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Research Article

Integrated Leafcurl Disease Control on Tobacco Plants in Klaten, Central Java

Pengendalian Terpadu Penyakit Kerupuk pada Tanaman Tembakau di Klaten, Jawa Tengah

Heri Widarta[1, 2], Sedyo Hartono[2]*, Sri Sulandari[2], Cahyo Hertanto[3], & Erna Anastasia[3]

[1]Agricultural Quarantine Major Service of Semarang
Jln. M. Pardi No. 7, Tanjung Emas, Semarang, Central Java 50174

[2]Department of Crop Protection, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora 1, Bulaksumur, Sleman, Yogyakarta 55281

[3]PT Perkebunan Nusantara X
Jln. Pramuka No. 1 Klaten, Central Java 57411

**Corresponding author. E-mail: sedyo_h@yahoo.com*

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ABSTRACT

Vorstenlanden tobacco is the best product of PT Perkebunan Nusantara X (PTPN X) Klaten, Central Java that is commonly produced into high economic value cigars. During the planting season of 2010/2011, there was an epidemy of leaf curl disease that caused billion rupiahs financial loss. Several efforts had been done, including the use of pesticides, but the result had not been satisfactory. Therefore, this research aimed to identify the pathogen of tobacco leaf curl disease and conduct integrated control system using three combinations in the nursery [A (biological agents treatment, plastic screenhouse, without physical barrier); B (biological agents treatment, plastic screenhouse + screen plot, with physical barrier of single screen, and sprayed insecticide); C (biological agents treatment, plastic screenhouse + single screen plot, with physical barrier of double screens, and fogging using white oil + insecticide)] and planting area [A (physical barrier of single screen and sprayed insecticide); B (physical barrier of single screen, sprayed insecticide, and sanitation of broad leaf weed + eradication of infected plant); C (physical barrier of double screens, fogging white oil + insecticide, and sanitation of broad leaf weed + eradication of infected plant)]. The result showed that leaf curl disease that occured at PTPN X Klaten was caused by Begomovirus, based on PCR result using Krusty and Hommer primers for *Begomovirus* Coat Protein gene. The most effective integrated disease control of leaf curl disease in the nursery was C combination, that consisted of biological agents treatment on the seedlings medium, screenhouse covered by plastic and double screens that was combined with physical barrier of double screens around the field, and fogging using white oil+insecticide of pyrethroid active ingredient. The most effective integrated disease control of leaf curl disease in the field was the same C combination, that consisted of the use of physical barrier of double screens, environmental sanitation of weeds around the field and eradication of infected plants and fogging using white oil + insecticide of pyretroid active agent.

Keywords: *Begomovirus*, integrated disease control, leaf curl disease of tobacco, white oil

INTISARI

Tembakau vorstenlanden merupakan produk andalan PT Perkebunan Nusantara X (PTPN X) Klaten, Jawa Tengah sebagai bahan baku cerutu yang bernilai ekonomi tinggi. Pada musim tanam 2010/2011 telah terjadi epidemi penyakit keriting atau kerupuk tembakau dengan kerugian mencapai milyaran rupiah. Upaya pengendalian yag telah dilakukan dengan mengandalkan pestisida tidak memberikan hasil yang memuaskan. Oleh karena itu, penelitian ini ditujukan untuk mengidentifikasi patogen penyebab penyakit kerupuk tembakau dan melakukan pengendalian secara terpadu dengan menggunakan tiga macam kombinasi yang dilakukan di pembibitan [A (perlakuan agens hayati, penyungkupan plastik, tanpa barier fisik); B (perlakuan agens hayati, penyungkupan, barier fisik tunggal, dan penyemprotan insektisida); C (perlakuan agens hayati, penyungkupan, barier fisik ganda, dan aplikasi white oil*)] dan di lahan pertanaman [A (barier fisik tunggal dan insekstisida); B (barier fisik tunggal, insektisida, dan sanitasi gulma); C (barier fisik ganda,* white oil + *insektisida, dan sanitasi gulma)]. Hasil penelitian menunjukkan bahwa penyakit kerupuk tembakau di PTPN X Klaten disebabkan oleh* Begomovirus *berdasarkan hasil PCR menggunakan primer Krusty dan Hommer untuk gen* Coat Protein Begomovirus*. Pengendalian terpadu penyakit kerupuk di pembibitan yang paling efektif adalah kombinasi C yang terdiri dari perlakuan agens hayati pada media bibit, penyungkupan rangkap plastik + waring ganda dipadukan dengan barier fisik berupa waring ganda sekeliling lahan dan aplikasi* white oil + *insektisida berbahan aktif piretroid dengan cara* fogging*. Demikian juga pengendalian terpadu penyakit kerupuk di lapangan yang paling efektif adalah kombinasi C yang terdiri dari perlakuan agens hayati pada media bibit, sanitasi gulma di sekitar lingkungan pertanaman dan eradikasi tanaman sakit, penyungkupan rangkap plastik + waring ganda dipadukan dengan barier fisik berupa waring ganda sekeliling lahan dan aplikasi* white oil + *insektisida berbahan aktif piretroid dengan cara* fogging*.*

Kata kunci: Begomovirus*, pengendalian terpadu, penyakit kerupuk tembakau,* white oil

INTRODUCTION

Vorstenlanden Tobacco is one of the best and high economic value plantation commodity to be produced as a good cigar. The vorstenlanden tobacco is cultivated by PTPN X Klaten in two different ways, i.e. Shade Grown Tobacco (SGT) which uses screen (insect net) to reduce the intensity of sun light to 70%, and cultivation of Open Field Tobacco (*na-ougts*) which is similar to the traditional cultivated tobacco. Since 2010, the SGT vorstenlanden tobacco cultivation has been reported to be infected by *Tobacco leaf curl virus* (ToLCV). The financial loss due to the virus reaches 45 to 65%, around 6 billion Rupiah.

The leaf curl disease of tobacco in Indonesia was previously found in Jember, East Java. It was caused by TLCV of *Begomovirus* genus (Hidayat *et al.*, 2008), followed by the report of Aji (2012) who stated that the leafcurl disease on tobacco at Klaten, Central Java was caused by *Tomato yellow leafcurl virus* (TYLCV). Recently, Trisno *et al.* (2014) reported the leafcurl disease was also happened in West Sumatera, which was caused by *Pepper yellow leafcurl Indonesia virus* (PYLIndV). Vorstenlanden tobacco is a new host of Begomovirus which previously there are some reports regarding epidemic of yellow leaf curl on chili and tomato in Central Java (Sulandari *et al.,* 2001; Sumardiyono *et al.,* 2003; Hartono, 2008). Begomovirus has a wide host range and the disease is rapidly spreading through whitefly vectors of *Bemisia tabaci* (Nakhla & Maxwell, 1998).

The control of Begomovirus disease has been conducted so far with the use of insecticide to reduce population of *B. tabaci*. However the vector is already resistant to the insecticide that makes of the control is less successful. Therefore, this research is aimed to conduct integrated control of leaf curl disease using various combination of disease control, from nursery to planting area in PTPN X Klaten.

MATERIALS AND METHODS

Field Survey

Field survey was conducted to observe the various symptom of leave curl disease, calculated disease incidents and observed the endemic and non-endemic area at PTPN X Klaten. Sampling was conducted to identify the causal agent of the disease using PCR technique.

Virus Identification Using PCR Technique

Total number of DNA was extracted from the sample using Nucleon PHYTOpure plant DNA extraction kit (Amersham Pharmacia Biotech, Buckinghamshire, England) according to factory guideline. The part of coat protein (CP) gene was amplified by PCR using pair of universal primers that was designed as Krusty and Hommer (Krusty: CCNMRDGGHTGTGARGGNCC and Homer: SVDGCRTGVGTRCANGCCAT). The primers were designed to amplify the targeted CP gene of Begomovirus (Revill *et al.* 2003). *Taq* polymerase was used in 50 µl of PCR reaction mix containing 1 µl DNA total, PCR buffer for Taq polymerase, 0.2 mM dNTP, and 25 pmol for each primers. Initial denaturation was done at 94°C for 2 minutes, PCR was continued with 35 cycles as followed: 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute, and it was followed by final extention at 72°C for 3 minutes. The PCR result was analyzed using 1% agarose gel electro-phoresis in the TBE buffer and visualized with ethidium bromide staining.

Integrated Disease Control at the Tobacco Nursery

There were three combinations of integrated disease control at the tobacco nursery, listed in Table 1.

Integrated Disease Control in Tobacco Planting Area

There were three combinations of integrated disease control at the planting area, listed in Table 2.

Table 1. The integrated disease control at the nursery with three combinations

Integrated control at the nursery				
Combination A	Biological Agents	Plastic screenhouse plot	Without physical barrier around the field	
Combination B	Biological Agents	Plastic screenhouse + screen plot	With physical barrier of single screen around the field	Sprayed insecticide
Combination C	Biological Agents	Plastic screenhouse + single screen plot	With physical barrier of double screens around the field	Fogging White oil+Insecticide

Table 2. The integrated disease control at the planting area with three combinations

Integrated control at the planting area			
Combination A	With physical barrier of single screen around the field	Sprayed insecticide	
Combination B	With physical barrier of single screen around the field	Sprayed insecticide	Sanitation of broad leaf weed around the field and eradication of infected plant
Combination C	With physical barrier of double screens around the field	Fogging White oil + insecticide	Sanitation of broad leaf weed around the field and eradication of infected plant

Field Observation

The observation was conducted to calculate disease incidence (DI) in the field, using the equation of:

$$DI = \frac{a}{a + b} \times 100\%$$

Note:
DI = Disease Incidence
a = Number of infected plants
b = Number of healthy plants

RESULTS AND DISCUSSION

Field Survey

Field survey in area of PTPN X Klaten detected various symptoms of leaf curl disease. The symptom was found on young to mature plants, i.e. chlorosis and dwarf, stunted, some part or most of leaves curled (Figure 1). This disease associated with the existence of vector, i.e. *B. tabaci*, the only vector for Begomovirus. Field survey also identified endemic and non-endemic area of curl leaf disease. The endemic areas were including Gadungan, Ceporan, Ngering, Bakung, and Towangsan. The incident at endemic area was 25−80%. Meanwhile, the non-endemic area were Karanglo, Ngrundul, Gondang, and Sumberejo. The endemic area was a non-technical irrigation area where Palawija crops were grown and this crops were potential host for Begomovirus. Non-endemic area had technical irrigation, thus there was only rice field around the tobacco plantation.

Identification of Disease Caused Agent in Laboratory

The assay was conducted by PCR to detect Begomovirus. Conventional detection of the specific symptom on the tobacco could not ensure the infection of Begomovirus. The symptom was not sufficient to identify whether the plant infected by Begomovirus in the field or not, thus it is needed to do PCR for detection of Begomovirus of tobacco.

DNA amplification from extraction of 3 diseased plants using primers of Krusty/Hommer was successfully obtained DNA fragment about 504 bp (Figure 2). It was in comply with target of PCR primer Krusty/Hommer to amplified part of Coat Protein Begomovirus gene, also confirmed that there was leaf curl disease in Klaten which was caused by Begomovirus. Previously, Aji (2012) also reported that yellow and dwarf on the tobacco plant in Klaten, Central Java was also caused by *Tomato yellow leafcurl virus* (TYLCV). The latest confirmation towards leaf curl tobacco sample from Klaten was also caused by TYLCV–Kanchanaburi (Natsuaki *et al.*, 2015; personal communication). Trisno *et al.* (2014) reported that leaf curl disease at West Sumatera was caused by *Pepper yellow leafcurl Indonesia virus* (PYLCIndV). While Hidayat *et al.* (2008) reported the leaf curl disease at Jember, East Java was caused by *Tobacco leaf curl virus* (ToLCV). It could be concluded that leaf curl disease in Indonesia was caused by different species of Begomovirus.

Integrated Disease Control of Tobacco Nursery

There were three combinations of leaf curl integrated disease control. The use of biological agents on seedling medium of A, B, and C Combinations showed uniformity of healthy seedlings (Figure 3B). Nevertheless, A Combination with only plastic screenhouse was not too dense (Figure 3A), and physical barrier around the field, showed that 10−15% of the early planting to be infected based on initial field observation of the symptoms. B Combination with physical barrier of net around the field showed 5−10% of tobacco infection. Sprayed imidacloprid insecticide had no significant result. The best was C Combination, where the screenhouse was covered with plastic and double screens, combined with double screens physical barrier (Figure 3C) and fogging using white oil + insecticide of pyretroid active agent. It showed best result of more than 95% (Figure 3B).

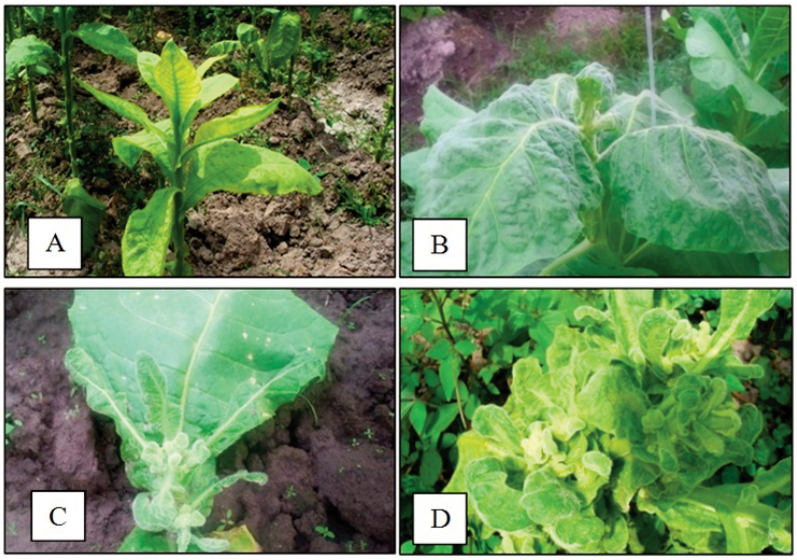


Figure 1. Symptoms of tobacco curl leaf disease: dwarf and leaf chlorosis (A); leaf folds and stunted (B); leaf malformation on the young plant and stunted (C); and leaf shrinking in irregular form on mature plant (D)

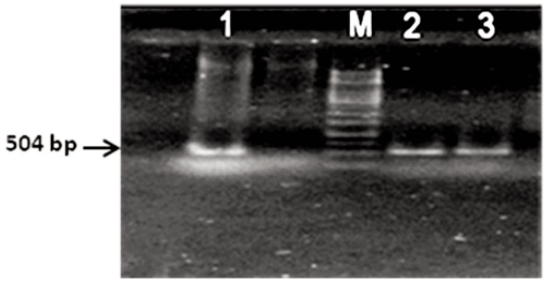


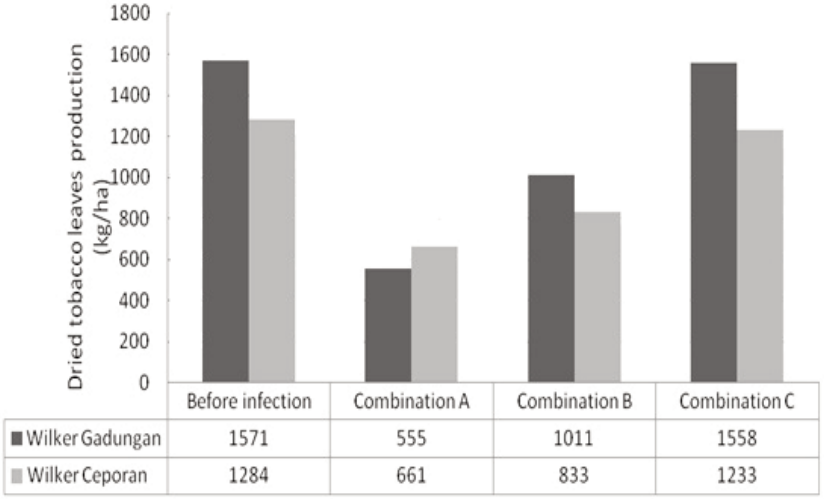
Figure 2. PCR test result of leaf curl of tobacco using primers of Krusty/Hommer to amplify *Begomovirus* which is visualized on agarose gel 1%: DNA marker (100 bp Ladder) (M); 1, 2, and 3, plant samples from 3 locations at Klaten

Figure 3. Integrated disease control of tobacco seedling: screenhouse of seedlings with plastic (A); the tobacco seeds are healthy (B); and physical barrier using double screens of 3 meters height around the field (C)

Integrated Disease Control of Tobacco in Planting Area

Integrated disease control on tobacco leaf curl at planting area was also conducted in three combinations. The A Combination at areas of PTPN X Klaten which was an endemic area, i.e.Wilker Gadungan and Ceporan, showed no significant result. That was 65% of dried tobacco leaves data prior to previous Begomovirus infection (Figure 4.). It was due to the use of single screen of SGT standard that had hole < 40 mesh, thus *B. tabaci* was easily entering tobacco field. Also the environmental sanitation was not free from broad leaf weeds and no eradication of infected plant during initial plantation that might become the source of virus transmission (Figure 5). Sprayed imidacloprid insecticide had not given a significant result on controlling the leaf curl disease. The B Combination, with good environmental sanitation, free from broad leaf weeds and there was infected plants eradication, showed not good result due to the use of single screen, thus the loss was still high, i.e., 45% than the previous production of dried tobacco (Figure 4).

The best combination of integrated control at planting area was the C Combination, with physical barrier of double screens and fogging using white oil+insecticide of pyretroid active agent. It gave significant value of dried tobacco leaves production of 1,558 kg/ha at Gadungan and 1,233 kg/ha at Ceporan. The both results showed the same number with previous result prior to the virus attack (Figure 4). The double screens covered by plastic around the field blocked *B. tabaci*. Besides, fogging using white oil+insecticide of pyretroid active agent showed good result. White oil was an insecticide to control various pest in the plantation area. It blocked respiratory tract, thus it had deadly effect to the bugs (Marques *et al.,* 2014).



	Before infection	Combination A	Combination B	Combination C
Wilker Gadungan	1571	555	1011	1558
Wilker Ceporan	1284	661	833	1233

Figure 4. Data of dried tobacco leaves production (kg/ha) from leaf curl endemic area prior to the virus infection and after the integrated disease control of A, B, and C combinations

(A) (B) (C)

Figure 5. Environmental sanitation of tobacco: *Ageratum sp*. as plant indicator of *Begomovirus* around tobacco planting area (A); broad leaf weeds outside and inside planting area of tobacco (B); and infected plant from leaf curl during its initial growth as source of inoculum on the disease spreading (C)

CONCLUSION

The curl leaf disease of tobacco at PTPN X Klaten was caused by Begomovirus based on PCR result using primers of Krusty dan Hommer for Coat Protein gene of Begomovirus. The most effective integrated disease control on curl leaf disease in the Nursery was C Combination, that consisted of biological agents treatment on the seedling medium, Screenhouse covered by plastic and double screens that was combined with physical barrier of double screens around the field, and fogging using white oil + insecticide of pyretroid active agent. The most effective integrated disease control of leaf curl disease in the planting area was C Combination, that consisted of the use of physical barrier of double screens, environmental sanitation of weeds around the field and eradication of infected plants, and fogging using white oil+insecticide of pyretroid active agent.

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