ragged on their perimeters
	5 days	10.6	Leaves are yellow, shrinked, and ragged on their perimeters, fanning on tillers
	7 days	10.6	Leaves are yellow, shrinked, and ragged on their perimeters, fanning on tillers
	10 days	10	Leaves are yellow, shrinked, and ragged on their perimeters, fanning on tillers

Table 3. Effect of acquisition period on yellow stunt severity

Resistant Status	Disease Severity (%) at Acquisition Period								Average
	3 hours	6 hours	12 hours	24 hours	3 days	5 days	7 days	10 days	
Imidacloprid-resisant	1*	1.1	1.1	1.4	1.3	1.6	1.6	1.8	1.35b
Susceptible	1.4	1.4	1.6	1.6	1.4	1.8	1.9	1.9	1.62a
Average	1.2d	1.25d	1.35cd	1.45 bcd	1.35d	1.7 abc	1.75ab	1.85a	

*: Numbers followed by different letters in the same row or column were significantly different based on a DMRT post-hoct test at α=0.05

so faster to become viruliferus (Hibino *et al.*, 1977). Based on the data showed BPH were able to transmit yellow stunt virus to rice plant in a short time The length of acquisition was depend on related to preference of the BPH on its host Ciherang was reported as a preference host for BPH (Suprihanto *et al.,* 2016). As a preference host, the BPH could suck nutrients as well as the virus in the host directly without needed for orientation time, so the condition is very effective as a vector.

Shorter acquisition time will increase the speed of rice yellow stunt disease dispersion. BPH preferences also depend on the host condition. BPH are more attracted to infected plants than healthy ones due to the ability of infected plants to emit attractive volatiles (Wang *et al.*, 2018).

On various treatments, it appears that resistant BPH has a longer incubation period than susceptible ones. It was closely related to viral titer in its insect. Feeding ability on resistant BPH would be decrease because of their fitness. Iswanto *et al.* (2019) and Liu and Han (2006) reported that imidacloprid-resistant BPH fitness decrease drastically compared to susceptible BPH. High viral titer will affect the incubation period and the severity of the symptoms severity.

Length of acquisition period between BPH imidacloprid-resistant and susceptible would affect disease severity (Table 3). Longer acquisition time caused more severe symptoms and shortens the time for the symptoms to appear (Figure 1 and 2).

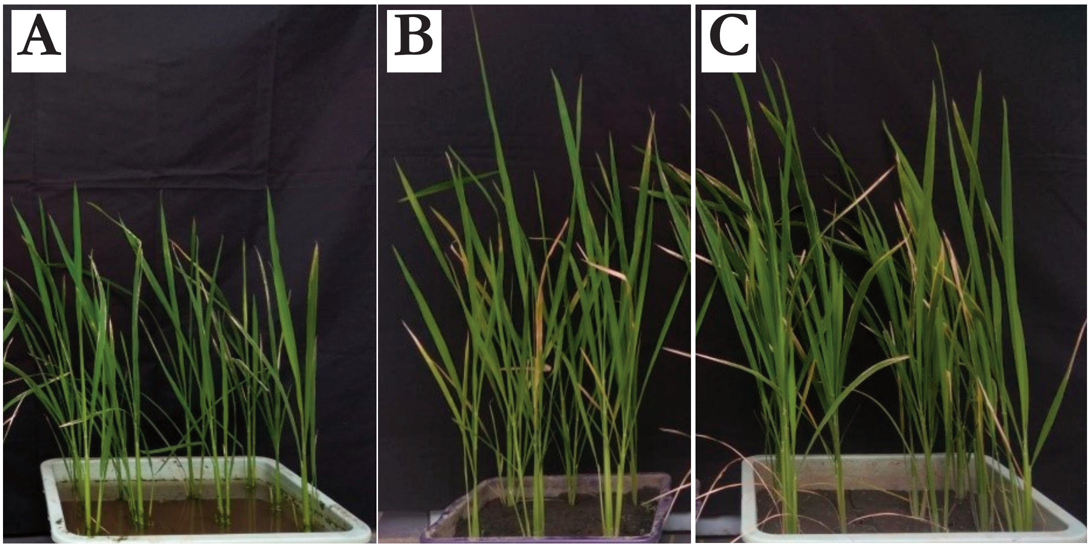

Figure 1. **Rice stunt yellow disease symptoms after 28 days after inoculation; (A). acquisition time of 10 days by imidacloprid-resistant insects; (B). acquisition time of 3 hours by imidacloprid-resistant insects; (C). control

** the height and vigour of plants showed difference

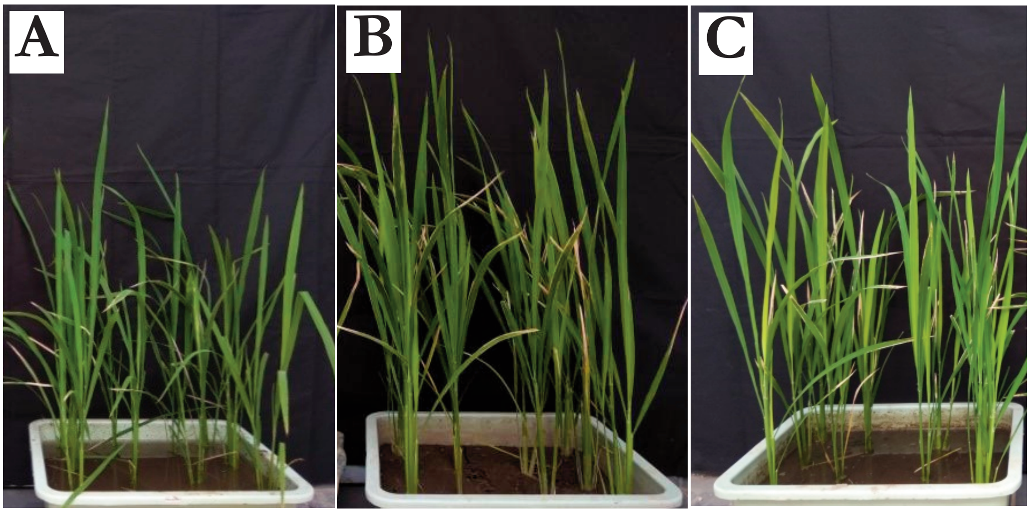

Figure 2.**Rice stunt yellow disease symptoms on 28 days after inoculation; (A). acquisition time of 10 days by susceptible insects; (B). acquisition time of 3 hours by susceptible insects; (C). control

** the height and vigour of plants showed difference

Acquisition time of 5 days can effectively transmit virus and was not significantly different from other treatments. Virus's titer inside plants affect incubation time and the severity of symptoms. This was shown in our results (Figure 3).

Effects of Inoculation Time of Susceptible and Imidacloprid-Resistant BPH on Transmission of Rice Yellow Stunt Disease Virus

Inoculation time of 30 minutes was already able to cause imidacloprid-resistant BPH to be able to transmit virus. Susceptible BPH were more effective in transmitting virus. Disease incidence and severity increased as inoculation periods became longer (Table 4.).

At 2 weeks after inoculation, susceptible BPH caused 60–100% disease incidence and 15–25% disease severity, while imidacloprid-resistant BPH caused disease incidence of 40–70 % and disease severity of 10–18%. Longer inoculation time increases the concentration of viruses transmitted into plants. Based on these results, both imidacloprid-resistant and susceptible BPH were potential vectors due to

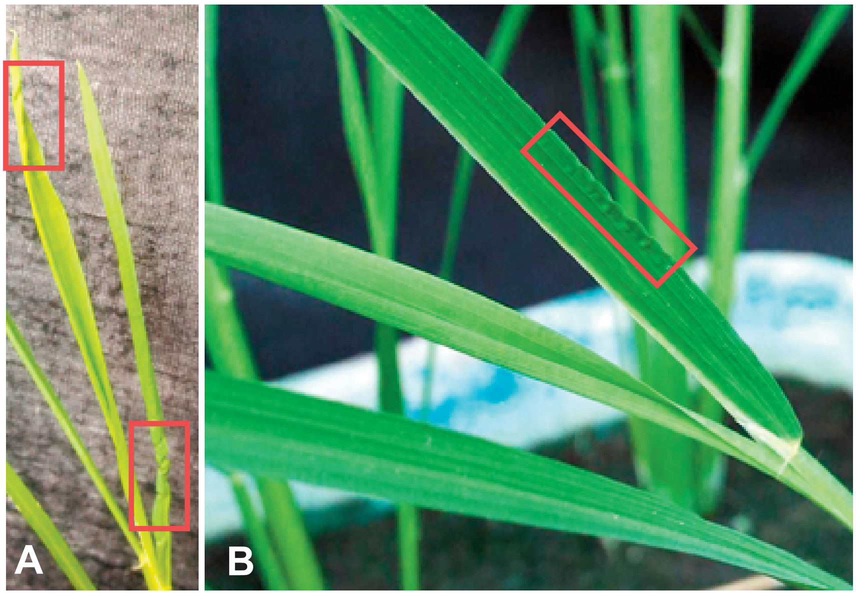

Figure 3. Variation of disease symptoms on 28 days after inoculation; (A). Twisted leaf tips and bases after BPH infestation; (B). Ragged leaf perimeters and galls

Table 4. Effect of inoculation period on the symptoms yellow stunt at 4 weeks after innocolation

Resistant Status	Inoculation Period	Incubation Period (days)	Symptoms Variation
Imidacloprid-resistant	30 minutes	12.87	Yellow and shrinked leaves
	60 minutes	11.62	Yellow and shrinked leaves
	3 hours	11.7	Yellow and shrinked leaves
	6 hours	11.6	Yellow and shrank leaves
	12 hours	10.7	Leaves are yellow and shrank, fanning tillers, and ragged leaves
	24 hours	10.5	Leaves are yellow and shrank, fanning tillers, and ragged leaves, gall appear at leaf bases
Susceptible	30 minutes	12.9	Leaves are yellow and shrank, fanning tillers, and ragged leaves
	60 minutes	12.2	Yellow and shrinked leaves
	3 hours	10.6	Leaves are yellow and shrank, fanning tillers, and ragged leaves
	6 hours	10.2	Leaves are yellow and shrank, fanning tillers, and ragged leaves
	12 hours	10.8	Leaves are yellow and shrank, fanning tillers, and ragged leaves
	24 hours	10.1	Leaves are yellow and shrank, fanning tillers, and ragged leaves

their ability to transmit virus after inoculation time for 30 minutes (Table 4). Shorter inoculation periods have been reported by Hashim and Ang (1984) where BPH were able to inoculate RRSV to healthy plants within 10 minutes. The data was important for the epidemiology studies of yellow stunt disease that caused by the mixed of RGSV and RRSV.

The longer the inoculation periods, the higher the disease severity (Table 5). Disease severity caused by susceptible BPH were significant higher compared to imidacloprid-resistan BPH. This may be due to the ability of susceptible BPH to ingest more nutrition and virus from inoculum sources. Iswanto *et al.* (2019) and Liu and Han (2006) reported that imidacloprid-resistant BPH fitness drecrease drastically compared to susceptible BPH. In contrast to BPH which is not susceptible, the time required for inoculation is longer. Helina *et al.* (2018) reported double infection of RGSV and RRSV that attacked Ciherang variety had incubation 10 days after inoculation (DAI) meanwhile for Situ Bagendit variety was 14 DAI. Disease severity increased as inoculation periods were longer (Figure 4 and 5).

Table 5. Effect of inoculation period on yellow stunt severity

Resistant Status	Disease Severity (%) at Inoculation Period						Average
	30 minutes	60 minutes	3 hours	6 hours	12 hours	24 hours	
Imidacloprid-resistant	1*	1	1.3	1.3	1.7	1.8	1.35b
Susceptible	1.8	1.8	1.9	1.7	1.8	1.9	1.8 a
Average	1.4c	1.4c	1.6abc	1.5bc	1.75ab	1.85a	

*: Numbers followed by different letters in the same row or column were significantly different based on a DMRT post-hoct test at α=0.05

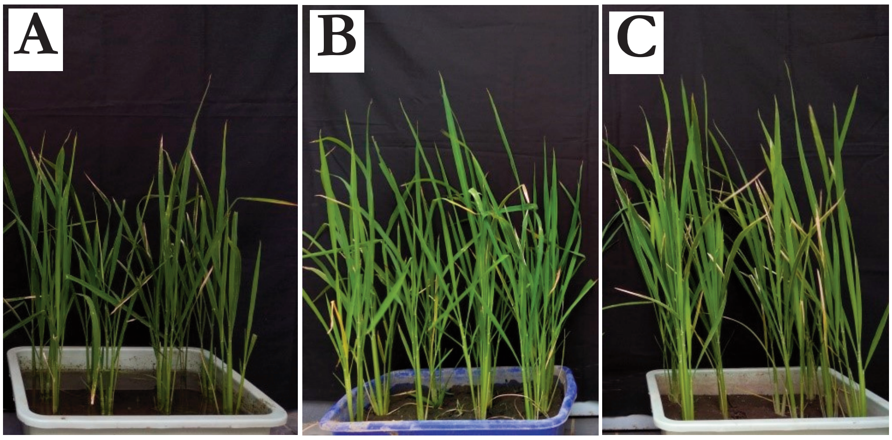

Figure 4 . **Rice stunt yellow symptoms on 28 days after inoculation; (A). Inoculation treatment for 24 hours of imidacloprid-resistant brown plant hoppers; (B). Inoculation treatment for 30 minutes of imidacloprid-resistant plant hoppers; (C). Control

** the height and vigour of plants showed difference

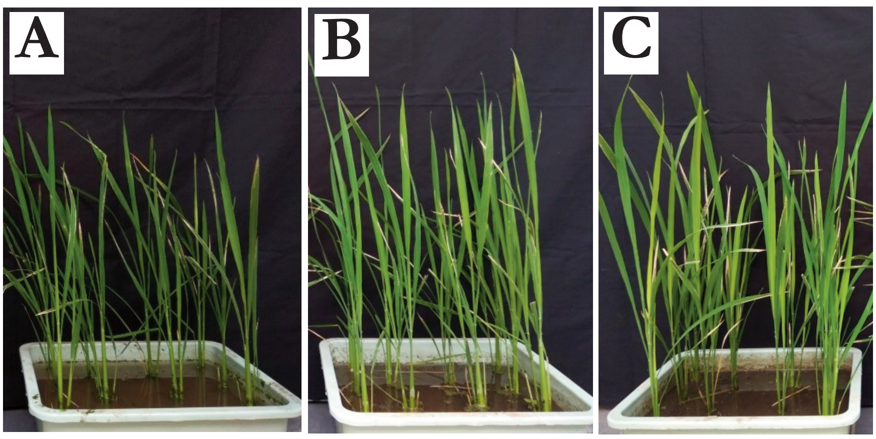

Figure 5. **Yellow stunt symptoms on 28 days after inoculation; (A). Inoculation treatment of 24 hours of susceptible brown plant hoppers; (B). Inoculation treatment for 30 minutes of imidacloprid-susceptible plant hoppers; (C). Control

** the height and vigour of plants showed difference

Effect of the Number of BPH Imidacloprid-Resistant and Susceptible to Rice Yellow Stunt Disease Transmission

Symptoms appeared sooner as the number of BPH increased (Table 6) due to the increase of viruses transmitted into plants. It is noteworthy, that even one BPH was enough to transmit this virus. This challenge the economic thresholds used when BPH are considered as pest and demonstrates the merit of different threshold when they are considered as vectors and inoculum sources exist in the field.

One susceptible BPH could be more effective in transmitting virus than imidacloprid-resistant BPH based on disease incidence and severity of the disease (Table 7). Although by single imidacloprid-resistant or susceptible BPH was enough to transmit virus, the increase of BPH numbers increased severity of symptoms on plants (Figure 6 and 7). Helina *et al.* (2018) also reported that 1–2 BPH were able to transmit RGSV or RRSV in field tests, but the incubation period for each plant was different depending on plant resistance and their virulences.

The number of BPH significanty affected the incidence and severity of the disease even though their resistance state had not effects. At high numbers, BPH may cause hopperburns and damage plants, meanwhile it only requires one BPH to transmit the virus. Therefore, monitoring of BPH and yellow stunt inoculums in the fields is essential.

Effect of Viruliferous BPH Imidacloprid-Resistant and Susceptible for its Lifespans

Imidacloprid-resistant BPH had shorter lifespans than susceptible BPH (Figure 8). Faster mortality occurred at imidacloprid-resistant BPH at day at 2–10 compared to susceptible of BPH that died at day 10–12. Observations on days 10–12, the number of resistant and susceptible BPHs that survived was still 60%, but on the following day, the number of resistant and sensitive BPH that died was more numerous and faster than those with sensitive ones. The number of resistant BPH that died reached 100% on the 16th day while sensitive BPH could survive up to 21 days. This may be due to physiological factors and cytological effects of BPH due to the effects of insecticides. In resistant BPH, there will be a decrease in fitness, survival, fecundity, number of eggs and their life span (Liu *et al.*, 2012; Iswanto *et al.,* 2019). As previously mentioned, fitness of resistant BPH are lower than susceptible ones. However, it needed to be aware that resistant BPH are still able to reproduce in the field and cause damage (Matsumura *et al.,* 2008).

Table 6. Effect of brown plant hopper (BPH) numbers to yellow stunt symptoms at 4 weeks

Resistant Status	Number of BPH	Incubation Period (days)	Symptoms Variation
Imidacloprid-resistant	1	9.8	Leaves are yellow and shrank, fanning tillers, and ragged leaves
	2	9.9	Leaves are yellow and shrank, fanning tillers, and ragged leaves
	3	7.3	Leaves are yellow and shrank, fanning tillers, and ragged leaves
	5	6.1	Leaves are yellow and shrank, stunted plants
Susceptible	1	9.4	Leaves are yellow and shrank, fanning tillers, and ragged leaves
	2	9.4	Leaves are yellow and shrank, fanning tillers, and ragged leaves
	3	7.1	Leaves are yellow and shrank, fanning tillers, and ragged leaves
	5	6.3	Leaves are yellow and shrank, fanning tillers, and ragged leaves

Table 7. Effect of brown plant hopper (BPH) numbers on yellow stunt severity

Resistancy	BPH numbers				Average
	1	2	3	5	
Imidaclorpid-resistant	1.8*	2	2	3.2	2.25
Susceptible	1.9	2	2	3	2.225
Average	1.85b	2.00b	2.00b	3.10a	

*: Numbers followed by different letters in the same row or column were significantly different based on a DMRT post-hoct test at α=0.05

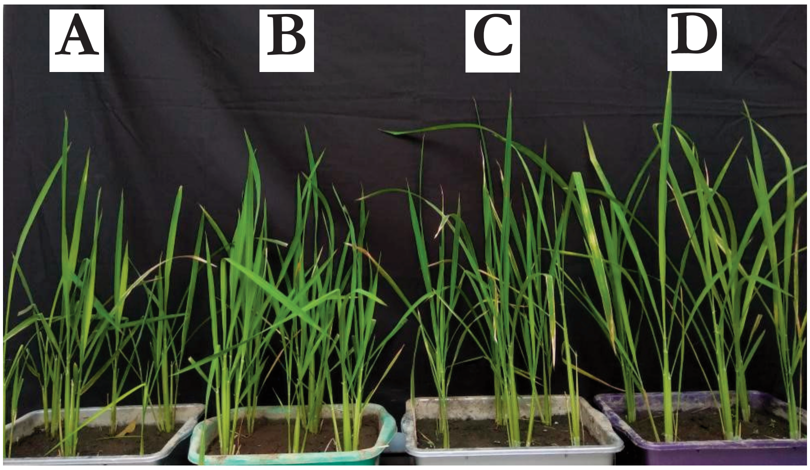

Figure 6. **Yellow stunt symptons on 28 days after inoculation; (A). treated with 5 imidacloprid-resistant individuals; (B). 3 imidacloprid-resistant individuals; (C). treated with 2 imidacloprid-resistant individuals; (D). treated with 1 imidacloprid-resistant individuals

** the height and vigour of plants showed difference

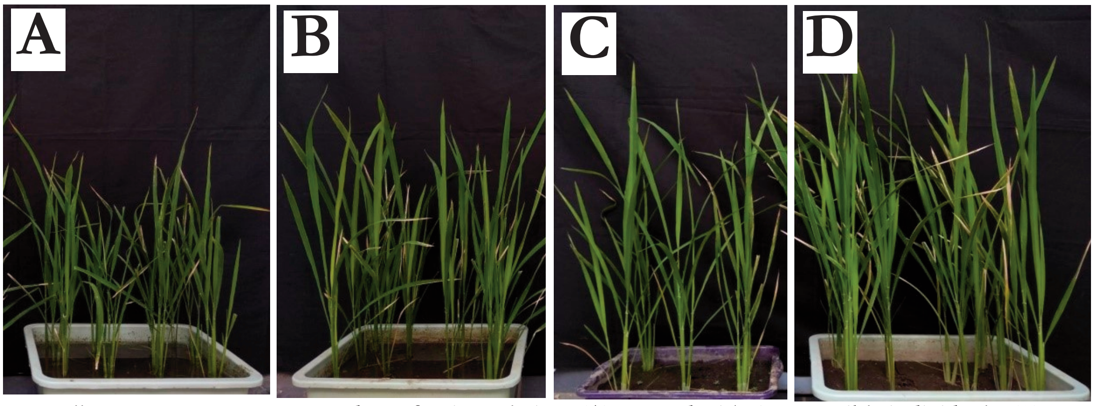

Figure 7. **Yellow stunt symptons on 28 days after inoculation; (A). treated with 5 susceptible individuals; (B). treated with 3 susceptible individuals; (C). treated with 2 imidacloprid-susceptible individuals; (D). treated with 1 imidacloprid-susceptible individuals

** the height and vigour of plants showed difference

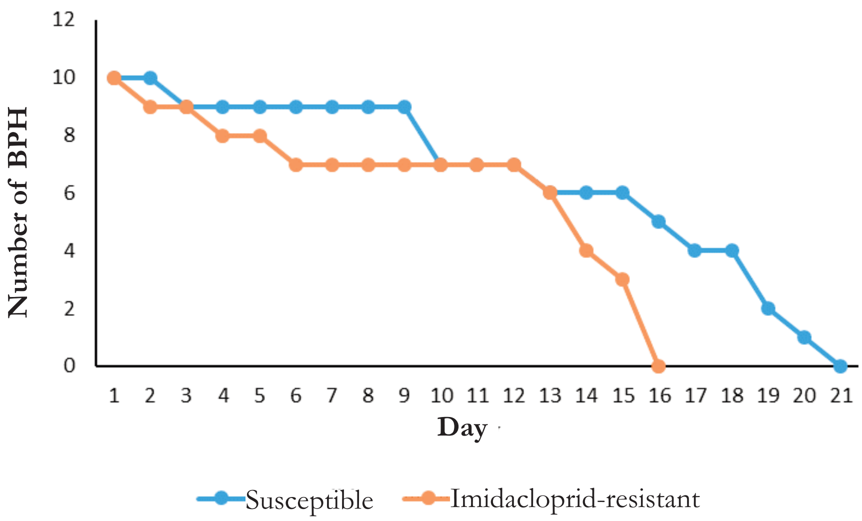

Figure 8. Lifespan of 3rd instar of imidacloprid susceptible and resistant brown plant hopper (BPH)

Density population of BPH in the field needs to be aware in relation to the role of BPH as a pest and as a vector. Even though the BPH population in the field was low, if there were some rice yellow stunt disease symptom, weeds, and infected plant debris in the field, it would be as an inoculum source of the the disease. BPH would more attracted on diseased plant than on healthy one for breeding site and also feeding area in the field. The condition could increase the mobility of BPH in the field and will simultaneously transmit the disease.

CONCLUSION

Based on incubation periode, symptom variation and disease severity, susceptible BPH were more effective in transmitting yellow stunt disease to healthy plants compared to imidacloprid-resistant individuals. The results showed that both resistant and susceptible BPH to imidacloprid was able to transmit the virus to healthy plants. The acquisition period and inoculation period test showed the BPH could transmit the virus with the shortest acquisition time for 30 minutes followed 24 hours of inoculation, as well as the acquisition time of 10 days with the shortest inoculation time for 30 minutes. Single BPH susceptible or imidacloprid-resistant BPH were able to transmit virus to healthy plants. Lifespans of viruliferus BPH imidacloprid-resistant BPH were shorter than susceptible BPH.

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