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Study and Biological Manage of The Plant Virus Diseases

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ABSTRACT

Plant virology is the study of viruses that infect plants, impacting agricultural productivity and ecosystem health. Viruses cause a range of symptoms, including mosaic leaf patterns, stunted growth, and reduced crop yields, posing a significant threat to global food security. Understanding plant viruses involves the study of their structure, transmission mechanisms, and interactions with host plants. Research in plant virology is essential for developing sustainable agricultural practices and reducing the adverse effects of virus infections on plants, ultimately contributing to food and agricultural security. The purpose of this reference paper is to discuss and provide information on research that has been conducted, including methodology, results, and data interpretation, symptoms caused, and how to manage them, innovations in biotechnology and the development of virus-resistant crop varieties. Plant viruses can be identified with RT-PCR method. In this study, the primary pair used to amplify were universal *Potyvirus* primer and specific PRSV primer. Application of biopesticides *Trichoderma* sp., *Streptomyces* sp.showed that the treatment of Bioside before infestation (T1) had a high mortality value of 95% compared to the treatment after infestation (T2) which was 75%, whereas without biocide mortality was low at only 15% , there was curl disease in the control reaching 37% between chili plants in all treatments.

Keywords: Viral, infections, biotechnology, control, sustainable

Introduction

Plant diseases caused by viruses are still a subject of research interest and need to be studied continuously because viruses are submicroscopic objects that only replicate in cells, unlike other parasitic microorganisms. Virus infections in plants will cause a variety of symptoms that are different from other OPT (Plant Pest Organisms). Virus infections in plants will cause symptoms of stunting, dark green, and bright green striped mosaics. Symptoms of virus attacks on susceptible plants will cause clear symptoms, while in resistant plants the symptoms will not be visible (Zaitlin, & Palukaitis, 2000). Plant diseases caused by viruses are still a subject of research interest and need to be studied continuously because viruses are submicroscopic objects that only replicate in cells, unlike other parasitic microorganisms. Virus infections in plants will cause a variety of symptoms Virus transmission can occur due to vegetative reproduction and can also be transmitted by animals or other microorganisms as well as tall putri, transmission in these ways does not occur for other OPT (Sastry, 2013).

The contagiousness of the virus is very high so it quickly becomes an epidemic of plant disease. Outside the host set, the virus is a crystal that does not carry out cell metabolic activities. Plant viruses are primarily transmitted by vectors, mechanical damage, or through seeds (Tatineni & Hein, 2023). Managing plant viruses can be challenging, as there are often no direct treatments once the plant is infected. Managing Plant Viruses can be done by Vector Control: Managing insect populations that transmit viruses, Resistant Varieties: Using plant varieties that are resistant to specific viruses.,

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Sanitation: Cleaning tools, avoiding mechanical transmission, and removing infected plants., Quarantine: Restricting the movement of infected plants or plant materials. Cultural Practices: Implementing crop rotation, proper spacing, and avoiding excessive pruning during critical times (Hull, 2014).

Managing plant viruses is critical for maintaining crop health and ensuring agricultural productivity. Unlike bacterial or fungal diseases, viral infections in plants cannot be treated directly with chemical agents. Therefore, prevention, control, and mitigation strategies primarily focus on preventing virus introduction, reducing transmission, and breeding resistant plant varieties (Akhtar & Shahid, 2025). There is much more to be learned about viruses than has been reported. Information on plant viruses and their effects on crop production is so far inadequate in some countries, while laboratory studies of the ecology and epidemiology of viruses have been largely neglected. Research must fill this gap to keep pace with the virological consequences of ongoing changes in agriculture. Some of this research may be basic, covering only the properties of viruses in the hope of eventually finding direct control methods (Loebenstein & Katis, 2014). However, much research is needed aimed at solving immediate practical problems. The purpose of this reference paper is to discuss and provide information on research that has been conducted, including methodology of molecular Detection of Viruses, results, data interpretation, symptoms caused, and how to manage them by biological control., and innovations in biotechnology.

Material and Methods

Molecular Identification of PRSV with Reverse Transcription – Polymerase Chain Reaction (RT-PCR)

RNA Extraction

Extraction of total RNA was conducted based on the modified CTAB method described by Doyle and Doyle (1987). Leaf tissue (0.1 g) was grinded with liquid nitrogen and 500 µl of 10% CTAB buffer (CTAB, 0.1 M EDTA pH 8, 1 M Tris-HCl pH 8, 5 M NaCl, 1% β-mercaptoethanol) was added. The ground sample was transferred to 1.5 ml microtube and incubated in a water bath at 65 ºC for 30 minutes, with intermittent mixing. A total of 500 µl of Chloroform: Isoamyl alcohol (24:1) was added to the tube and vortexed to mix well. The microtube was centrifuged at 12,000 rpm for 10 minutes. The aqueous phase on top was transferred to a sterile 1.5 ml microtube, then added 3 M of sodium acetate (1/10 of the supernatant volume) and isopropanol (2/3 of the total volume). The mixture was homogenized and incubated at -20 ºC overnight. After incubation, the mixture was centrifuged at 12,000 rpm for 10 minutes, and the supernatant was discarded. The RNA pellets were washed by adding 500 µl of 70% ethanol, then centrifuged at 8,000 rpm for 5 minutes. The ethanol was discarded, and the tube was placed upside down on tissue paper to air dry for approximately 30 minutes. After drying, the RNA pellets were dissolved in 50 µl of TE buffer (10 mM Tris-HCl pH 8.0 + 1 mM EDTA).

cDNA Synthesis

Total RNA extraction product was used as a template for cDNA synthesis. The composition of reverse transcription (RT) consisted of 2 µl of RT buffer, 0.5 µl dNTP mix 10 mM, 0.5 µl Ribosafe inhibitor (Bioline), 0.5 µl Tetro cDNA transcriptase (Bioline), 0.5 µl Oligo $d(T)_{18}$ 10 mM, 4 µl ddH_2O, and 2 µl RNA template. The reverse transcription reaction was carried out at 42 °C for 60 minutes followed by 85 °C for 5 minutes in GeneAmp PCR system 9700 machine. The cDNA product was then used as a template at the amplification stage.

cDNA Amplification

Amplification was carried out using 2 separate primer pairs; universal *Potyvirus* primer and specific PRSV. The universal *Potyvirus* primers used were MJ-1 (5'-ATGGTHTGGTGTGYATHGARA AYGG-3')/ MJ-2 (5'-TGCTGCKGCYTTCATYTG-3') with a target amplicon size of 320 pb. The specific PRSV primers used were PRSV326 (5'-TCGTGCCACTCAAT CACAAT-3')/PRSV800 (5'-GTTACTGACACTG CCGTCCA-3') with a

target amplicon size of 475 bp (Marie-Jeanne *et al.* 2000; Mohammed *et al.* 2012). As much as 25 µl amplification reaction consisted of 12.5 µl My Taq Redmix 2x (Bioline), 1 µl 10 µM reverse primer, 1 µl 10 µM forward primer, 9.5 µl nuclease-free water, and 1 µl cDNA. The cDNA was amplified in GeneAmp PCR system 9700 machine with an amplification program according to the target (Table 1).

Table 1. PCR amplification program for detecting *Potyvirus* and PRSV

Target	Amplification program					Cycles
	Pre-denaturation	Denaturation	Annealing	Extention	Final extention	
Potyvirus	94 ºC; 3 min	96 ºC; 30s	61 ºC; 1 min	72 ºC; 1 min	72 ºC; 7 min	45
PRSV	94 ºC; 5 min	94 ºC; 30s	50 ºC; 1 min	72 ºC; 1 min	72 ºC; 7 min	35

DNA Visualization

DNA was electrophores on 1% Agarose in 0.5x *Tris-boric* EDTA (TBE) buffer. Samples were added with fluoroVuo nucleic acid dye (Smobio, Taiwan) (1:40,000) and then electrophoresis at 50 Volt for 50 minutes. DNA was visualized under an ultraviolet transilluminator and documented with digital camera.

Manage of Plant Viruses by Biological agents (BCAs)

Making of biological agents

Streptomyces sp and *Trichoderma* sp. was carried out by taking soil samples in the fields of shallot farmers in Pare, Kediri (with isolate code BMP). Soil sampling was carried out randomly at several points at a depth of 10-15 cm to maximize the potential of the soil containing Actinomycetes bacteria (Aminnullah, 2020). Dilution was carried out with a series of dilutions of 10^4 and 10^5 for *Trichoderma* sp. and 10^5 and 10^6 for *Streptomyces* sp., then inoculated on PDA (Potato Dextrose Agar) and GNA (Glucose Nutrient Agar) media, Preparing cultures of biological agents *Trichoderma* sp. (7 days old) and *Streptomyces* sp. (14 days old). The next step is to make a slice of the biological agent using a 0.5 cm diameter cork borer. The ratio of the biological agents *Trichoderma* sp. and *Streptomyces* sp. used is 5:1. Insert the slice into a 250 mL Erlenmeyer flask containing Glucose Potato extrac solution. The finally is to shake the mixture using a laboratory shaker at a speed of 60 per minute for 10 days and sterile water is used as a control.

Applications BCAs at vectors

Application of biopesticides *Trichoderma* sp., *Streptomyces* sp. before vector infestation was carried out using a 1L sprayer to peanut plants that were covered. Aphids were infested on the plants as many as 5 for 30 minutes. Biocide application after infestation of vector was carried out by infesting 5 aphids on long bean plants and spraying biocide. Plants were covered and gauzed to prevent infestation by other insects. The dose of Biocide is 100 ml/plant. Each treatment consisted of 4 plants as replications. Observations of aphid mortality on treatment plants were carried out at 24, 48, and 72 hours after treatment.

Applications of BCAs in Chili field

The application of biological agents in the field was carried out 2 times with different concentration treatments (3:1), 2 weeks and 4 weeks after planting. Research observations were carried out by observing the variables that determine the ability of biological agent inhibition, as follows: (2) Severity of the disease, carried out on the number of leaves attacked by the yellow and curly virus every 7 days until harvest (Anitha & Rabeeth, 2010).

Results and Discussion

Molecular identification of plant viruses

Plant viruses can be identified with RT-PCR method. In this study, the primary pair used to amplify were universal *Potyvirus* primer and specific PRSV primer. they amplify different genomic regions of the viral coat protein (CP) gene, which is known to have a high level of conservation among members of the Potyvirus genus. The CP gene functions in RNA encapsidation, vector transmission, pathogenicity, and cell-to-cell movement (Gonsalves et al., 2010).

The DNA fragment of 320 bp was successfully amplified from several field samples (Figure 1). This indicates that the field samples were infected by *Potyvirus*. The MJ1/MJ2 primer pair was designed to detect the Potyvirus CP gene, producing an amplicon of approximately 320 bp (Marie-Jeanne et al., 2000). These primers have been widely used to detect and identify viruses from the *Potyvirus* group, including *Bean yellow mosaic virus*, *Cowpea aphid-borne mosaic virus*, *Ornithogalum mosaic virus*, and *Wisteria vein mosaic virus* in vanilla plants (Grison et al. 2006), *Dasheen mosaic virus* (DsMV) in *Colocasia esculenta* in India (Babu et al., 2011), and PRSV in papaya plants from Nanggroe Aceh Darussalam (Hidayat et al., 2012).

Figure 1. DNA visualization of *Potyvirus* using universal primer of MJ-1/MJ-2. Sample from Malang (1&2), Kebumen (3&4), Purworejo (5&6), Bantul (7&8), and Bogor (9 – 12); M. 100 bp DNA Marker (Thermo Scientific)

Further confirmation was performed using the specific primer (PRSV326/PRSV800). Specific DNA fragment of 475 bp was successfully amplified from several field samples (Figure 2). The specific PRSV326/PRSV800 primer pair was designed to amplify a portion of the PRSV CP gene, producing an amplicon of approximately 475 bp (Mohammed et al., 2012). The same primer was used by Harmiyati et al., (2015), Listihani et al. (2018), and Farida et al. (2022) and was successfully to amplified PRSV from papaya and cucumber. The results of this RT-PCR confirm that the virus associated with ring spot disease in papaya from several papaya-growing centers in Java is PRSV and/or other members of the *Potyvirus* group.

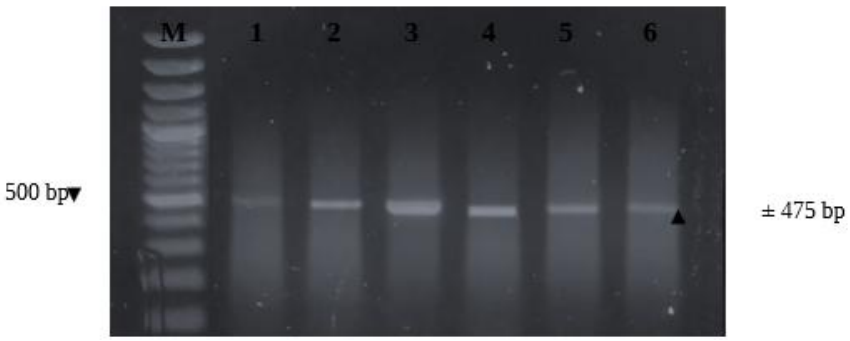

Figure 2. DNA visualization of PRSV using specific primer of PRSV 326/PRSV800. Sample from Malang (1), Bogor (2 & 3), Kebumen (4), Purworejo (5) and Bantul (6); M. 100 bp DNA Marker (Thermo Scientific)

Observation results showed that on average the treatment before infestation (T1) had a high mortality value of 95% compared to the treatment after infestation (T2) which was 75%, whereas

without biocide mortality was low at only 15%. This is thought to be due to the administration of biopesticides containing *Streptomyces* sp. and *Trichoderma* sp. able to act as a stomach poison.

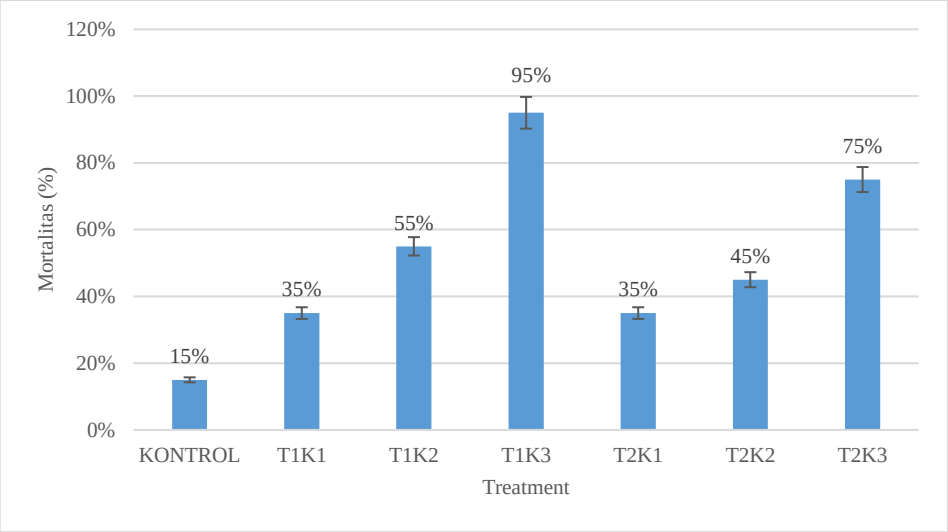

Figure 3. Mortality graph of *Aphis craccivora* Koch. for 3 days (Remarks: T1: before infestation, T2: after infestation, K1: *Streptomyces* sp. + *Trichoderma* sp. 100 cc and chitosan 0.6%, K2: *Streptomyces* sp. + *Trichoderma* sp. 100 cc and chitosan 0.9 %, K3: *Streptomyces* sp. + *Trichoderma* sp. 100 cc and chitosan 1.2%)

Mumba et al. (2020) stated that stomach poison works when pests eat plant parts that have been treated, in this case, biopesticides containing *Streptomyces* sp. and *Trichoderma* sp. The high mortality was also caused by the addition of chitosan to the biocide. The administration of chitosan is thought to be able to encourage the production of the chitinase enzyme, this is because *Streptomyces* sp. and *Trichoderma* sp. able to make chitinase enzymes where this enzyme can degrade chitin as a nutritional source (Haedar et al., 2017).

The results of multi-antagonist administration of *Streptomyces* sp. and *Trichoderma* sp. and humic acid. On tomato plants in Pare Kediri with the Regusol soil type, it showed that there was curl disease in the control reaching 37% and with a dose of 300 mL there was no curl disease, on chili plants in all treatments there were symptoms of curl but the lowest level of attack was at a dose of 300 mL (Figure 4).

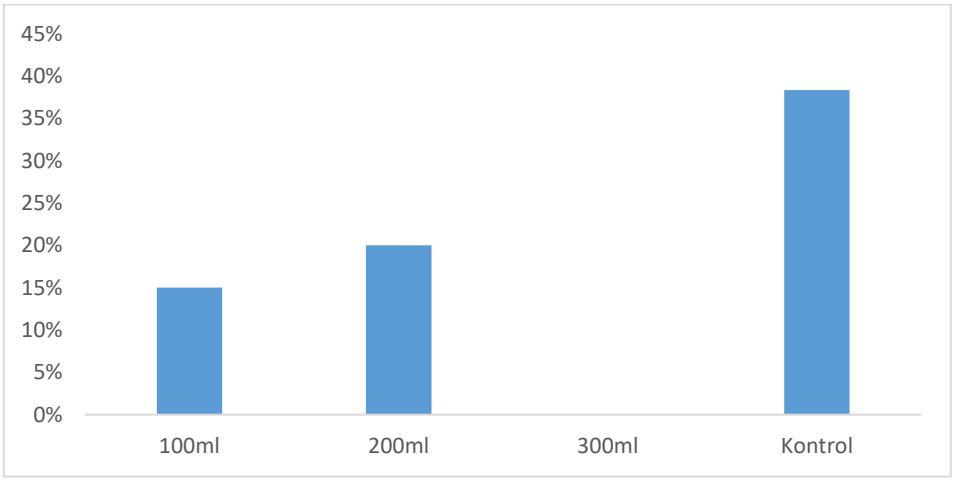

Figure 4. Average percentage of curl disease on chili and tomato plants after application of humic acid and multi antagonists *Streptomyces* sp and *Trichoderma* sp. in Pare

Conclusion

Plant viruses can be identified with RT-PCR method. In this study, the primary pair used to amplify were universal *Potyvirus* primer and specific PRSV primer. Application of biopesticides *Trichoderma* sp., *Streptomyces* sp.showed that the treatment of Bioside before infestation (T1) had a high mortality value of 95% compared to the treatment after infestation (T2) which was 75%, whereas without biocide mortality was low at only 15% , there was curl disease in the control reaching 37% and no curl disease, on chili plants in all treatments. The results of multi-antagonist aplications on tomato plants in Pare Kediri showed th,e control reaching 37% and with a dose of 300 mL in all treatments there were symptoms of curl but the lowest level of attack was at a dose of 300 mL.

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