Effect of Chemotherapy and Thermotherapy on the Virus Incidence Reduction in Tissue-Cultured Ilocos White Garlic (*Allium sativum* L.)

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ABSTRACT

Garlic (*Allium sativum* L.), an important crop in the Philippines, suffers from viral infections that limit local production by reducing yield and bulb quality. Garlic is propagated vegetatively; hence, the planting material from the previous harvest is planted for the next planting season, thereby viral transmission from generation to generation is inevitable. To address this, the study evaluated the virus elimination effect of chemotherapy and thermotherapy with meristem culture in Ilocos White garlic.

This study tested two virus elimination treatments – chemotherapy at 10, 20, 30, and 40mg/l acyclovir – an antiviral drug, and thermotherapy at 50°C for 1, 1.5, and 2 hours. Five viruses, namely, OYDV, SLV, GCLV, LYSV, and *Allexivirus,* were molecularly detected through RNA extraction and RT-PCR.

Results showed Ilocos White garlic harvested from MMSU were infected with only LYSV and *Allexivirus* out of five viruses screened. Chemotherapy using acyclovir reduced LYSV by 75% using 10mg/l and *Allexivirus* by 100% using 20 to 40mg/l. Thermotherapy at 1 and 1.5 hours reduced LYSV by 66.67% and 75%, respectively, and *Allexivirus* by 100% for 1, 1.5, and 2 hours. These results showed that LYSV is more virulent than *Allexivirus* and more resistant to treatments.

The coordinated approach of meristem culture with chemotherapy using acyclovir at 20mg/l and thermotherapy at 50°C for 1 hour showed potential virus elimination and reduction ability. Further optimization and combinations of these methods are recommended to achieve consistent and significant virus elimination in garlic essential in sustaining the local garlic industry.

Keywords*: Acyclovir, Garlic, Thermotherapy, Virus incidence reduction*

Introduction

Garlic (*Allium sativum* L.), locally known as “bawang”, is a member of the *Amaryllidaceae* family. It is known for adding aroma and flavor to several foods and dishes and is also known for is considerable therapeutic properties and noteworthy health benefits due to its bioactive compounds (Ansary et al., 2020). Because of its potential and significance in

numerous culture and industry, garlic is widely cultivated worldwide and is also popularly grown in the Philippines. The Ilocos Region (Region I) is deemed to be the country's top garlic producer among the other six garlic-producing regions, producing about two-thirds of the annual garlic production in the Philippines (Arcalas, 2024). Statistics revealed that 1,880 hectares of land in Ilocos Norte, 130 hectares in Ilocos Sur, and four hectares in Pangasinan are allotted for planting and production of local garlic (Micua, 2017). In 2022, most of the garlic produced in the Ilocos Region was contributed by Ilocos Norte with a share of 97.20% (Philippine Statistics Authority [PSA], 2023).

Despite being widely cultivated in the country, the importation of garlic remains high. About 95% of the garlic supply in the Philippine market was imported from foreign countries (Sevillano, 2017). The massive garlic importation was due to limited and insufficient local production. The local production of garlic has long been following a downtrend, with the garlic self-sufficiency ratio falling to a record low of 5.5% in 2022, as reported by the Philippine Statistics Authority. Possible reasons for the decline in garlic production include extreme weather conditions, insect pests, and diseases (Adorada et al., 2023).

Virus infection takes a large toll on the level of productivity of local garlic. The presence of viruses is due to the state of the garlic planting materials on hand. The crop is asexually and vegetatively propagated, where cloves from recent harvests are used for the next planting season. This practice makes garlic vulnerable to viral infection, and over the years, garlic planting materials have accumulated diseases. This results in a reduction in yield and varietal degeneration (Baranwal & Pramesh, 2015). The presence of viral infections and complexes in garlic has been found to cause reduction in plant emergence, plant height, production of marketable bulbs, and lower average mass of bulbs and cloves (Marodin et al., 2019). Among the garlic varieties cultivated in the country, Ilocos White garlic is the most common variety grown for commercial use and production (Business Diary Philippines, 2022). Local varieties produced in the country are known for being more pungent and aromatic compared to imported ones. However, due to the limited availability of locally produced garlic, an increase in its price in the market urges consumers to buy the cheaper and large-sized imported garlic (Rodriguez, 2023).

In response to the decreasing quantity of locally produced garlic and the quality of local garlic planting materials, virus elimination is one of the ways to revitalize the country's garlic industry through several virus elimination and virus incidence reduction treatments. This includes thermotherapy, chemotherapy, and tissue culture. Tissue culture is used in the production of clean planting materials in several crops. One type of tissue culturing is meristem culture, which involves the use of plant parts containing meristematic cells that have high metabolic activities, restricting the multiplication of viruses (Singh, 2023). Thermotherapy involves the use of heat to remove viruses from plant materials by inhibiting viral replication or by causing virus RNA degradation (Wang et al., 2018). Chemotherapy involves the use of chemicals such as acyclovir – an antiviral drug. Although acyclovir is primarily known for treating infections caused by herpes viruses, the potential of acyclovir in reducing viral infections in plants has been reported, specifically in peach, potato, sugarcane, and dahlia, among others.

Generally, this study aimed to assess the virus incidence reduction of chemotherapy using acyclovir and thermotherapy on tissue-cultured Ilocos White garlic. Specifically, it evaluated the effectiveness of chemotherapy and thermotherapy treatments in reducing virus incidence in tissue-cultured Ilocos White garlic; determined the optimal concentration of chemotherapy and duration of thermotherapy for an effective viral elimination; and determined the effect of chemotherapy and thermotherapy on the growth of tissue-cultured Ilocos White garlic.

The results of this study could provide researchers with information and reference points in exploring virus incidence reduction and virus elimination in local garlic. Furthermore, the results could be of great help in developing strategies to protect garlic crops, reduce losses for local garlic farmers, and revitalize the local garlic farming industry.

The study was limited to the evaluation and determination of the antiviral and plant growth effects of chemotherapy using acyclovir and thermotherapy using a water bath on tissue-cultured Ilocos White garlic. The study was also limited to the molecular screening of Shallot Latent Virus (SLV), Leek Yellow Stripe Virus (LYSV), Garlic Common Latent Virus (GCLV), Onion Yellow Dwarf Virus (OYDV), and *Allexivirus,* employing five molecular markers in the PCR tests.

Methods

Locale of the Study

From August 2024 to May 2025, thermotherapy and chemotherapy treatments, tissue culture, and virus indexing were conducted to develop a technique for reducing viral incidence in Ilocos White garlic. Samples were collected from the Garlic Research Center at Crops Research Laboratory (CRL). Laboratory procedures such as tissue culture were performed in the Tissue Culture Laboratory at CRL; RNA extraction and cDNA synthesis were conducted at Genomics and Genetic Engineering Laboratory (2GLab) of Center for Cellular and Molecular Medical Research (CMED) at the College of Medicine; and PCR and agarose gel electrophoresis were performed at the CHED-IG laboratory at the College of Arts and Sciences of Mariano Marcos State University, Brgy. 16-S Quiling Sur, City of Batac, Ilocos Norte.

Design of the Study

The study involved three thermotherapy treatments and four chemotherapy treatments. Specifically, thermotherapy treatments involved a 50°C water bath for 1, 1.5, and 2 hours. Chemotherapy treatments involved varying concentrations of acyclovir at 10, 20, 30, and 40mg/l.

The experiment was conducted using a Completely Randomized Design (CRD). For thermotherapy, a total of ten garlic bulbs were used. Eight cloves were extracted from each bulb, resulting in 80 cloves. These 80 cloves were randomly assigned across the four treatments, with two cloves allotted per treatment from each bulb, resulting in twenty cloves per treatment and two replications per bulb per treatment. For the chemotherapy, a total of ten garlic bulbs were used. Ten cloves were extracted from each bulb, resulting in 100 cloves. These 100 cloves were randomly assigned across the 5 treatments, with two cloves allotted per treatment from each bulb, resulting in 20 cloves per treatment with two replications per bulb per treatment.

Among the bulbs subjected to treatment, a subset of five bulbs each for chemotherapy and thermotherapy was randomly selected for virus indexing, with each bulb contributing a representative clove per treatment. In this context, the bulb was considered the biological replicate; however, independent experimental replication was not performed due to resource constraints. This represents a limitation that may affect the results; thus, it is suggested that future studies include independent experimental repeats to confirm reproducibility and reliability.

Plant Sample Preparation

Selected Ilocos White garlic bulbs were separated into cloves, and their skin was manually peeled off. Cloves were surface sterilized by washing with powdered soap and sterile distilled water through vigorous shaking by hand, followed by rinsing with sterile distilled water thrice. Additionally, cloves were further sterilized using fungicide as described by Benke et al. (2023). A 0.1% Tween 20 (Ajax Finechem) solution was poured into flasks with cloves and subjected to vigorous shaking in a laboratory shaker for 15 minutes. Afterward, the fungicide solution was decanted, and cloves were rinsed with sterile distilled water. Final sterilization was performed using 20% commercial bleach solution with vigorous shaking in a laboratory shaker for 15 minutes. The bleach was decanted, and the cloves were rinsed with sterile distilled water thrice.

Culture Media Preparation

The study utilized the MS (Murashige and Skoog, 1962) media composed of macronutrients, micronutrients, vitamins, and iron sources to induce the growth of meristem cultures. However, it is noted that the media lacks myo-inositol and pyridoxine hydrochloride. Specifically, it contains 100 ml/L of MS I (Macronutrients), 10 ml/L of MS II (Micronutrients), 10 ml/L of MS III (Organics/Vitamins), and five ml/L MS IV (Iron source). The media was also supplemented with 30 g/L (3%) sucrose, 4.5 g/l (0.45%) agar powder, growth regulators; 300 µl/L of indole 3-acetic-acid (IAA), and 2,000 µl/L of 6-(y,y,-Dimethylallylamino) purine (2-iP).

For the thermotherapy setup, the prepared media were transferred to test tubes at 10 ml volume, secured with cotton plugs, and were autoclaved at 121°C for 15 minutes. For the chemotherapy setup, the bulk of the prepared media was autoclaved for sterilization at 15 psi for 15 minutes. After which, inside a laminar flow hood, the powdered acyclovir in varying concentrations was dissolved in the medium. 10 ml of the medium containing the antiviral agent was then dispensed into sterile test tubes using a micropipette with sterile tips. Test tubes were then sealed with sterile cotton plugs and incubated for at least 3 days prior to use.

Heat Treatment, Meristem Excision, and Tissue Culture

After surface sterilization of cloves, cloves intended for thermotherapy were immediately exposed to a water bath at 50°C for 1, 1.5, and 2 hours prior to meristem excision. On the other hand, cloves intended for chemotherapy were directly subjected to meristem excision. Meristem was excised from the cloves inside a sterile laminar airflow chamber using sterile scalpels and forceps, avoiding contamination. The isolated meristems were transferred and inoculated into the prepared culture medium, positioning them with the roots

facing downwards to facilitate growth. Tissue cultures were kept inside a growth chamber for one month (30 days) under a 16-hour light/8-hour dark photoperiod.

Clove and Leaf Sample Collection for Molecular Procedures

The study utilized the excess clove samples as the source for baseline data for virus detection, while leaf samples one month after tissue culture cultivation served as the source for post-treatment data. For chemotherapy, excess cloves after meristem excision were immediately sealed and stored in a freezer for further molecular virus detection procedures. However, for thermotherapy, excess cloves from heat-treated samples were no longer viable for molecular detection of viruses for baseline data, as exposure to heat may have altered the presence of viral infection. Due to this, untreated cloves (control) served as the source for baseline data for each bulb.

RNA Extraction

To determine the presence of viral infection in the pre- and post-treated samples, total RNA was isolated from clove samples for baseline data, and from the leaf samples for post-treatment data. RNA extraction was carried out following a conventional method with protocol adapted from the Invitrogen user guide (2016) with modifications (Catalog Nos. 15596026 and 15596018). Approximately 100mg of clove tissues were homogenized in liquid nitrogen using a sterile, pre-chilled mortar and pestle. The cells were lysed using RNAgents® Denaturing Solution (Promega) and incubated for 5 minutes at room temperature, followed by centrifugation at 12,000 x g for 2 minutes at 4°C. Phase separation was done by isolating the supernatant and adding 5M NaCl and chloroform, then centrifuging at 12,000 x g for 10 minutes at 4°C. The aqueous phase after phase separation was added with absolute isopropanol and centrifuged at 12,000 x g for 10 minutes at 4°C for RNA precipitation. The total RNA precipitate, in the form of a white gel-like pellet, was washed using 75% ethanol and centrifuged at 10,000 x g for 1 minute at 4°C. The supernatant was discarded, and the remaining RNA pellet was air-dried for 5 to 10 minutes and further resuspended in 20µl RNAse-free water (Vivantis). Concentration of isolated RNA samples was identified using ScanDrop 2 Spectrophotometer (Analytik Jena), and RNA concentration was further normalized to 0.5µg with the addition of ultra-pure water. Samples with low RNA concentration were not normalized and were no longer subjected to cDNA synthesis and PCR.

cDNA Synthesis

Reverse transcription was performed on 3µl of total RNA extracted from cloves and leaf samples using Vivantis 2-Step RT-PCR Kit in a 20µl reaction volume. First, the RNA-Primer mixture containing 3µl of total RNA, 1µl of random hexamer, 1µl of 10mM dNTPs Mix, and 5µl of nuclease-free water was incubated at 65°C for 5 minutes and chilled on ice for 2 minutes. Then, 10µl cDNA synthesis mixture consisting of 4µl of 5X Buffer M-MuLV, 0.5µl of M-MuLV reverse transcriptase 29 (200 units/µl), and 5.5µl of nuclease-free water was added to the RNA primer mixture, amounting to a total of 20µl cDNA product.

PCR Amplification

PCR was performed using Vivantis 2-Step RT-PCR Kit with an optimized protocol. A total of 2µl of cDNA template was added to 8µl of PCR reaction mixture, which consisted of 1µl

of 1x ViBuffer A, 0.4µl of 2mM MgCl2, 0.1µl of 0.1mM dNTPs mix, 0.2µl of 2mM forward primer, 0.2µl of 2mM reverse primer, 0.1µl of 0.05 units Taq DNA polymerase, and 6µl of nuclease-free water. The PCR amplification was carried out starting with pre-denaturation at 94°C for 2 minutes, followed by 35 cycles of a PCR profile consisting of denaturation at 94°C for 30 seconds; primer-dependent annealing at 60°C, 58°C, 55°C,56 - 57°C, and 57 – 58°C for OYDV, LYSV, *Allexivirus*, GCLV, and SLV, respectively, for 30 seconds; and extension at 72°C for 30 seconds. This was followed by a final extension at 72°C for 5 minutes. PCR amplification utilized the primers developed from the study by Nam et al. (2015) for the amplification of OYDV, LYSV, *Allexivirus*, GCLV, and SLV.

Agarose Gel Electrophoresis

The amplified PCR products were separated through gel electrophoresis on a 1% agarose gel (1 gram of Agarose powder [1st Base] dissolved in 100ml 0.5x TBE Buffer) stained with 5µl of nucleic acid gel stain (GelRed, Biotium) in 0.5x TBE Buffer. 3µl of PCR products, mixed with loading dye, were loaded and electrophoresed at 100 volts for 30 to 45 minutes. Bands were visualized in a Gel Imaging and Analysis System (Biobase), and fragment sizes were determined by comparison with a 1Kb and 100bp DNA ladder (Vivantis).

Data Collection & Analysis

The survival rate of the Ilocos White garlic after treatment and tissue culture was obtained by manually recording the number of regenerated explants. This determined the ability of the tissue-cultured Ilocos White garlic to withstand and tolerate the chemotherapy and thermotherapy treatments. To determine and calculate the plant survival rate per treatment, the following formula was used:

$$Survival\ rate\ (\%) = \frac{Number\ of\ regenerated\ plantlets}{Number\ of\ initiated\ explants} \times 100$$

The virus incidence reduction of each treatment was determined through the baseline and post-treatment data collected. The virus incidence reduction reflects the extent to which the treatment was able to decrease the level of viral infection in the samples compared to the levels prior to treatment. Virus incidence reduction rate for chemotherapy treatments was calculated using the formula below:

$$Virus\ Incidence\ Reduction\ (\%) = \frac{Number\ of\ negative\ post-treatment\ samples}{Number\ of\ positive\ pre-treatment\ samples} \times 100$$

Moreover, the growth rate of treated plants was also recorded by manually measuring the plant length and leaf width (in cm) prior to leaf harvest after a month of cultivation. The data gathered on the growth of treated plants was laid out in a Completely Randomized Design (CRD) and subjected to One-Way Analysis of Variance (ANOVA) to assess whether there were statistically significant differences in plant length and leaf width across the different treatments. Pairwise comparisons were conducted in the presence of statistically significant ANOVA results using the Least Significant Difference (LSD) test. The analyses were performed using IBM SPSS Statistics, version 27.

Results and Discussion

Survival Rate

Over the one-month cultivation period, the survival of the garlic plantlets was recorded. The survival of plantlets within the thermotherapy and

chemotherapy treatments was indicated through observable growth, such as shoot and leaf development from the inoculated tissues.

As presented in Table 1, a total of 72 out of 80 plantlets survived the thermotherapy treatment, corresponding to an overall survival rate of 90%. Among the varied treatments, plantlets exposed to 50°C for 1 hour had the highest survival rate of 100%. This is followed by heat exposure for 1.5 hours, with a 90% survival rate, while both control and 2-hour heat exposure showed equal survival rates at 85%. These results suggest that thermotherapy combined with meristem tissue culture is a viable approach to potentially reduce viral infection in garlic without compromising plant survival. These results are consistent with the study by Sulistio et al. (2015), indicating that moderate heat treatment at around 37°C to 45°C for a period was effective in suppressing viruses without great loss of plant viability. Benke et al. (2023) also reported the successful virus elimination with low loss of plant survival by using a combined thermotherapy and meristem tissue culture treatment.

Table 1. Survival rate of Ilocos White garlic treated with thermotherapy at 50°C at different durations of exposure.

Treatment	Number of regenerated plantlets										Total	% Survival
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10		
Control	1	1	2	2	2	1	2	2	2	2	17	85.00
1 hour	2	2	2	2	2	2	2	2	2	2	20	100.00
1.5 hours	2	2	2	2	1	1	2	2	2	2	18	90.00
2 hours	1	2	2	0	2	2	2	2	2	2	17	85.00
Total											72	90.00

[B] *Bulb*

Table 2 shows the effect of varying acyclovir concentrations on the survival of the tissue-cultured Ilocos White garlic. A total of 99 Ilocos White garlic plantlets survived the acyclovir treatment out of the 100 initially inoculated. As presented in Table 2, treatments using 10, 20, and 40mg/l of acyclovir consistently exhibited complete plantlet survival, which equates to a 100% survival rate. The same result was observed with plantlets in the control group. On the other hand, a slightly lower survival rate for plantlets treated with 30mg/l acyclovir was observed. Only 19 out of 20 plantlets survived after 30mg/l acyclovir treatment, which equates to a 95% survival rate. This may be associated with the level of concentration, in which higher concentrations typically slow down plant growth or may cause phytotoxicity, as observed in the study by Nerway et al. (2020). The data gathered on the survival of the tissue-cultured Ilocos White garlic after acyclovir treatment suggests that the antiviral drug had minimal impact on the survival of the plantlets. Thus, the application of acyclovir at concentrations between 10 – 40mg/l was considered safe, as it did not interfere with the ability of the plantlets to regenerate after tissue-culture inoculation.

Plant Growth Rate

After a month of explant cultivation, the plant length and leaf width of each plant sample were measured and recorded. It is noted that contaminated samples during the cultivation period had no recorded plant length or leaf width, as measuring was no longer possible. This is because tissue culture contamination often makes

cultured tissues unfit for healthy plantlet development, often leading to pathogen infection or plantlet death.

Table 2. Survival rate of Ilocos White garlic treated with chemotherapy at varying acyclovir concentrations.

Treatment	Number of regenerated plantlets										Total	% Survival
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10		
Control	2	2	2	2	2	2	2	2	2	2	20	100.00
10mg/l	2	2	2	2	2	2	2	2	2	2	20	100.00
20mg/l	2	2	2	2	2	2	2	2	2	2	20	100.00
30mg/l	1	2	2	2	2	2	2	2	2	2	19	95.00
40mg/l	2	2	2	2	2	2	2	2	2	2	20	100.00
Total											99	99.00

[B]*Bulb*

Table 3 presents the statistical analysis of the effects of thermotherapy treatments on the growth of tissue-cultured Ilocos White garlic. Based on statistical analysis, plant length differed significantly among the different treatments ($p < 0.001$), while leaf width did not show any significant differences ($p = 0.543$). As shown in Table 3, there is a significant decrease in plant length in samples exposed to longer treatment durations (1.5 and 2 hours). This suggests a trade-off between antiviral effectiveness and plant growth. This result was consistent with other studies reporting the need to balance treatment intensity and plant growth, as prolonged exposure to high temperatures may cause stress in plant tissues (Torres et al., 2000; Ramírez-Malagón et al., 2006).

Table 3. Statistical analysis of the effect of thermotherapy treatments on the growth of tissue-cultured Ilocos White garlic.

Treatment	No. of explants	Plant length (cm)	Leaf width (cm)
Control	17	26.76^{a}	0.38^{a}
1 hour	20	26.05^{ab}	0.40^{a}
1.5 hours	19	20.74^{bc}	0.34^{a}
2 hours	17	15.72^{c}	0.36^{a}
	F-value	10.71^{**}	0.72^{ns}
	p-value	<0.001	0.543

Means with the same superscript are not significantly different.
***Significant at 0.01 level; *Significant at 0.5 level; [ns]Not significant*
[F-value]F-test statistic; [p-value]significance value

Table 4 shows the statistical analysis of the effect of varying acyclovir concentrations on the growth of tissue-cultured Ilocos White garlic. It can be observed that plantlets treated with 30mg/l acyclovir exhibited the greatest mean plant length, while plantlets treated with 10mg/l acyclovir exhibited the widest leaf width. However, statistical analysis reveals that there are no significant differences in plant length ($p = 0.660$) and leaf width ($p = 0.524$) among the varying acyclovir concentrations. These

findings suggest that the varying concentration of acyclovir did not significantly affect the growth and the specific morphological characteristics of the tissue-cultured Ilocos White garlic. This result aligns with the study by Kudělková et al. (2017), where the effects of antiviral compounds, including acyclovir, against peach viruses were examined. Findings show that acyclovir was effective in eliminating viruses and that it did not significantly affect plant growth parameters, including plant length and width.

Table 4. Statistical analysis of the effect of acyclovir treatments on the growth of tissue-cultured Ilocos White garlic.

Treatment	No. of explants	Plant length (cm)	Leaf width (cm)
Control	13	24.93	0.44
10mg/l	11	26.33	0.53
20mg/l	15	25.64	0.44
30mg/l	13	26.46	0.47
40mg/l	13	24.21	0.39
	F-value	0.605ns	0.810ns
	p-value	0.660	0.524

[ns]Not significant; [F-value]F-test statistic; [p-value]significance value

Virus Incidence Reduction

Among the 10 bulb samples subjected to treatment and tissue culture, only 5 bulbs for thermotherapy and chemotherapy setups were randomly selected for virus indexing. Each bulb has its corresponding clove representative per treatment. The bulb was used as a biological replicate, since it was presumed that cloves from the same bulb have more or less exposure to the same type of virus. It is noted that for thermotherapy, pre-treatment samples were untreated cloves or those used for the control setup, where no heat exposure was applied, while for chemotherapy, pre-treatment samples used were excess cloves after meristem excision. Upon viral detection through PCR, OYDV, GCLV, and SLV were not detected among the pre-treatment and post-treatment samples for both thermotherapy and chemotherapy. This is indicated by the absence of amplicons on the expected base pair sizes upon running the PCR products on gel electrophoresis, indicating that all samples were free from these types of viruses. On the other hand, Leek Yellow Stripe Virus (LYSV) and *Allexivirus* were both detected from samples subjected to thermotherapy and chemotherapy.

An existing thermotherapy protocol in MMSU involves the exposure of garlic cloves to a hot water bath at 50°C for 2 hours. The protocol's reported efficiency is at 53% for the elimination of OYDV and LYSV (Pascua et al., 2012). In the current study, a much shorter duration of exposure to heat treatment (1 and 1.5 hours) was evaluated to determine whether a comparable or improved virus incidence reduction could be achieved at a reduced duration of thermotherapy.

Table 5 displays the summary of the prevalence of both LYSV and *Allexivirus* on tissue-cultured Ilocos White garlic treated with thermotherapy and the corresponding virus incidence reduction rate of each treatment shown in Figure 1. All untreated samples or those

in the control group tested positive for both LYSV and *Allexivirus,* thus the 0% virus incidence reduction rate. For samples exposed to heat treatment, a 66.67%, 75%, and 0% virus incidence reduction rate against LYSV was observed for 1-, 1.5-, and 2-hour duration of exposure, respectively. Longer duration of exposure to heat treatment suggests that a higher virus incidence reduction rate can be achieved as observed between 1- and 1.5-hour duration of exposure; however, a sudden decline in virus incidence reduction rate was observed at 2-hour duration of heat exposure, as shown in Figure 1. This decline at 2 hours aligns with the principle described by Magyar-Tábori et al. (2021) that prolonged exposure to elevated temperatures can cause heat-induced physiological damage to plant tissues, reducing regeneration capacity and potentially limiting thermotherapy effectiveness. Additionally, amplicons from PCR products appeared as thicker bands on control samples compared to the amplicon bands from thermotherapy-treated samples. This suggests that LYSV is more concentrated in the control samples and less in the treated samples, indicating a possible reduction in viral load, though total virus elimination was not achieved. On the other hand, a 100% virus incidence reduction rate was observed against *Allexivirus* at exposure to heat for 1 hour, and consistently reduced virus incidence at 100% when using longer durations of heat exposure (1.5 and 2 hours).

The results obtained from this study show that for LYSV, moderately increasing the duration of exposure to heat treatment from 1 to 1.5 hours improved virus incidence reduction, with reduction rates of 66.67% and 75%, respectively, which are higher than the 53% of the garlic samples tested negative from LYSV reported by Pascua et al. (2012) employing 2 hours duration of exposure. Thus, these findings suggest that shorter duration of exposure to heat treatment (1 and 1.5 hours) can already provide substantial virus incidence reduction against LYSV and achieve complete elimination of *Allexivirus,* potentially offering a more time-efficient alternative to the existing 2-hour thermotherapy protocol.

However, one of the limitations of the study is that the thermotherapy-treated cloves were not the actual cloves used for baseline virus detection; instead, untreated cloves were used since clove samples subjected to a hot water bath may already have an altered presence of viral infection. The researchers resorted to this approach because, prior to the conduct of the study, it was presumed that cloves from the same bulb have more or less exposure to the same type of virus, and the bulb was used as a biological replicate. This approach may introduce bias since viruses in vegetatively propagated garlic are often distributed unevenly among individual cloves within the same bulb, resulting in natural variation in viral incidence and concentration prior to treatment (Cremer et al., 2021). This was further discovered in the study during the individual virus indexing of each clove. Still, the absence of direct baseline testing on treated cloves remains a limitation that may partly reflect starting differences in virus levels. Thus, in future work, it is suggested to create a protocol in which baseline data can be acquired directly from clove samples subjected to thermotherapy in order to strengthen the interpretation of virus elimination results.

Table 5. Summary of positive pre-treatment samples, negative post-treatment samples, and the virus incidence reduction rate of varying thermotherapy treatments.

Treatment	LYSV			*Allexivirus*		
	PR	PO	Virus incidence reduction rate (%)	PR	PO	Virus incidence reduction rate (%)
Control	4	0	00.00	4	0	00.00
1 hour	3	2	66.67	3	3	100.00
1.5 hours	4	3	75.00	4	4	100.00
2 hours	2	0	00.00	2	2	100.00

[PR]Number of positive pre-treatment samples; [PO]Number of negative post-treatment samples; [LYSV]Leek Yellow Stripe Virus

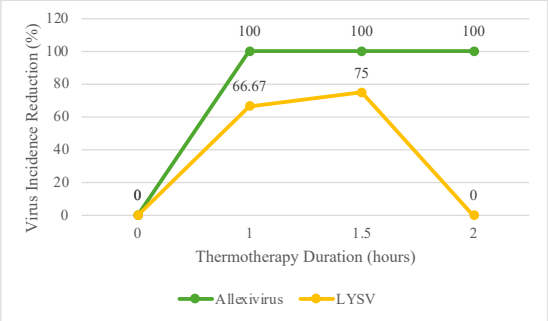

Figure 1. Virus incidence reduction in heat-treated Ilocos White garlic at 50°C for different time durations.

On the other hand, virus elimination in plants through chemotherapy involves the use of antiviral drugs, one of which includes ribavirin. Although virus elimination efficiency using ribavirin is promising, several studies have reported that the growth of explants exposed to ribavirin treatment has been reduced. A study by Benke et al. (2023) reported that garlic plants treated with ribavirin through tissue culture exhibited inferior growth. This result also conforms to that of Karjadi et al. (2022), who reported that the addition of ribavirin in MS media reduced the percentage growth of potato explants. With the conflicting virus elimination efficiency and plant growth effects of ribavirin, the current study utilized acyclovir for chemotherapy. Acyclovir, a much affordable antiviral drug than ribavirin, has shown promising results in eliminating viral infection in several crops. This includes the results reported by Pavelkova et al., (2017) reporting that acyclovir was efficient in eliminating peach viruses; Rizk et al., (2021) demonstrating the effectiveness of acyclovir at different concentrations (200, 400, and 800mgl^{-1}) in reducing the concentration of Potato Virus Y; and Dewanti et al., (2016) showcasing the potential of acyclovir at 20 and 40mgl^{-1} concentration in the 100% elimination of Sugarcane Mozaic Virus.

In the current study, the effect of acyclovir on the incidence of virus in tissue-cultured Ilocos white garlic was evaluated. Table 6 summarizes the presence of LYSV and *Allexivirus* on samples treated with varying concentrations of acyclovir, and the rate of virus incidence reduction is presented in Figure 2. For *Allexivirus,* all acyclovir treatments exhibited the ability to reduce virus incidence. Particularly, a higher virus incidence reduction rate (100%) was observed at treatments with higher acyclovir concentrations (20, 30, and 40mg/l) compared to the 66.67% and 60% reduction rates observed at control and 10mg/l treatments, respectively, as shown in Figure 2. This suggests that

acyclovir is effective in suppressing or eliminating *Allexivirus* at higher concentrations. In contrast, lower concentrations, such as 10mg/l, the prevalence of virus after treatment indicates partial but not complete virus suppression. These results conform with those of Kidulile et al. (2018), stating that higher concentrations (30 and 40mg/l) of chemicals, specifically salicylic acid and ribavirin – chemicals used for virus elimination studies – were the most effective concentrations for the elimination of East African Mosaic Virus and Dahlia Mosaic Virus, suggesting that virus elimination efficiency of chemical treatments is influenced by concentration levels. For LYSV, a less pronounced result was observed. As shown in Table 6, virus incidence reduction was observed at certain acyclovir concentrations, specifically at 10mg/l, exhibiting a 75% reduction rate. However, most treatment groups showed only minor virus incidence reduction or none at all. The lower efficacy of the treatment against LYSV may be associated to high initial virus titre as observed on the PCR products (Figure 3) with thicker amplicon bands compared to that of the *Allexivirus.* The study by Benke et al. (2023) also reported that failure to eliminate certain viruses may possibly be due to the high initial titre of the virus. Moreover, this interpretation aligns with the study by Conci et al. (2002), who reported that the relative

concentration of LYSV in garlic differs based on several parameters, including sampling time, cultivar, and region, and that virus concentration fluctuates throughout the crop cycle and bulb storage.

The observed virus incidence reduction using acyclovir aligns with the report of Panattoni et al. (2013), indicating that most of the antiviral compounds used in plant virus elimination belong to inhibitors of neuraminidase (NA), inosine monophosphate dehydrogenase (IMPDH), and inhibitors of S-adenosylhomocysteine dehydrogenase (SAH). Antivirals used in plant virus elimination are not viricidal in action, but they work by inhibiting viral replication, which plays an important role in halting the spread of viral infection in plants (Rizk et al., 2021). In this study, acyclovir effectively lowered viral incidence in tissue-cultured Ilocos White garlic, offering a more cost-effective alternative to other antiviral drugs such as ribavirin, given ribavirin's limited local availability in the local market. Locally accessible and more affordable acyclovir supports practical use without compromising plant stability, as evidenced by non-significant growth rate differences between control and chemotherapy-treated garlic plants.

Table 6. Summary of positive pre-treatment samples, negative post-treatment samples, and the virus incidence reduction rate of varying acyclovir treatments.

Treatment	LYSV			*Allexivirus*		
	PR	PO	Virus incidence reduction rate (%)	PR	PO	Virus incidence reduction rate (%)
Control	4	1	25.00	3	2	66.67
10mg/l	4	3	75.00	5	3	60.00
20mg/l	3	0	00.00	3	3	100.00
30mg/l	4	0	00.00	3	3	100.00
40mg/l	4	1	25.00	3	3	100.00

[PR]Number of positive pre-treatment samples; [PO]Number of negative post-treatment samples; [LYSV]Leek Yellow Stripe Virus

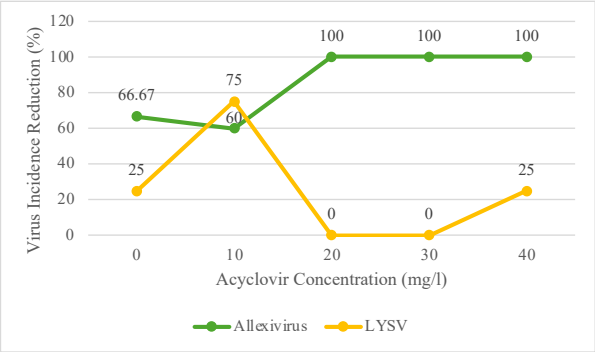

Figure 2. Virus incidence reduction in Ilocos White garlic treated with different concentrations of acyclovir.

Overall, results for both thermotherapy and chemotherapy treatments against *Allexivirus* were more pronounced than in LYSV, suggesting that under the tested conditions, LYSV appeared less responsive to the virus elimination treatments. In contrast to the results of the present study, Mang et al. (2022) and Benke et al. (2023) reported that *Allexivirus* was more difficult to eradicate than potyviruses, including LYSV. However, Benke et al. (2023) also reported that the difficulty in eradicating *Allexivirus* may be associated with the high initial titre of the virus in the samples. In the case of the current study, the initial virus titre of LYSV may have also affected its resistance to the virus elimination treatments, while *Allexivirus* could have had a lower initial titre, which accounts for its higher virus incidence reduction rate.

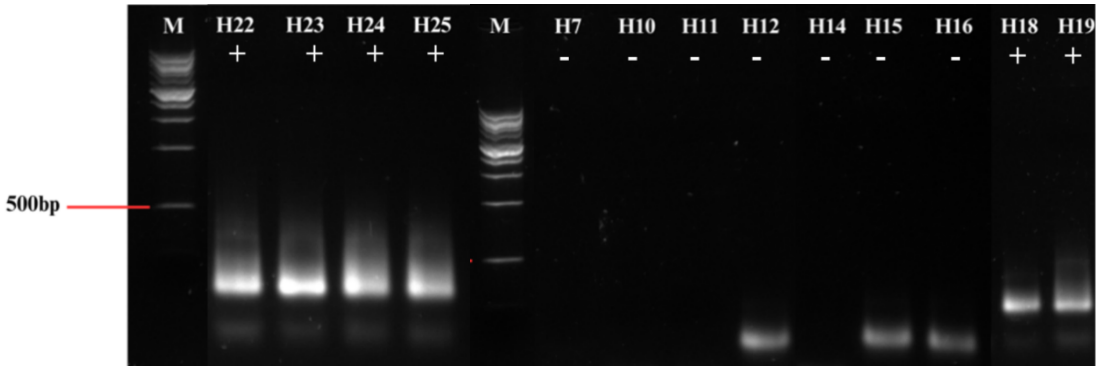

Figure 3. PCR products for the amplification of LYSV. M: 1 kb DNA ladder, H22 – H25: negative controls, H7 – H19: positive controls exposed to heat treatment in varying time durations.

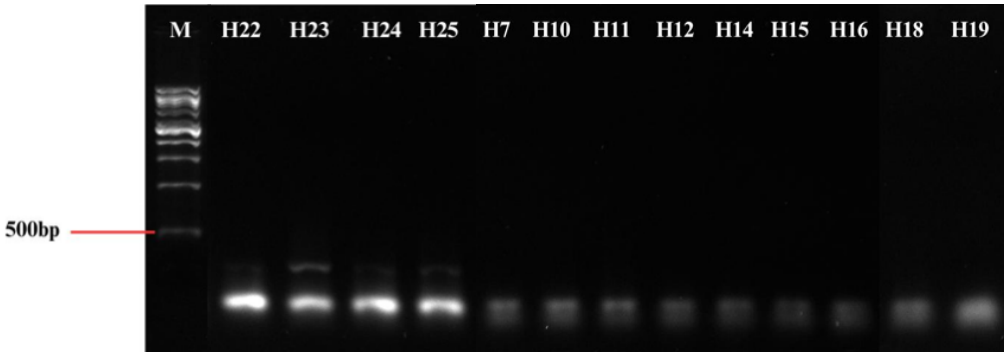

Figure 4. PCR products for the amplification of *Allexivirus*. M: 1 kb DNA ladder, H22 – H25: negative controls, H7 – H19: positive controls exposed to heat treatment in varying time

durations.

Figure 5. Garlic plantlets positive for LYSV and *Allexivirus* one month after application of treatments.

Additional Findings

During the virus indexing of each clove, some cloves from the same bulb tested negative for the virus being detected, while the remaining cloves tested positive. As shown in Figure 6, 8 out of 42 cloves tested negative for *Allexivirus,* comprising 19.05% of the total cloves tested. On the other hand, 10 out of 39 cloves tested negative for LYSV, which accounts for 25.74% of the total cloves tested. This result suggests that not all cloves coming from the same bulb contain the virus. This indicates that there is a differential viral load distribution within a bulb. This result is consistent with studies reporting unequal distribution of viral infection in plants. A highly uneven distribution of the Tomato spotted wilt virus (TSWV) was recorded in dahlia bulbs in a study by Asano et al. (2017). Differential organ infection by viruses has also been reported in garlic. In a study by Ramírez-Malagón et al. (2006), the presence of potyviruses such as Onion Yellow Dwarf Virus (OYDV) and Leek Yellow Stripe Virus (LYSV) was reported to have differential distribution in all organs of the same plant. Additionally, their results revealed that several cloves tested positive for potyvirus, while others from the same bulb tested negative. This finding in the present study could potentially help in speeding up the delivery of virus-free planting materials to farmers.

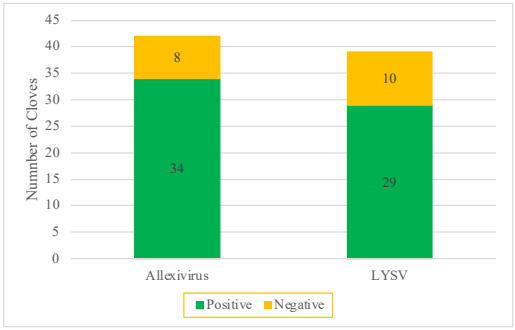

Figure 6. Total differential viral load for *Allexivirus* and LYSV in the Ilocos White garlic cloves.

Conclusion

The results of this study show that chemotherapy using acyclovir and thermotherapy using a water bath

combined with meristem tissue culture showed potential in reducing the presence of viral infection, specifically LYSV and *Allexivirus,* in tissue-cultured Ilocos White garlic. Among the treatments involved in this study, the treatment that balances survivability, growth, and virus incidence reduction rate in tissue-cultured Ilocos White garlic was considered. As results showed, thermotherapy at 50°C for 1 hour is optimum for virus incidence reduction for both LYSV and *Allexivirus*. On the other hand, chemotherapy using acyclovir is optimum at 20mg/l for the complete removal of *Allexivirus.* The optimum duration of thermotherapy and concentration of chemotherapy are determined by their ability to effectively reduce virus incidence without compromising the growth and development of the tissue-cultured garlic. Moreover, the growth of tissue-cultured Ilocos White garlic is significantly the same as the control at different chemotherapy concentrations and thermotherapy at 50°C for 1 hour. Lastly, the results of the study determined the presence of differential viral loads per bulb. Cloves from the same bulb do not have the same viral load.

Anchored on the findings, the combined use of 20mg/l acyclovir and 50°C thermotherapy for 1 hour should be explored for their combined potential in reducing viral incidence in tissue-cultured Ilocos White garlic. The distribution of viral loads in different parts of the garlic plant at a specific stage and time after harvesting should also be assessed, as well as screening for cloves that do not contain viruses to potentially speed up the distribution of virus-free planting materials to local garlic farmers. Finally, the conduct of a field trial is further suggested to assess and determine the stability of the tissue-cultured Ilocos White garlic with reduced viral incidence.

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