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Chemotherapeutic Effects of the Extracts of Allium cepa and Allium sativum on Infectivity of Tobacco Mosaic Virus

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CHEMOTHERAPEUTIC EFFECTS OF THE EXTRACTS OF *ALLIUM CEPA* AND *ALLIUM SATIVUM* ON INFECTIVITY OF TOBACCO MOSAIC VIRUS

BY

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VITA

The author, Min-Lan Chang, daughter of D. L. Chang and J. J. Chang, was born on December 20, 1954 in Seoul, Korea.

She received a secondary education from Overseas Chinese School in Korea, and she graduated in 1972 from Cathedral High School for Girls in San Francisco, California.

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In September, 1977, she enrolled in Loyola University in Chicago graduate school, under the direction of Dr. A. S. Dhaliwal, as his research assistant and laboratory instructor. In the summer, 1980, she presented a paper at 'Botany 80' convention at the University of British Columbia on "Chemotherapeutic Effects of the Extracts of Allium Species on TMV Replication," under the sponsorship of Professors A. S. Dhaliwal and W. C. Cordes.

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INTRODUCTION

Lwoff (1957) proposed that viruses are strictly intracellular and potentially pathogenic entities. With an average size of .1 um and a minimum of 10^{-5} fold concentration, viruses are capable of infecting animals, plants, bacteria, algae and fungi. Most living creatures can suffer from viral disease.

Efforts have been made throughout the world to find the suitable antiviral agents. A large number of compounds have been synthesized as potential antiviral agents but only a few have showed antiviral activity. These have a very limited clinical application. Both inhibitors of RNA synthesis (antinomycin D, 8-azaguanine, thiouracil), and inhibitors of protein synthesis (guanidine carbonate and chlormaphenicol) have been reported to have antiviral effects. But to date there is no chemical agent available which could destroy viruses without a destructive effect on the host. Plants that are known to have medicinal value have been credited for having antiviral activity, but no conclusive studies on these have been reported.

The purpose of the present study was to investigate the antiviral effects of the extracts from two plant species Allium cepa and Allium sativum on the replication of Tobacco Mosaic Virus (TMV), which was preceded by a bioassay test to examine its infectivity on ten day-old primary leaves of

Phaseolis vulgaris cv pinto.

I. REVIEW OF LITERATURE

The following is a review of the literature of previous investigators and researchers (demonstrated the effects of chemical agents on the replication of viruses in living cells.)

A. Characteristics of Virus Replication in Living Cells

1. Structural Features of Viruses

Early in 1935 the electron microscope revealed the structural details of verion, the rod shaped TMV was successfully crystallized by Stanley (1935) (Figure 1).

Casper (1963) estimated that the length of the TMV virion was 2980 Å and that the average molecular weight of TMV RNA was 2.05×10^6 daltons.

Gierer and Schramm (1956) used a phenol procedure to denature protein and to isolate TMV nucleic acid from TMV, and reported that purified RNA was infectious.

Yamazaki and Kaesberg (1963) reported the isolation of the protein coat of brome mosaic virus using 1M calcium chloride.

Frankel-Conrat (1956) demonstrated the reconstitution of TMV RNA from one strain of virus with protein from another strain and showed that this reconstituted virus was

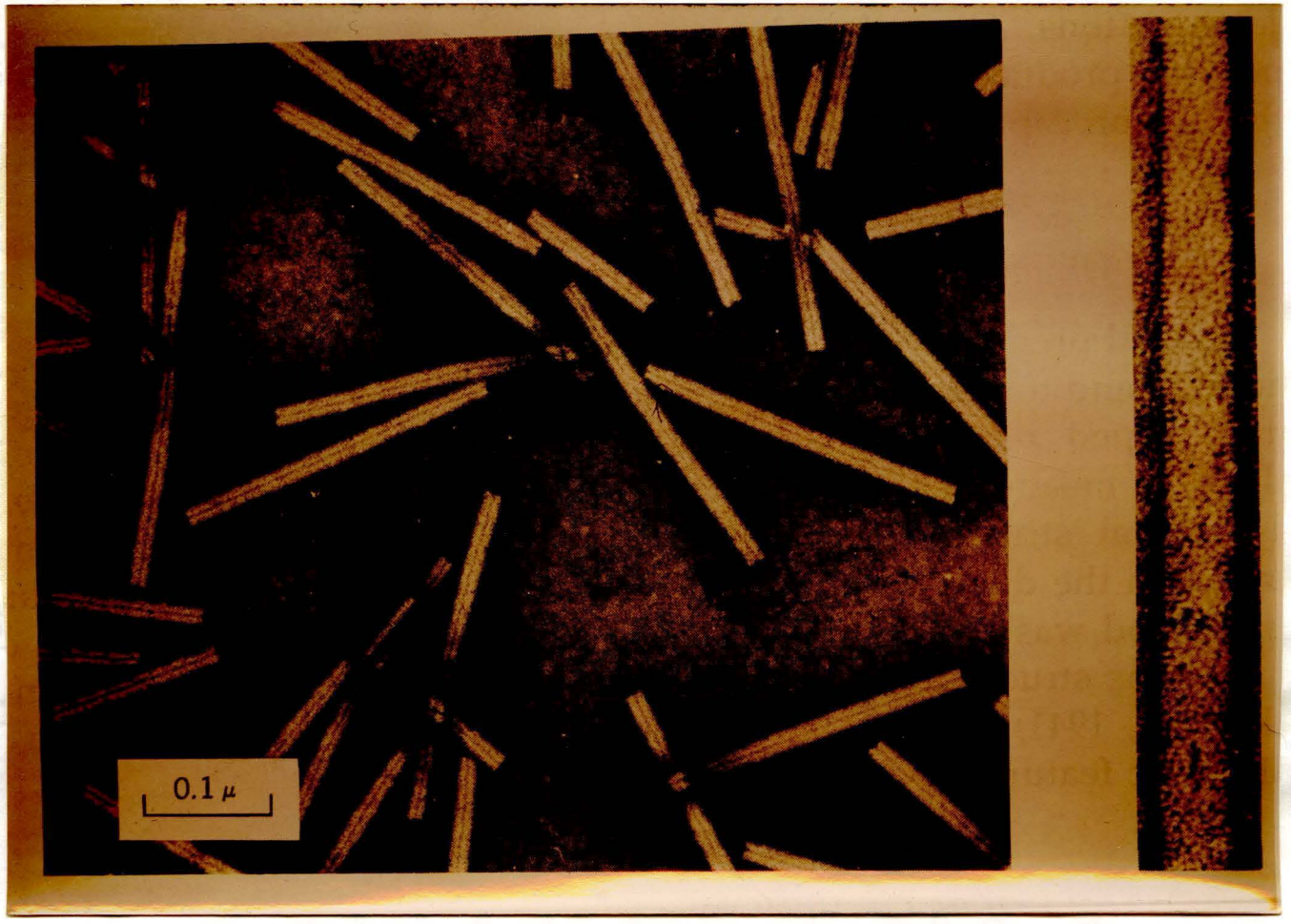


Figure 1. Purified Tobacco Mosaic Virus was shown by phosphotungstic acid negative contrast. It has average 3980 Å in length. One segment is seen end-on, showing the axial hole.

infectious.

Luria and Darnell (1967) stated that viruses were elements of nucleic acid that replicate inside the living cell using cellular synthetic mechanisms and cause the synthesis of specialized elements that can transfer the viral genome to other cells.

Motoyoshi and Oshima (1968) demonstrated that isolated tobacco leaf cells could be infected with TMV by mechanical stirring. Takebe et al. (1968) also isolated mesophyll cells from TMV-infected tobacco plants and found that isolated cells supported the replication of virus.

2. Virus Isolation and Local Lesion Assay

Bawden (1964) demonstrated that 10 day-old bean leaves produce local lesions if inoculated with tobacco necrosis virus. Bean leaves older than 10 days are less susceptible ;o virus. Schein (1965) found that bean leaves were low in susceptibility to TMV when they were fully unfolded but susceptibility increased when the leaves were expanded about one third.

Venekamp and Mosch (1964) have described a procedure for isolation of several viruses by means of columns of cellulose powder combined with a mixture of polyethylene glycol, dextran, glucose and salts.

Suitable buffers of ionic strengths for viruses can

preserve the infectivity. Tomlinson (1963) used phosphate buffer which failed to give infectious preparation of Lettuce Mosaic Virus, while borate buffer gave highly infectious preparation. Thornberry (1935) reported that diabasic phosphate salt in the inoculum greatly increased infectivity of TMV. This has been repeatedly confirmed.

Holmes (1931) stated that the starch content of the cells in the lesion may differ from that in the uninfected cell. At the beginning of a photosynthetic period and of the end of dark period, lesions may contain more starch than at other times.

Wu et al. (1969) suggested that very small sizes of necrotic lesion produced by TMV in pinto may be due to the rapid deposition of cellulose in the necrotic cells with the spread of virus.

Diener and Jenifer (1964) reported that if Chinese Cabbage was grown in nitrogen deficient culture, it showed well defined purple local lesions, which were more readily detected than chloratic lesions produced by the common strains of Tobacco Mosaic virus.

3. Metabolism of Plants Infected With Viruses

Langenberg and Schlegel (1967) reported that the TMV distribution could be conveniently detected by using I^{125} labelled specific antibody. Specific antibody binding was

seen in the nucleus 6 hours after inoculation. Some specific binding also occurred in the cytoplasm at that time.

In an attempt to obtain direct evidence on the timing of the uncoating process Reddi (1966) inoculated tobacco leaves with intact TMV labeled with P^{32}. He followed the time course occurrence of the appearance of RNA that was sensitive to ribonuclease degradation. About 1% was sensitive at zero time. About 2.5% was detected at 3 hours after inoculation. A significant rise (about 5%) appeared at 6 hours, after which the rate of increase decreased.

Israel and Ross (1967) observed an increased amount of endoplasm (i.e. reticulum-associated ribosomes) in the regions of local lesions, induced by TMV.

Matthews (1970) described the physiological changes in virus-infected plants. These were: a) decrease in the rate of photosynthesis, b) increase in respiratory rate, c) increase in the activity of metabolic enzymes, d) decrease in the response to plant growth regulators.

The physiologically active gas, ethylene, was detected in plants infected with potato virus Y. Intact leaves from healthy plants produced practically no gas (Ross and Williamson, 1951).

Merrett and Sunderland (1967) found that increased respiration in Xanthi tobacco leaves showed necrotic local lesions carries respiration through both the glycolytic and pentose phosphate pathways.

Fry and Matthews (1963) followed the change in total RNA content of tobacco leaf epidermis. The mechanical inoculation with TMV induced a rise and fall in RNA content over the first few hours. At about 6-8 hours after inoculation there was a 10% rapid rise of RNA content per unit of epidermis. This rise occurred at least 3-5 hours before a rise in infectivity was noticed.

B. Environmental Factors Affecting the Infectivity of Virus

Matthew (1970) stated that greenhouse-grown plants had greatest susceptibility when they were grown and used under the following conditions: 1) mineral nutrition and water supply that do not limit growth; 2) moderate to low light intensities; 3) a temperature in the range of 18C-30C in virus and host; 4) inoculation carried out in the afternoon.

Bawden and Roberts (1948) demonstrated that shading the bean plants for about 24 hours before inoculation increased the susceptibility to TMV. High light intensities may change the kind of lesion produced by potato virus X in Glutinosa globosa, and may also lead to the appearance of spontaneous virus-like lesions (Francki, 1967).

Preincubation of Tobacco and Bean plants at somewhat higher temperature than normal before inoculation increases susceptibility (Gonzalez and Pound, 1963). Temperature may also affect the speed and efficiency of grant transmission

for ring-spot virus in Prunus tomentosa at an optimal temperature of 30°C, at either higher or lower than 30°C temperature, the graft transmission for ring-spot virus marked fall in efficiency (Fridlund, 1967a).

Kimmins (1967) reported that bean plants in an atmosphere with high relative humidity before inoculation with tobacco necrosis virus increased their susceptibility compared with plants held at lower humidities.

Kassanis (1953) investigated the effects of various levels of nitrogen, phosphorus and potassium on susceptibility of tobacco N. glutinosa plants to strains of TMV. A deficiency of these nutrients may inactivate the TMV replication.

Bald and Samuel (1934) investigating tomato spotted wilt virus, found that the rate of loss of infectivity was increased by aeration and retarded in an atmosphere of nitrogen.

Increased levels of carbon dioxide in the atmosphere reduced the susceptibility of bean plants to tobacco necrosis virus (Kalmus and Kassanis, 1944).

The effect of time of day of inoculation by tobacco necrosis virus in beans was investigated by Matthews (1953a), who reported that the highest number of lesions were produced between 2 and 4 P.M.

In the winter, plant host produced substantial numbers of necrotic local lesions, as reported by Bhargava (1951), than

in any other seasons.

C. Effects of Physical Agents Inhibiting the Replication of Viruses

Goodheart (1969) explained three features of viral inactivation: a) denaturation of nucleic acid results in strand separation due to breakage of the hydrogen bonds between the two strands; b) ability to inhibit the virus may also be affected by molecular weight of the virus; c) each stage of host cell metabolism has different capability for sustaining viral infection.

Zelle (1954) demonstrated the action spectra of ultra-violet light; the most efficient wavelength for inactivation is around 2,600 Å.

Freifelder (1965) demonstrated convincingly that x-irradiation breaks one or both strands of the DNA molecule. The effect of x-rays on TMV showed that viscosity of RNA decreased as x-ray dose increased (Lauffer, 1956).

Effects of various pH's on tomato spotted wilt virus (TSWV) infectivity was reported by Best (1968). TSWV was inactivated below pH 5 and above pH 10. The nature of the protein coat and RNA played a significant part in the pH stability between 7 and 8.

Huguelet and Hooker (1966) found that periods of darkness immediately after inoculation delayed the appearance

of lesion by about the length of 14 days dark period and number of lesions appearing was reduced.

Nienhaus and Yarwood (1963) reported that heating one primary leaf of pinto bean on the plant to a temperature of 70°C for 20 seconds killed the leaf and increased number of lesion production. Comparison to 50°C for 1 min. heat treatment.

D. Effect of Chemotherapeutic Agents on the Replication of Virus

Matthews (1951) demonstrated the effects of substituted purines on development of virus infection. In 1952 he showed that guanazola interfered with incorporation of guanine in the virus, and at the same time adenine or guanine could promote replication of the virus. He named this 'reversible activity.'

Schneider (1954) investigated the effect of purines and stated that 8-azaadenine and 8-azaguanine inhibited TMV multiplication.

Commoner (1951) reported the inhibition of the biosynthesis of TMV by thiouracil. This inhibitory effect was partially reversed by uracil that blocked the synthesis of TMV by interfering with some aspect of uracil metabolism.

Nichols (1953) demonstrated that in the absence of thiouracil more virus was produced in light than in dark.

In 1954, Hare and Lucas, using proteins from bovine milk and blood serum, discovered that casein and crude whey protein fraction at pH 9-11 were strong inhibitors of TMV.

In 1954 Linder and Kirkpatrick showed that stone fruit and TMV was markedly inhibited by chloramphenicol, thiouracil and guanazola, as measured by number of local lesions produced in leaves of cucumber plants and tomato seedlings.

In 1968 Hirai et al. isolated blasticidine from streptomyces and reported that inhibits amino acid C^{14} on peptid chain in cytoplasmic, chloroplast and mitochondrial proteins of TMV infected tobacco leaves. They also stated that due to the protoplasmic protein inhibition of TMV RNA was also reduced after infection.

Verma (1968) reported that when guanidine carbonate was introduced to the leaf cell at early stages of infection it prevented the synthesis of RNA and protein, possibly by blocking the formation of virus-induced RNA polymerase.

Dhaliwal and Rudd (1976) reported that Phaseolus vulgaris grown in a medium containing manganese was three times more susceptible than the control plants. Bean plants grown in a medium containing dimethylsulfoxide with manganese were twice as susceptible as the controls.

Horne and Waterson (1960) used ether treatment on some parainfluenza viruses and found it dissolved the virus coat.

Strong salt solutions such as ammonium sulfate tend to reduce the infectivity of rod-shaped viruses by causing

aggregation of particles (Pirie, 1954).

From the results of kinetic studies conducted by Cartwright etc. (1956) on the loss of infectivity of TMV, it was shown that the inactivation of a single virus particle could be brought about by reaction with one formaldehyde molecule.

The organic acids at concentrations around 0.1M, when sprayed on the leaves of bean plants before or up to about 2 hours before inoculation, greatly reduced the number of local lesions produced by a tobacco necrosis virus (Matthews and Proctor, 1956).

Urea, known as a protein denaturant, is thought to act by weakening both hydrogen bonding and hydrophobic interactions in proteins, and causes a loss of infectivity of TMV (Kauzmann, 1959).

E. Effect of Antibiotics on the Replication of Viruses

Schuster and Schramm (1958) followed the rate of loss of infectivity and the rate of deamination of adenine, guanine and cytosine. They concluded that deamination of one nucleotide in about 3000, without splitting the RNA chain, was sufficient to inactivate the whole TMV RNA.

Hakala (1959) showed bromodeoxyridine, when substituted for thymidine in DNA, inhibited the growth of Rous sarcoma virus. That growth of RSV can be inhibited by actinomycin D

and mitomycin D was reported by Bade in 1965. Barry (1962) stated that actinomycin D, blocking the function of cellular DNA, affected the replication of two myxoviruses (influenza and New Castle disease virus). In 1965 Fisher etc. stated that actinomycin D prevented the initiation of the poliovirus RNA replication.

Kirkpatrick (1954) reported that chloramphenical was active against the multiplication of some varieties of gram-positive and gram-negative bacteria genus, which includes streptococcus, staphlococcus, escherischia bacillus.

F. Inhibitory Effects of Plant Extracts on the Replication of Virus

The presence of inhibitors in plant extracts has been known since Duggar and Armstrong (1925) described the inhibition of TMV infectivity by extracts of Phytolacca decandra. Thornberry (1935) found that the degree of inhibition depended on the concentration of tannic acid and on the time in contact with the virus.

Bawden and Kleczkowski (1945) found that extracts of leaf, stem and root of strawberries contained material, presumably tannin, that precipitated proteins from extracts and led to noninfectious TMV on N. glutinosa.

Kuntz and Walker (1947) described two inhibitors in spinach juice; one was absorbed by charcoal, the other was

removed by adding $CaCl_2$ and filtering. It was known as exalate which probably prevented replication of TMV.

Sap from Phytolacca esculenta was found to have an inhibitory effect on TMV infection. Chemically it was found to be glycoprotein (Kassanis and Kleczkowski, 1948).

Francki (1964) reported that extracts of cucumber leaves reduced infectivity 100 times when mixed with purified cucumber mosaic virus. The inhibiting material precipitated the virus.

Extracts of leaves from pepper, geranium and jimson weed inhibited the development of local lesions induced by TMV inoculum on pinto leaves (Apablaza and Bernier, 1972).

Patil (1973) used extracts of cucumber leaf, rose leaf and diluted milk as inhibitors and inactivators to control virus (TMV). Extracts of these plants have over 89% inhibitory effect, whereas treatment from diluted milk resulted in complete reduction in local lesions.

In 1970 Dhaliwal etc. demonstrated the inhibition of TMV infectivity by extracts from Allium species. Attempts were made to isolate inhibitory components from the extracts by centrifugation forces. The sap was centrifuged at 6000 rpm and it was found that both the pellet and the supernatant had inhibitory effects on TMV infectivity.

In 1971 Chen isolated a compound from roots of pea plant which was an effective inhibitor of E. coli multiplation and some other bacteria.

Abdou (1972) reported the antimicrobial activity of the crude juice of Allium species on E. coli, Salmonella typhi and Bacillus subtilis. Later in the year, he demonstrated that extracts of Allium species contained protein, salts, carbohydrates, fats and vitamins with antimicrobial activities which can prevent the disease, causing certain bacteria.

METHODS AND MATERIALS

A. Isolation of Tobacco Mosaic Virus

A common strain of TMV (PV-135) was obtained from American type culture collection, Rockville, Maryland. Thirty day old plants of Nicotiana tabacum cv turkish were inoculated with TMV and the plants were incubated for 60 days. The infected leaves (Figure 2) from the inoculated plants were harvested and macerated in a Waring blender with 10% polyethylene glycol (PEG) and 4% sodium chloride. Macerated plant material was passed through a cheese cloth to separate fibrous material from the sap. The sap was centrifuged (at 1,000 G for 20 minutes) in an IEC (International Equipment Co.) clinical centrifuge. The supernatant was then passed through a microcrystalline cellulose column to isolate the virus by using 10% PEG and 4% NaCl as solvents in the chromatagraph (Figure 3). The impurities were removed with 5% PEG and 2% NaCl, and the virus from the column was eluted with the mixture of 5% PEG and 0% NaCl. The 5-0 solution then was centrifuged in a Sorvall centrifuge for 10 minutes at 6,000 rpm. The supernatants were discarded and the pellets were suspended in pH 7 buffer solution. Buffers were prepared by mixing 0.1M of Dibasic potassium phosphate (K_2HPO_4) and 0.1M of monobasic potassium phosphate (KH_2HPO_4)

Figure 2. Systematically infected Nicotiana Tobaccum cv Turkish leaves by Tobacco Mosaic Virus during last 60 days incubation.

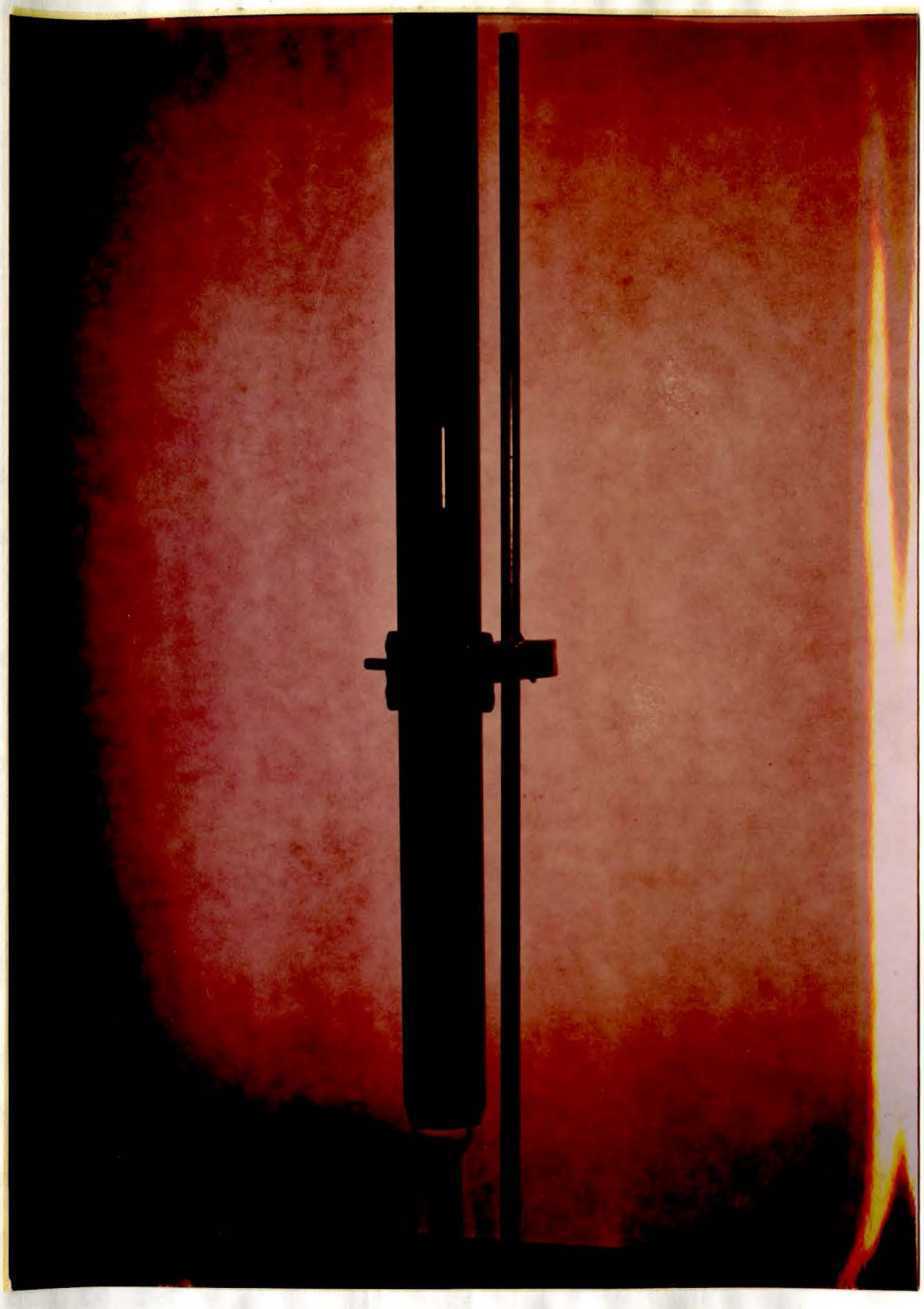


Figure 3. Microcrystalline cellulose column to eluting purify TMV by chromatography.

pH 7.2. The buffer containing virus was centrifuged in an ultracentrifuge (Sorvall oil Turbine Drive #2) at 40,000 rpm for one hour. The pellets obtained with ultracentrifuge were dissolved in buffer. This was the pure virus and was refrigerated for future use.

B. Cultivation of Allium Cepa and Allium Sativum

The bulbs of Allium cepa (onion) and Allium sativum (garlic) were grown in pots (6 X 8 in) by using sandy loam potting soil and horticultural vermiculite (Figure 4 , 5). The bulbs after planting were kept in a growth chamber at 30°C and 70% relative humidity (RM) and supplied with constant artificial light (2,000 Lux). After 25 days of growth all the pots with the bulbs were transfered to the greenhouse where 25°C and 50% RH were maintained with naturally supplied light.

C. Preparation of Extracts from Allium Species

After 30 days of growth the leaves of Allium plants were harvested. Leaves were washed thoroughly (Figures 6 and 7), then cut into pieces and ground with pestle and mortar. This ground material was then passed through two layers of cheese cloth to remove fibrous material from the sap. The sap from the bulbs was also extracted in the same manner, except the bulbs for this purpose were harvested 120 days after

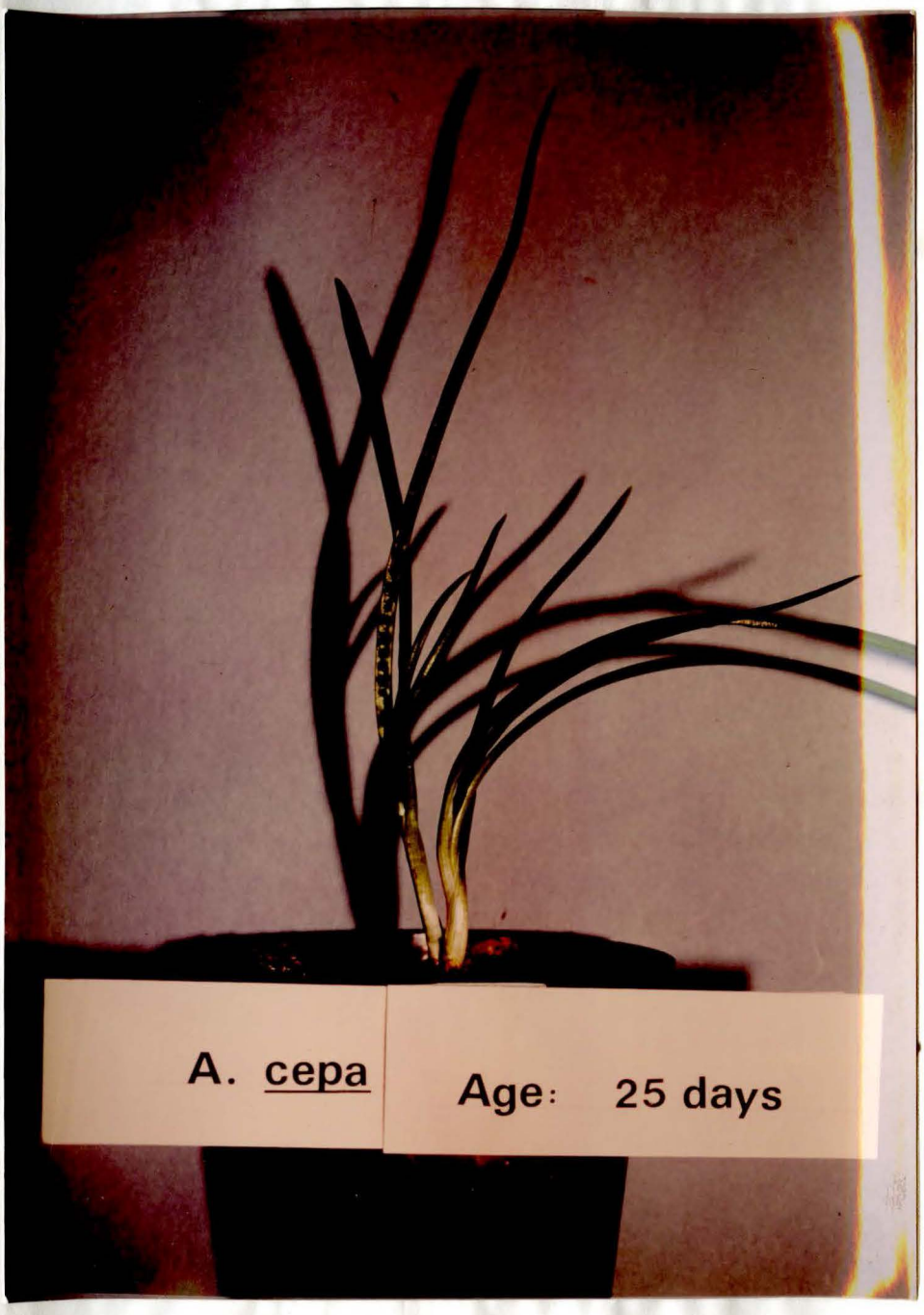


Figure 4. Extensive growth of leaves was shown from bulb of Allium cepa after 25 days growth period. This was cultivated under moderate light intensity, constant supply of H_2O and basic soil nutrient in the soil.

Figure 5. Extensive growth of leaves was shown from bulb of Allium sativum after 25 days growth period. This was cultivated under moderate light intensity, constant supply of H_2O and basic soil nutrient in the soil.

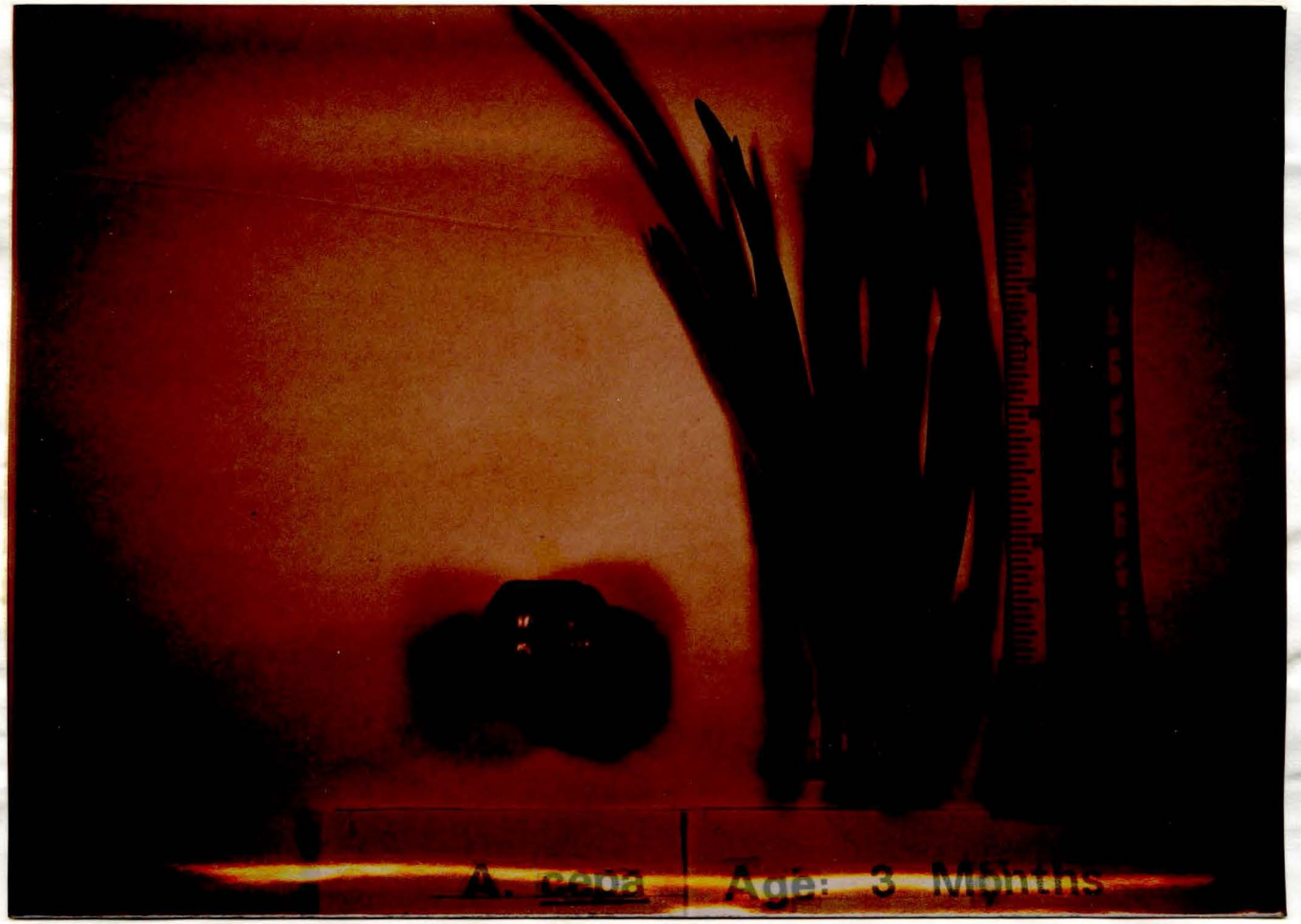


Figure 6. The leaf and bulb of Allium cepa were able to be collected at the same time by end of third month period. They then may be ready for preparation of extracts.

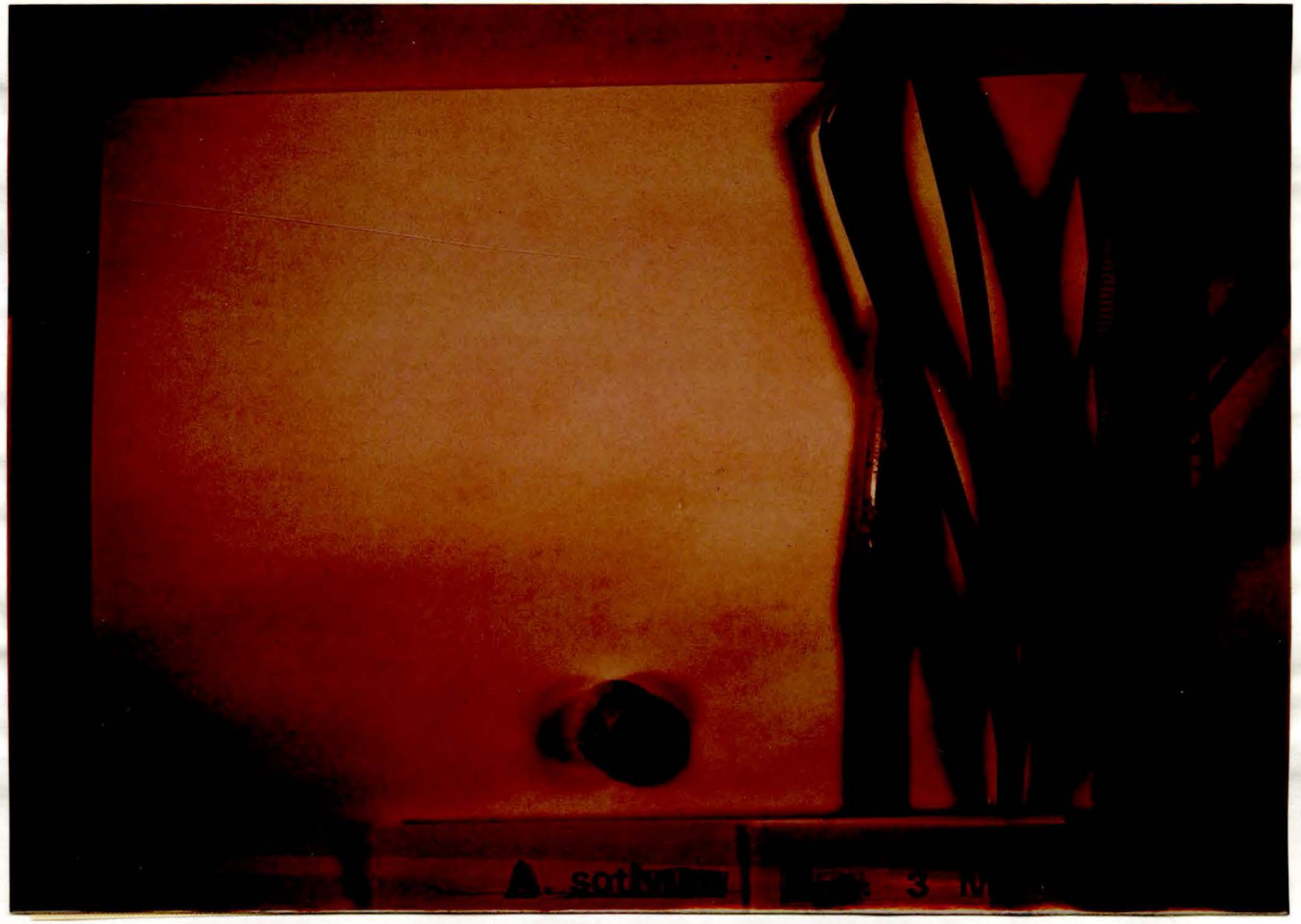


Figure 7. The leaf and bulb of Allium sativum were able to be collected at the same time by end of third month period. They then may be ready for preparation of extracts.

planting.

D. Preparation of Assay Host

Seeds of Phaseolus vulgaris cv pinto were germinated in trays (6 X 20 X 30 in) using sandy loam soil in the growth chamber at 30°C and 50% RH. When seeds germinated, the trays were removed from the growth chamber and placed in the greenhouse for further growth. At the end of 10 days the fully expanded primary leaves of pinto beans were harvested. The leaves were given hot water (45°C) treatment for 1 minute, and the midribes of leaves were cut off. Half leaves were placed randomly on 1% agar trays. The leaves were blotted dry and dusted with 320 mesh carborundum by placing the tray in a wooden chamber especially built for this purpose.

E. Bio Assay of Sap-Treated TMV

The modified bioassay procedure of Dhaliwal (1970) was used. The virus was diluted to 10^{-4} with pH 7 buffer to give 50 to 100 lesions per half leaf. This dilution was used in all the experiments. The concentrated sap from both leaves and bulbs of the species A. cepa and A. sativum were diluted tenfold with distilled water.

The initial dilution was prepared by mixing 1 ml of sap with 9 ml of water. Saps diluted 10^{-1} to 10^{-5} were used for TMV treatment. Each of five dilutions of plant extracts and

TMV were mixed with a Vortex genie mixer, then assayed every 15 minutes for 1 hour. After 1 hour the mixtures were assayed every hour for 5 hours. These samples were assayed for infectivity on six half leaves of assay host P. vulgaris.

F. Assay Procedure

The method of Lamborn and Cochron (1969, 1971) was used to inoculate bean leaves with sap treated TMV. A drop of treated TMV with sap was placed at the tip of carborundum dusted half leaves with a Pasteur pipette. The leaves were then placed on a foam sponge (4 X 2 X 1 in.), which gives a cushion support to the leaves and limits the damage at the time of inoculation. The leaves were inoculated by passing cushion supported leaves under an ultrasonic probe (using #4 power Level, Branson sonifier cell distruptor) (Figure 8). The pressure of the leaf (using #4 power) against the probe, the speed of an inoculation passage and the power of the ultrasonic energy may influence the sensitivity of the bioassay. Therefore, even pressure and constant speed of an inoculation were started from tip towards the base of the leaf.

G. Incubation of Inoculated Leaves

Each set of six inoculated half-leaves were placed between two paper strips and were incubated in air-tight

plastic containers at 30°C for 18 hours. These incubated leaves were then placed on 1% agar in trays covered with glass plates and were then incubated for another three days at 25°C under 200 ft candles light intensity. Viruses-caused lesions at the end of this incubation were counted with a binocular microscope (Figure 9 and 10). To determine the inhibitory effect of saps the data were analyzed statistically (Appendix A and B).

H. Separation of Inhibitory Compound by Dialysis

Attempts were made to fractionate the sap by dialysis in order to separate the particular inhibitory compounds of the sap which might be responsible for the inhibition of the TMV infectivity. The sap mixed with 50% H_2O was centrifuged at 15,000 rpm for 2 hours in the ultracentrifuge. The supernatant was then placed in the dialysis tubing. This dialysis tubing was placed in the beaker (Figure 11) containing the same volume of distilled water as volume of supernatant of sap in the tube. The dialysis tube in the beaker was stirred constantly with the magnetic stirrer (F. H. Sargeant & Co.) at 5°C to prevent contamination and to promote better distribution of dialysis. The dialysates were collected every 12 hours and retentates were dialysed again with the addition of the same amount of H_2O as before. At the end of 48 hours both retentate and dialysate were tested

for their inhibitory effects on TMV. The pellets of dialysed supernatant were also tested for their possible inhibitory effect for TMV infection.

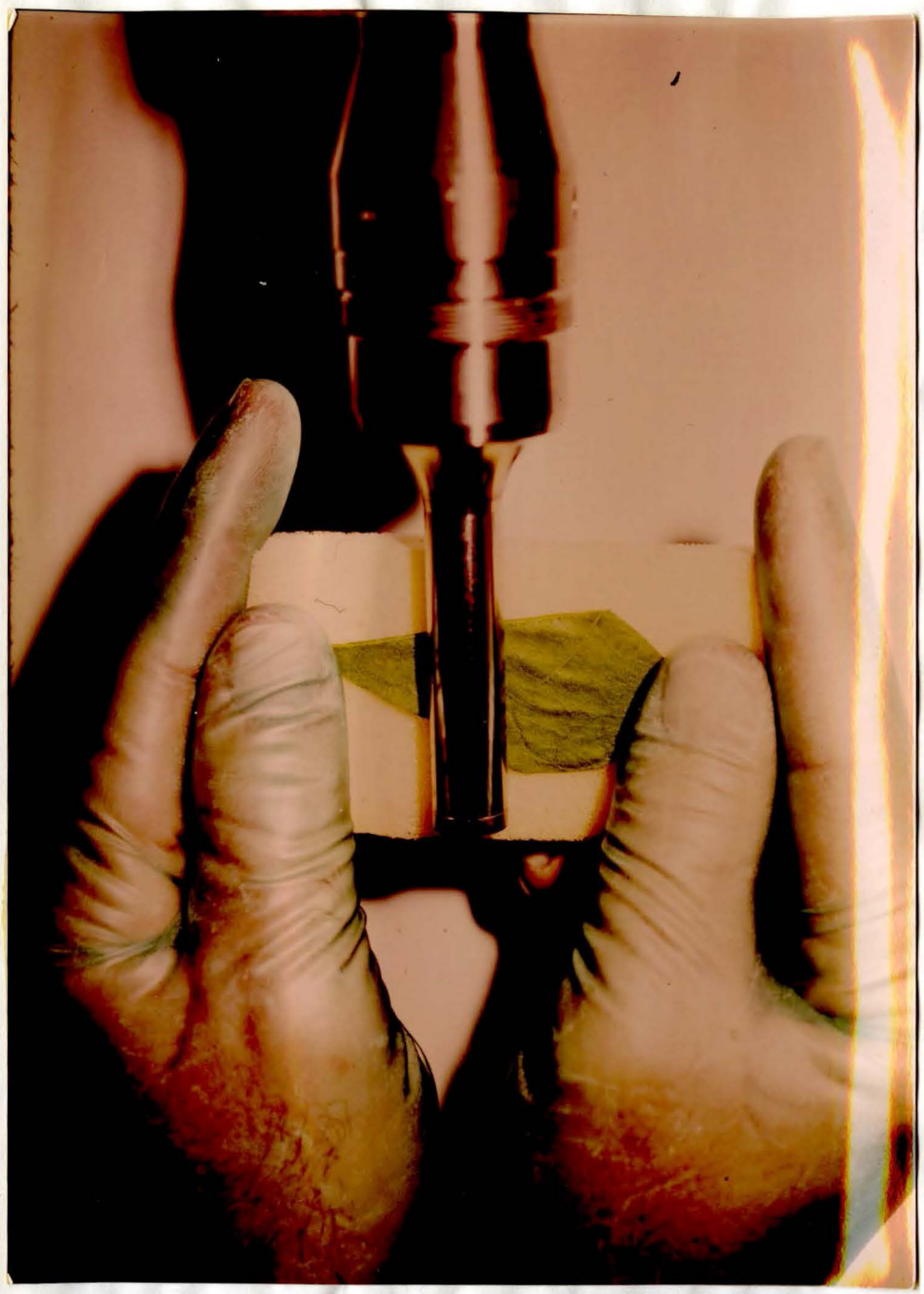


Figure 8. Ultrasonic inoculation of a half leaf of Phaseolus vulgaris cv pinto, with extract treated TMV. Leaves were pass through under the sonic probe with even pressure.

Figure 9. A sample of inoculum was inoculated on six half leaf, these then incubated under 2000 Lux light intensity for 35 days, where lesions started to appear.

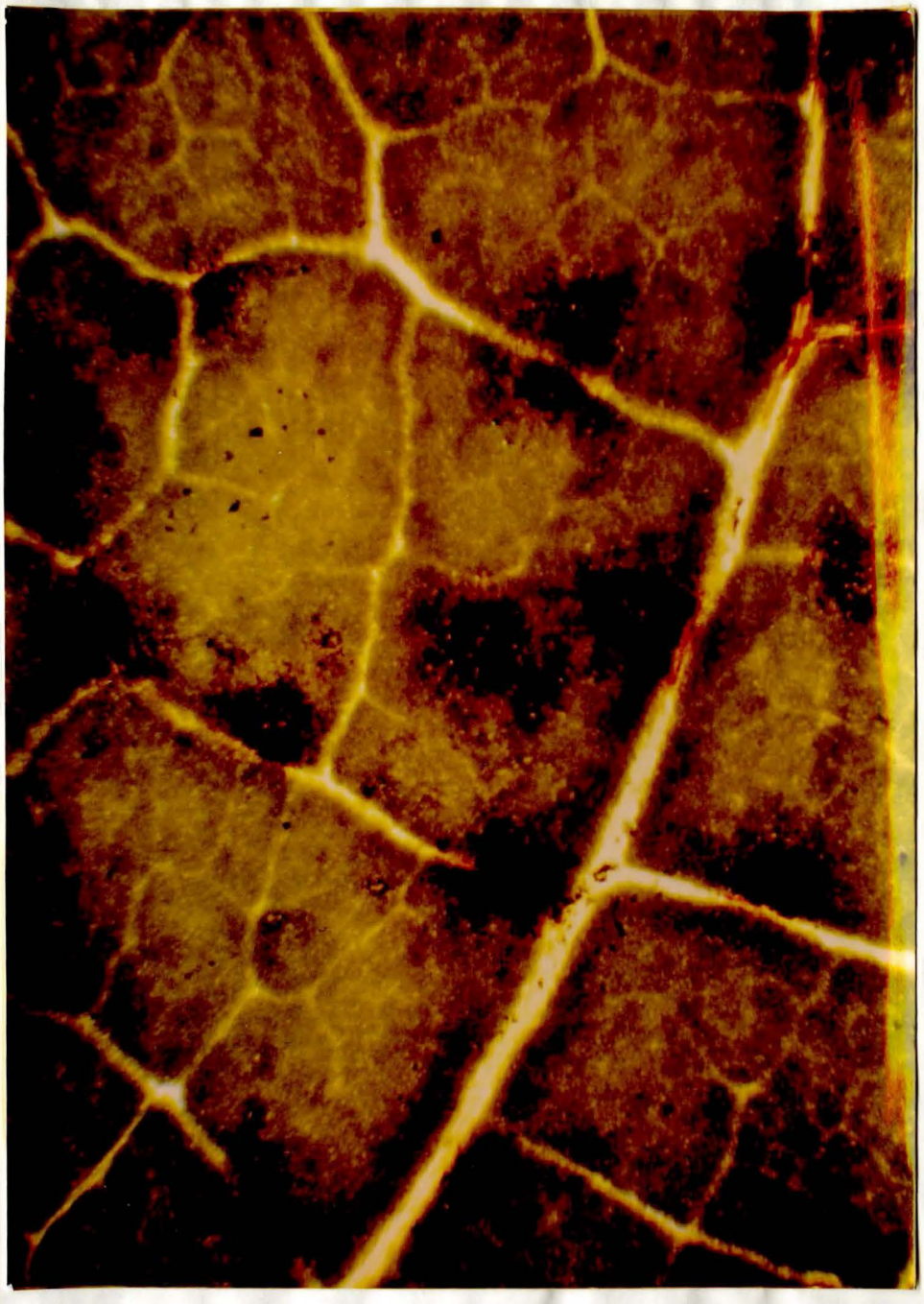


Figure 10. Local lesion produced by non extracts treated TMV in Phaseolus vulgaris cv pinto during 3-5 day incubation time.

Figure 11. Inhibitory extracts leaves and bulbs were fractionated by dialysis. Dialysate were constantly stirred by magnetic stirrer in 5°C refrigeration.

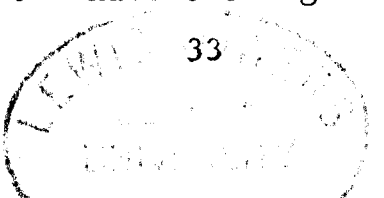
RESULTS AND DISCUSSION

A. Effect of the Extracts of Allium Cepa Leaves and Bulbs on the Replication of TMV

Leaf extracts were prepared by using pestle and mortar with distilled water and tenfold dilutions were made to test the inhibitory effects on TMV. The infectivity was tested on 6 half leaves of pinto beans. It was noted that all concentrations of the extracts were inhibitory at all incubation times. At zero time the highest inhibitions were noticed in the dilution of 10^{-3} (Figure 12). The next highest inhibition was noticed at the extract dilution of 10^{-1} and then in the order of 10^{-4}, 10^{-5}, and 10^{-2}. The lowest inhibitory effect was at 10^{-2} dilution.

The incubation time, 60 minutes, most affected the infectivity of TMV at dilutions of 10^{-1}, 10^{-2}, and 10^{-4}. Dilution 10^{-3} lost its inhibitory effect with time of incubation, dilution 10^{-4} gained its effectiveness with incubation time, whereas the concentration of the leaf extracts in the dilution of 10^{-1}, 10^{-2} and 10^{-4} reduced the infectivity of TMV to the level of about 99%.

The diluted bulb extracts had slightly greater inhibitory effect on TMV than did the leaf extracts. The dilutions 10^{-1} and 10^{-2} have the highest inhibitory effect at



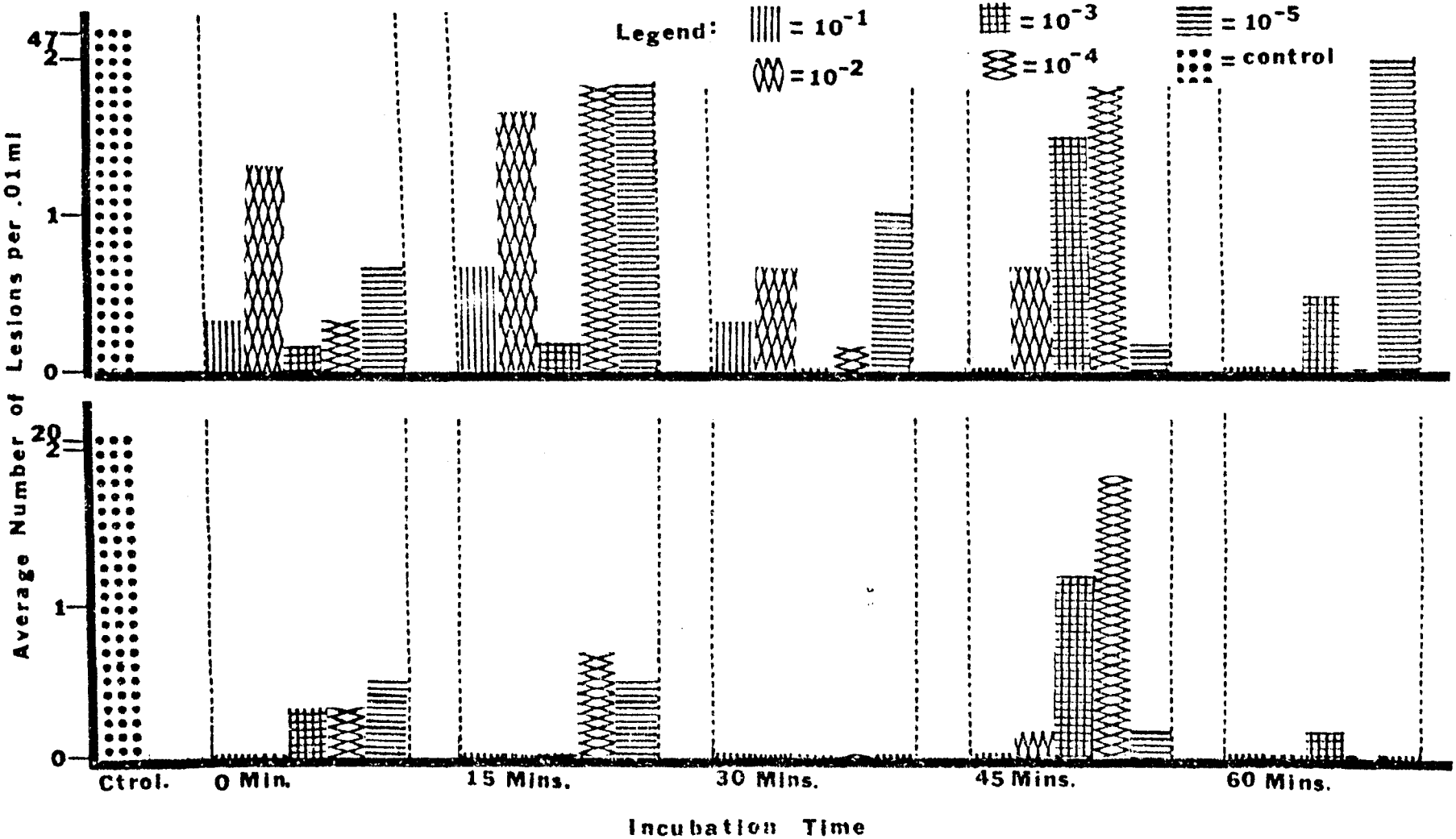


Figure 12. Assessment of the inhibitory effect of Allium cepa leaf (top) and bulb (bottom) extracts on the replication of Tobacco Mosaic Virus by local lesion assay on six half leaves of Phaseolus vulgaris cv pinto.

Incubation Time	Extracts Dilution 10^{-x}	No. of Lesion from six .01 ml sample/half leaf	Avg. No. of Lesions per half leaf	No. of lesions from six .01 ml sample/half leaf	Avg. No. of Lesions per half leaf
Minute	X =	Leaf extract Treated TMV		Bulb extract Treated TMV	
0	1	0 0 0 1 1 0	0.33	0 0 0 0 0 0	0.00
	2	1 4 1 2 0 0	1.33	0 0 0 0 0 0	0.00
	3	1 4 1 2 0 0	0.17	0 2 0 0 0 0	0.33
	4	1 0 0 0 1 0	0.33	0 0 2 0 0 0	0.33
	5	1 1 1 1 0 0	0.67	0 0 1 2 0 0	0.50
15 min.	1	1 0 0 2 0 1	0.67	0 0 0 0 0 0	0.00
	2	1 3 4 2 0 0	1.67	0 0 0 0 0 0	0.00
	3	0 0 0 0 1 0	0.17	0 0 0 0 0 0	0.00
	4	1 1 2 2 1 4	1.83	0 0 0 2 2 0	0.67
	5	5 1 1 1 3 0	1.83	2 0 0 0 0 1	0.50
30 min.	1	0 0 0 2 0 0	0.33	0 0 0 0 0 0	0.00
	2	0 0 0 0 2 2	0.67	0 0 0 0 0 0	0.00
	3	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.00
	4	0 1 0 0 0 0	0.17	0 0 0 0 0 0	0.00
	5	0 0 0 4 0 2	1.00	0 0 0 0 0 0	0.00
45 min.	1	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.00
	2	3 0 1 0 0 0	0.67	0 0 0 0 1 0	0.17
	3	2 2 0 1 1 0	1.00	0 0 2 3 1 1	1.17
	4	0 0 0 0 0 11	1.83	4 4 0 0 3 0	1.83
	5	0 0 0 0 0 1	0.17	0 0 0 1 0 0	0.17
60 min.	1	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.00
	2	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.00
	3	1 0 0 0 2 0	0.50	0 1 0 0 0 0	0.17
	4	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.00
	5	0 1 2 3 2 4	2.00	0 0 0 0 0 0	0.00
Control	10^{-4}	45 42 23 50 103 20	47.17	26 41 11 20 41	20.5

Table I: Assessment of the inhibitory effect of Allium cepa leaf and bulb extracts on the replication of Tobacco Mosaic Virus by local lesion assay on six half leaves of Phaseolus vulgaris cv pinto.

all inoculation times of bulb extracts. Yet there were a decreased inhibitory effect in the extract diluted by 10^{-3}, 10^{-4} at 45 min. inoculation time.

The leaf extract (Figure 13) was tested at one hour intervals for 5 hours. At 2 hours incubation time the highest inhibition was at the dilution of 10^{-3} then in the order of 10^{-5}, 10^{-2} and 10^{-4}. At 5 hours incubation time, the highest inhibitory effect on TMV was at the dilution of 10^{-2}, 10^{-4}, and 10^{-1}, and lowest inhibitory effect was at 10^{-3} and 10^{-5} dilutions.

When bulb extracts of the dilution 10^{-1} were tested, they reduced infectivity on TMV by 98%. 10^{-2} dilution slightly lost the inhibitory effect at the end of 2 hours and 5 hours incubation period. At the end of 5 hours incubation time the same level of infectivity was observed both 10^{-2} as well as 10^{-4} dilution. The extracts dilution of 10^{-4} at the end of 2 hours and 4 hours incubation times was highly effective. Although 10^{-5} dilution was less effective among all the five dilutions, it showed the most inhibition in 4 hours and 5 hours of incubation times.

Of these two given figures of Allium cepa, the inhibitory effect of the extracts (I and II) of A. cepa may be explained by the inhibitors in the extracts prevented the replication of virus either by preventing the uncoating of the virus, once extracts had an excess in the cell, or they somehow did not let the RNA link with the host cell ribosomes

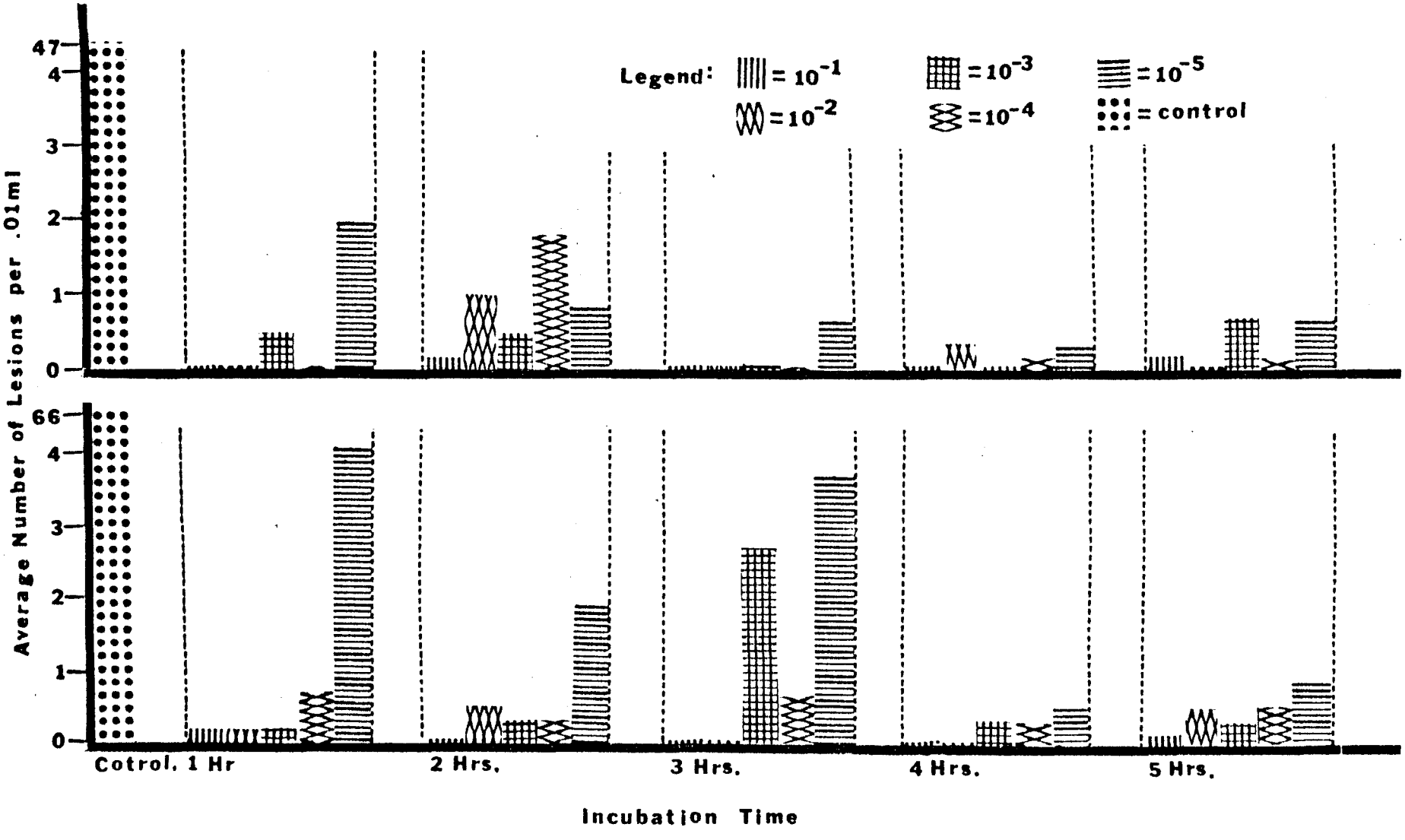


Figure 13. Assessment of the inhibitory effect of Allium cepa leaf (top) and bulb (bottom) extracts on the replication of Tobacco Mosaic Virus by local lesion assay on six half leaves of Phaseolus vulgaris cv pinto.

Incubation	Extracts Dilution	No. of lesion from six .1 ml sample/ half leaf	Avg. No. of Lesions	No. of lesion from six .1 ml sample/ half leaf	Avg. No. of Lesions
Hours	X =	Leaf extract reated TMV		Bulb Extract reated TMV	
1	1	0 0 0 0 0 0	0.00	0 0 0 1 0 0	0.17
	2	0 0 0 0 0 0	0.00	0 1 0 0 0 0	0.17
	3	1 0 0 0 2 0	0.50	0 0 1 0 0 0	0.17
	4	0 0 0 0 0 0	1.83	1 0 0 0 1 0	0.67
	5	0 1 2 3 2 4	2.00	0 2 0 7 11 4	4.00
2	1	2 1 3 0 1 0	1.17	0 0 0 0 0 0	0.00
	2	0 0 5 0 1 1	1.00	0 1 0 0 0 2	0.50
	3	2 0 0 0 0 1	0.50	0 2 0 0 0 0	0.33
	4	0 0 2 0 0 9	1.83	1 0 0 0 1 0	0.33
	5	0 0 0 0 0 3	1.83	0 2 2 2 5 0	1.83
3	1	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.00
	2	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.00
	3	0 0 0 0 0 0	0.00	0 2 0 5 7 2	2.67
	4	0 0 0 0 0 0	0.00	1 1 2 0 0 0	0.67
	5	0 1 1 0 0 2	0.67	0 0 18 1 1 2	3.17
4	1	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.00
	2	1 0 1 0 0 0	0.33	0 0 0 0 0 0	0.00
	3	0 0 0 0 0 0	0.00	0 0 2 0 0 0	0.33
	4	0 0 1 0 0 0	0.17	0 0 0 1 1 0	0.33
	5	0 0 0 0 0 2	0.33	0 0 0 2 0 1	0.50
5	1	0 0 0 0 0 1	0.17	0 0 0 1 0 0	0.16
	2	0 0 0 0 0 0	0.00	2 0 0 1 0 0	0.50
	3	0 0 0 0 1 3	0.67	0 0 1 1 0 0	0.33
	4	0 0 0 0 0 1	0.17	0 2 0 0 1 0	0.50
	5	0 0 2 2 0 0	0.67	1 0 0 2 1 1	0.83
Control	Untreated TMV 10^{-4}	45 42 23 50 103 20	47.17	4 121 45 81 55 93	66.5

Table II: Assessment of the inhibitory effect of Allium cepa leaf and bulb extracts on the replication of Tobacco Mosaic Virus by local lesion assay on six half leaves of Phaseolus vulgaris cv pinto.

to form polysomes for the synthesis of functional proteins.

B. Effect of the Extracts of Allium sativum Leaves and Bulbs on the Replication of TMV

In Figure 14, at 15 minutes incubation time, the highest inhibition on TMV was noted from all the five dilutions of leaf extracts.

At 30 minutes incubation time, the effectiveness of 10^{-2} and 10^{-4} dilution on TMV did not change, whereas 10^{-1} dilution lost most of its effect followed by 10^{-3} and 10^{-5}. As close to 60 minutes incubation time the effect of all the dilutions was increased except 10^{-2}.

The dilution of bulb extracts from 10^{-2} to 10^{-5} increased the inhibitory effect on TMV until 60 minutes incubation period. The dilution of 10^{-3} lost inhibition at 45 minutes incubation and 10^{-1} dilution gradually lost inhibitory effect from 15 minutes to 60 minutes incubation times.

In Figure 15, the leaf extracts inoculated with TMV were incubated for 5 hours, and samples were taken every one hour then examined. The infectivity of TMV on 6 half leaves of pinto bean. At 2 hour incubation time the highest inhibition was noticed in all the five dilutions. The extract dilutions of 10^{-1}, 10^{-3} slightly increased the inhibitory effect on TMV at 5 hours of incubation period. There was gradual loss of the inhibitory effect on TMV in the order of

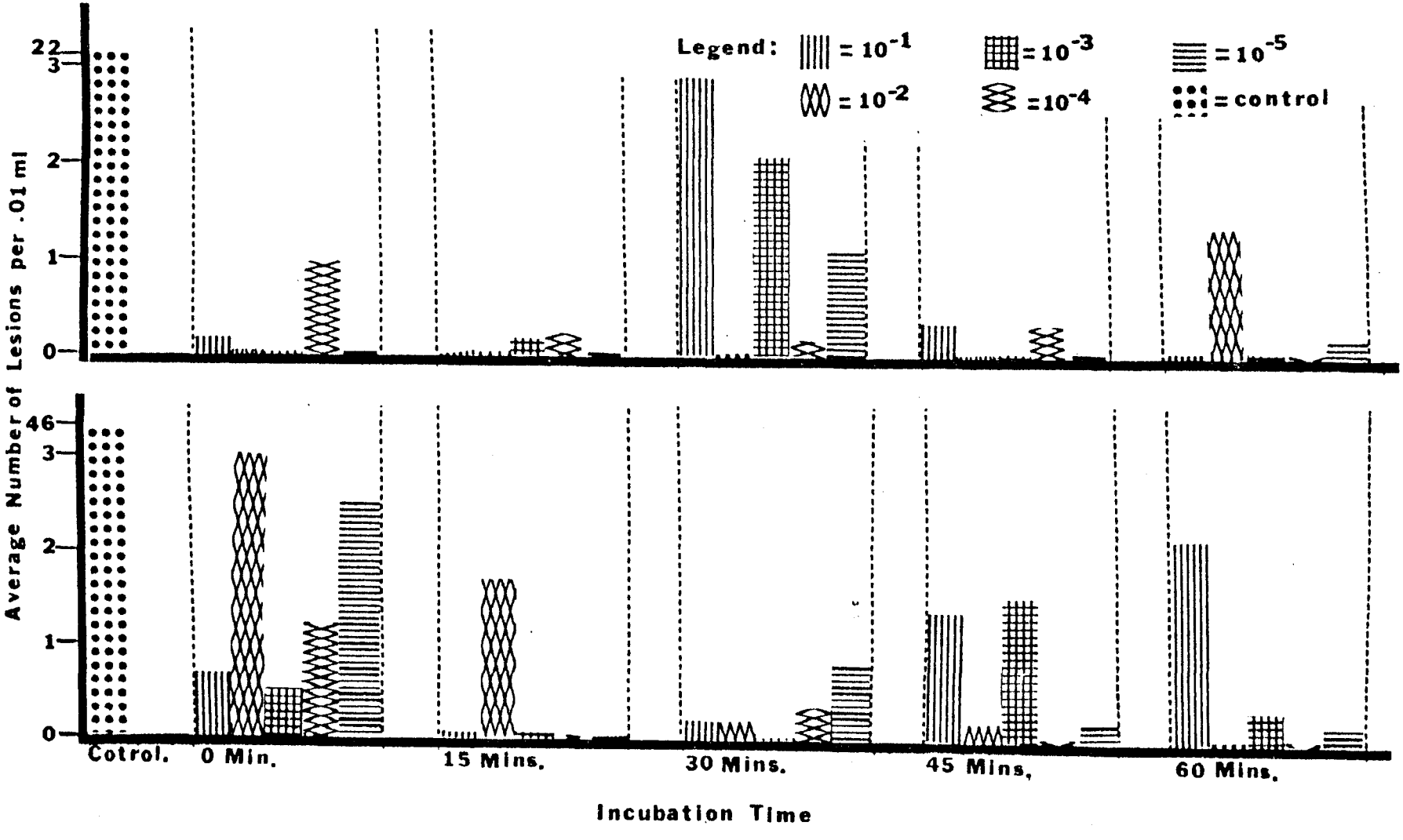


Figure 14. Assessment of the inhibitory effect of Allium sativum leaf (top) and bulb (bottom) extracts on the replication of Tobacco Mosaic Virus by local lesion assay on six half leaves of Phaseolus vulgaris cv pinto.

Leaves Incubation	Extracts Dilution	No. of lesion from six .1 ml sample/ half leaf	Avg. No. of Lesions	No. of lesion from six .1 ml sample/ half leaf	Avg. No. of Lesions
Minutes	X =	Leaf extract Treated TMV		Bulb extract Treated TMV	
0	1	0 0 0 0 0 1	0.17	0 0 0 1 3 4	0.67
	2	0 0 0 0 0 0	0.00	17 0 0 0 1 0	3.00
	3	0 0 0 0 0 0	0.00	0 0 0 3 0 0	0.50
	4	3 0 0 0 0 3	1.00	4 0 1 2 0 0	1.17
	5	0 0 0 0 0 0	0.00	0 0 0 0 3 12	2.50
15	1	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.00
	2	0 0 0 0 0 0	0.00	0 2 0 8 0 0	1.67
	3	0 1 0 0 0 0	0.17	0 0 0 0 0 0	0.00
	4	13 0 0 0 0 1	2.33	0 0 0 0 0 0	0.00
	5	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.00
30	1	0 0 0 0 19 0	3.17	0 0 1 0 0 0	0.17
	2	0 0 0 0 0 0	0.00	0 0 0 0 0 1	0.17
	3	0 0 0 0 1 12	2.17	0 0 0 0 0 0	0.00
	4	0 1 0 0 0 0	0.17	2 0 0 0 0 0	0.33
	5	0 0 0 0 7 0	1.17	1 1 0 0 0 3	0.83
60	1	0 0 0 0 0 0	0.00	0 4 2 0 0 0	2.17
	2	0 0 6 2 0 0	1.33	0 0 2 2 0 9	0.00
	3	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.33
	4	0 0 0 0 0 0	0.00	0 2 0 0 0 0	0.00
	5	0 0 0 1 0 0	0.17	0 1 0 0 0 0	0.17
Control	Untreated TMV 10^{-4}	4 5 26 41 3 56	22.7	60 81 27 75 92	46.83

Table III: Assessment of the inhibitory effect of Allium sativum leaf and bulb extracts on the replication of Tobacco Mosaic Virus by local lesion assay on six half leaves of Phaseolus vulgaris.

10^{-4}, 10^{-2}, and 10^{-5} dilution in the same incubation period. As shown in Figure 15 leaf extracts showed slightly more inhibitory effect over bulb extracts. Bulb extracts of 10^{-1}, 10^{-2} lost inhibitory effect in 3 to 5 hours incubation time. The 10^{-3} dilution showed the same level of inhibition between 4 to 5 hours of incubation times, whereas 10^{-4} dilution gradually lost inhibitory effect on TMV. 10^{-5} dilution was most inhibitory on TMV at 4 hours incubation time, and became less inhibitory on TMV at 5 hours incubation period.

Infectivity of TMV did not seem to depend on the dilutions of the extracts (Figure 1 to 4). Such highly concentrated extracts may not have inhibited as much as less concentrated extracts. All five extract dilutions prepared were able to inhibit the virus replication over 90%. The inhibition might be due to the fact that the inhibitors in the extracts might either be blocking or competing with virus receptor sites on leaf surface or altering the host cell physiologically, so that they no longer were receptive to the virus.

C. Effect of the Extracts in Supernatant (Retentate, Dialysates) and Pellets on the Replication of TMV

The nature of inhibitory effect of Allium species on TMV could be explained on the basis that the extracts of both leaves and bulbs contained the large molecular weight

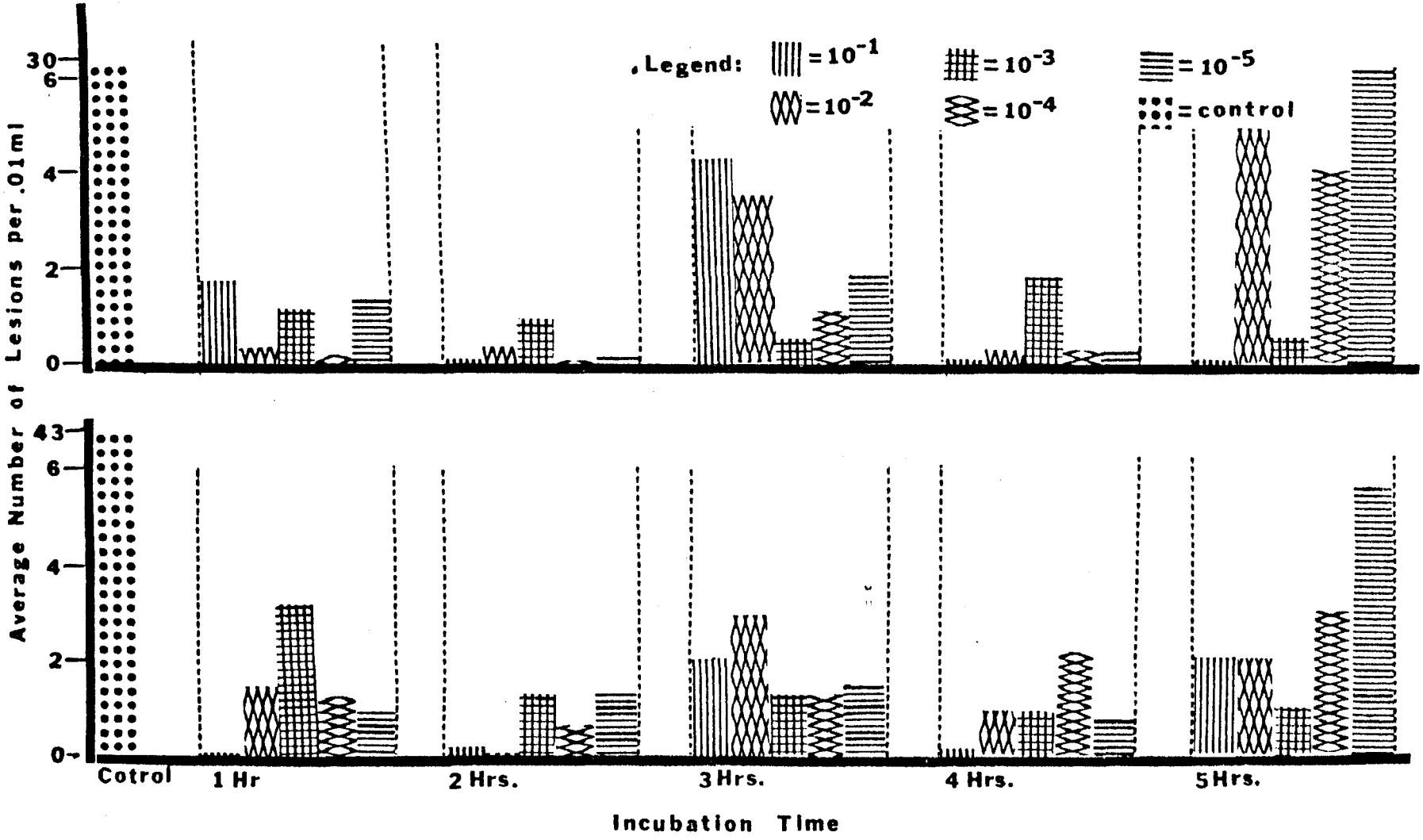


Figure 15. Assessment of the inhibitory effect of Allium sativum leaf (top) and bulb (bottom) extracts on the replication of Tobacco Mosaic Virus by local lesion assay on six half leaves of Phaseolus vulgaris cv pinto.

Leaves Incubation	Extracts Dilution	No. of lesion from six .1 ml. sample/ half leaf	Avg. No. of Lesions	No. of Lesion from six .1 ml sample/ half leaf	Avg. No. of Lesions
Hours	X =	Leaf Extract treated TMV		Bulbs Extract Treated TMV	
1	1	0 0 0 1 0 0	0.16	0 0 0 0 0 0	0.00
	2	0 0 0 0 2 0	0.33	0 3 0 2 3 1	1.50
	3	1 3 0 0 3 0	1.16	7 2 4 4 1 1	3.17
	4	0 1 0 0 0 0	0.16	0 0 7 1 0 0	1.33
	5	0 5 0 0 2 1	1.33	1 2 1 2 0 0	0.00
2	1	0 0 0 0 0 0	0.00	0 0 0 0 0 0	0.17
	2	0 0 0 2 0 0	0.33	1 1 0 1 3 0	1.00
	3	1 2 1 1 1 0	1.00	0 0 7 0 1 0	1.33
	4	0 0 0 0 0 0	0.00	3 0 0 0 0 1	0.67
	5	0 0 0 0 1 0	0.16	6 0 1 0 0 1	1.33
4	1	0 0 0 0 0 0	0.00	0 1 0 0 0 0	0.17
	2	0 0 0 1 1 0	0.33	0 2 4 0 0 0	1.00
	3	0 4 3 0 3 1	1.83	1 1 0 0 0 4	1.00
	4	0 0 2 0 0 0	0.33	2 2 3 1 4 1	2.17
	5	0 0 1 0 0 1	0.33	1 3 0 0 0 1	0.67
5	1	0 0 0 0 0 0	0.00	0 1 6 3 0 2	2.00
	2	3 4 0 0 11 11	4.83	0 3 1 1 7 0	2.00
	3	0 0 3 0 0 0	0.50	0 0 3 2 1 0	1.00
	4	3 3 1 2 9 6	4.00	0 10 0 0 1 7	3.00
	5	7 26 3 2 0 0	6.33	6 11 11 1 3 2	5.17
Control	Untreated TMV 10^{-4}	40 3 11 40 24 58	30.67	47 30 39 36 43 67	43.67

Table IV. Assessment of the inhibitory effect of Allium sativum leaf and bulb extract on the replication of Tobacco Mosaic Virus by local lesion assay on six half leaves of Phaseolus vulgaris cv pinto.

substances which settled in pellets. The substances in the pellets had over 99% inhibitory effect on TMV (Figures 16 and 17 . These large molecular substances might have acted at the initial stages of the inhibition process, which blocked the receptive sites of the host cell or the protein coat of the virus itself. Therefore extract treated TMV particles could not start the multiplication.

The first 12 hours dialysate of the bulb extracts of two Allium species Cepa and Sativum had greater inhibitory effect on TMV than 48 hours retentate, whereas the 48 hours retentate of leaf extracts and 12 hours dialysate varied in their inhibitory effect on TMV replication. Longer than the dialysis time as 24 hours followed by 48 hours dialysate reduced infectivity of TMV. This might be due to dilution of extracts by addition of distilled H_2O a 12 hour intervals. These small molecular weight substances from dialysate of supernatant may have acted on the later stages of the inhibition process. Since these small molecules entering into the leaf host may reach the sites at which virus multiplied, at the same time the small molecules of the inhibitors might also alter metabolism of the host cell so that virus only could replicate to a limited degree.

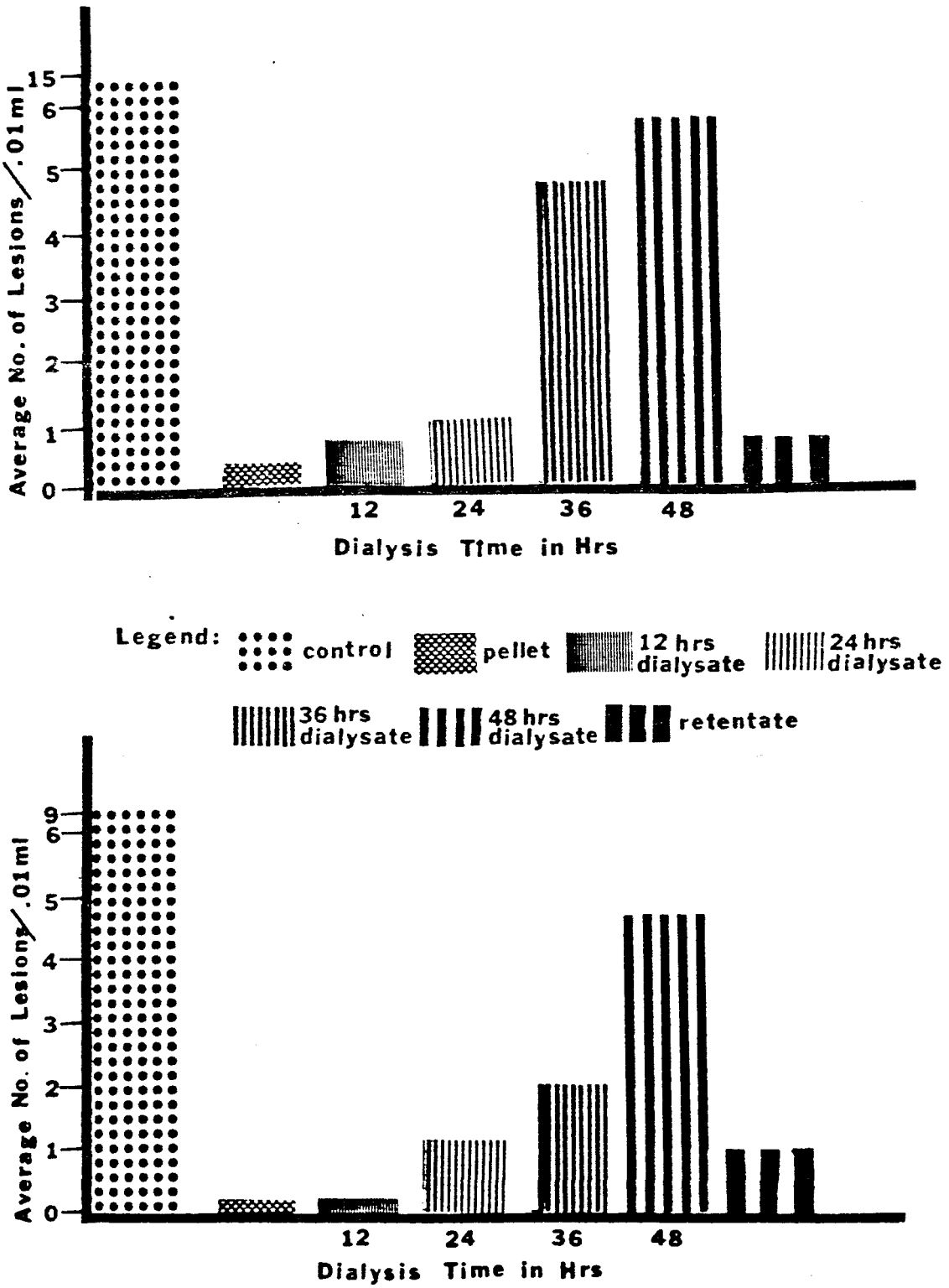


Figure 16. Bio-assay of the inhibitory effect of Allium cepa leaf (top) and bulb (bottom) extracts on the replication of TMV by local lesion assay using the dialysis technique.

Extracts of Centrifugation	Dialysis Time	No. of lesion from six .01 ml sample/ half leaf	Avg. No. Lesions
Treated Leaves Extracts on TMV			
Supernatant First Dialysate	12 hr.	0 0 1 1 1 1	0.67
Second Dialysate	24 hr.	3 0 2 0 1 0	1
Third Dialysate	36 hr.	1 0 26 0 1 0	4.66
Fourth Dialysate	48 hr.	5 4 2 4 5 14	5.66
Fourth Retentate		2 0 0 2 1 0	0.83
Pellet		0 0 0 2 0 0	0.33
Control: Untreated TMV (10^{-5})		21 15 15 12 11 16	15.00
Treated Bulbs Extracts on TMV			
Supernatant First Dialysate	12 hr.	1 0 0 0 0 0	0.17
Second Dialysate	24 hr.	0 2 2 1 1 1	1.17
Third Dialysate	36 hr.	0 3 0 1 1 7	2
Fourth Dialysate	48 hr.	4 8 9 5 1 1	4.67
Fourth Retentate		0 1 0 3 1 2	1.17
Pellet		0 0 0 1 0 0	0.17
Control: Untreated TMV (10^{-5})		14 29 4 4 3 2	9.33

Table V: Bio-assay of the inhibitory effect of allium cepa leaf and bulb extracts on the replication of TMV by local lesion assay using the dialysis technique.

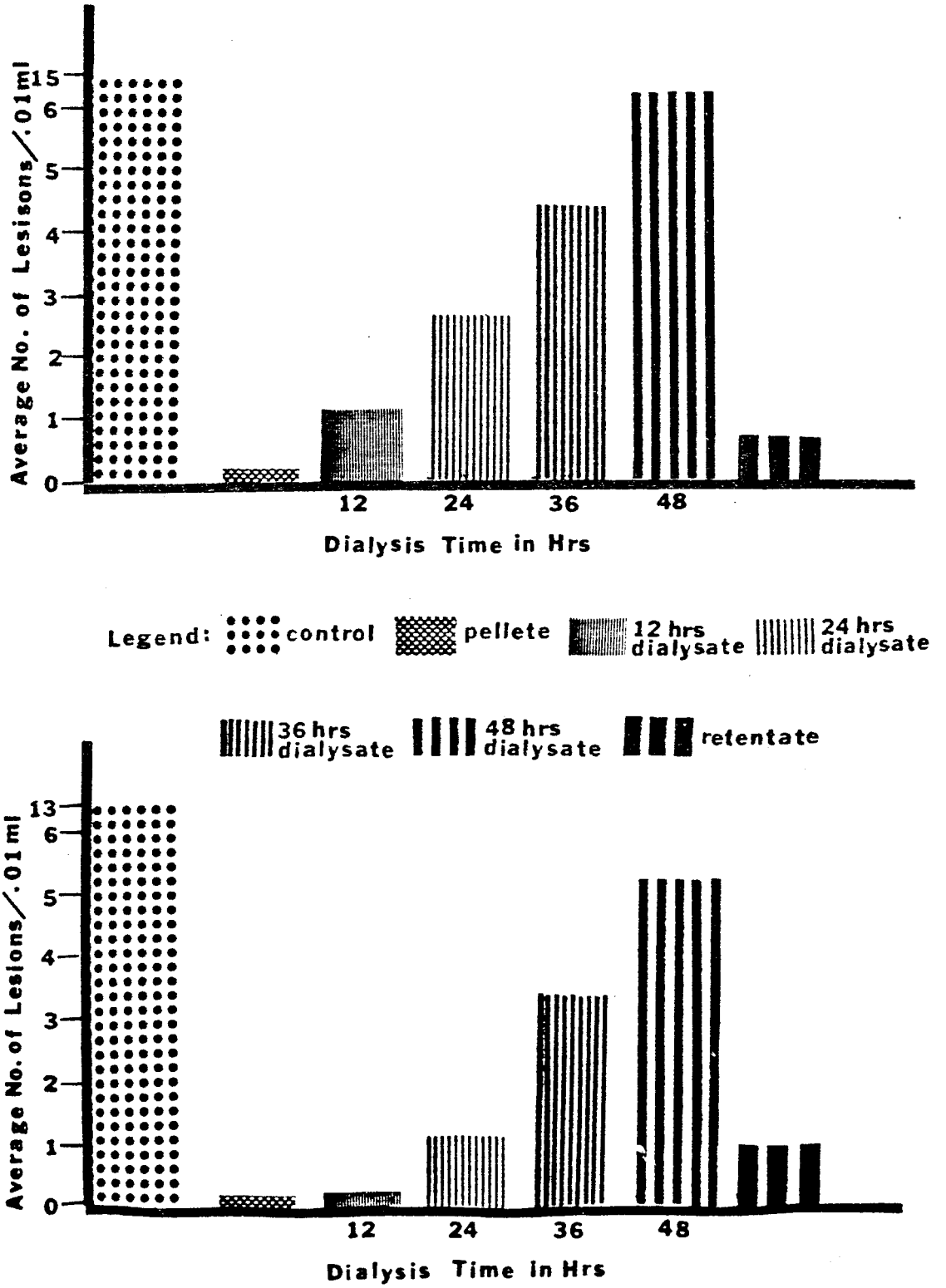


Figure 17 . Bio-assay of the inhibitory effect of Allium sativum leaf (top) and bulb (bottom) extracts on the replication of TMV by local lesion assay using the dialysis techni

Extracts of Centrifugation	Dialysis Time	No. of lesion from six .01 ml sample/ half leaf	Avg. No. Lesions
Treated Leaves Extracts on TMV			
Supernatant First Dialysate	12 hr.	0 0 0 0 0 7	1.16
Second Dialysate	24 hr.	2 11 3 0 0 0	2.66
Third Dialysate	36 hr.	3 6 1 8 4 3	4.33
Fourth Dialysate	48 hr.	2 8 7 2 2 1 0	5.16
Fourth Retentate		0 0 1 0 0 4	0.83
Pellet		0 0 1 0 0 0	0.17
Control: Untreated TMV (10^{-5})		7 15 13 14 6 19	14.8
Treated Bulbs Extracts on TMV			
Supernatant First Dialysate	12 hr.	0 0 0 0 0 0	0.17
Second Dialysate	24 hr.	4 0 1 0 2 0	1.17
Third Dialysate	36 hr.	1 5 2 7 2 3	3.33
Fourth Dialysate	48 hr.	1 0 3 5 22 0	5.16
Fourth Retentate		3 2 0 1 0 0	1
Pellet		0 0 0 0 0 1	0.17
Control: Untreated TMV (10^{-5})		11 21 15 7 8 16	13.00

Table VI; Bio-assay of the inhibitory effect of Allium sativum leaf and bulb extract on the replication of TMV by local assay using the dialysis technique.

V. SUMMARY AND CONCLUSION

Objective and Purpose

Early Chemotherapeutic pathologists have demonstrated a great interest in the effect of the various types of chemicals on the inhibition of viral infection. To this date there is no chemical agent available which could be used to protect plants and animals from all virus diseases. However there are some potential chemicals which have been reported by Matthews (1951), who found that guanazolo of purine inhibits virus multiplication by interfering with the guanine of virus. In the same year Commoner and Mercer (1951) demonstrated that thiouracil blocks the synthesis of Tobacco Mosaic Virus (TMV).

On the other hand plants that are known to have medicinal value, have been credited for having antiviral activity. No conclusive studies have been reported.

The purpose of this investigation was to determine if extracts of plant were inhibitory to the infections of Tobacco Mosaic Virus. Bio assay procedure was used to test the infectivity of TMV on the ten day old primary lesions of Phaseolus vulgaris cv pinto.

Application of Materials

A common strain of Tobacco Mosaic Virus (PV-135)

Seeds and bulbs of Allium cepa and Allium sativum

Sandy loam soil, vermicular soil

Seeds of Phaseolus vulgaris, cv Pinto

Sonifier cell distruptor, ultrasonic probe

320 mesh Carborundum

Blender, mortar and pestle, cheese cloths

Binocular microscopes and lesion counter

Preparation of Inhibitory Extracts

Extracts of Allium cepa and Allium sativum were prepared by grinding leaves and bulbs with the pestle and mortar, then passing the samples through two layers of cheese cloth. Extracts were diluted 10^{-1} and 10^{-5} and mixed with 10^{-4} dilution of TMV in the ratio of 9:1. Controls were prepared by mixing distilled water with 10^{-4} dilution of TMV, to give on average of 50 lesions forming unit per half leaf. Treated extracts were tested on 6 half leaves (0.01 ml of innoculum per half leaf) of 10 day old Phaseolus vulgaris using ultrasonic apparatus (Lamborn and Cochran 1969).

Inoculated leaves were incubated in air-tight containers in the dark for 18 hours and the under fluorescent light 200 ft.c. intensity for 72 hours. The lesions were counted under binocular microscope. The results indicated the degree of inhibitory effect on lesion

production did not seem to depend on dilution of extracts in given incubation times. However, the extracts of both leaves and bulbs of Allium species did inhibit the replication over about 90%.

Separation of Inhibitory Extracts

Attempts were made to fractionate the extracts by ultracentrifugation at 15,000 rpm for 2 hours and dialysis procedure to separate the particular compound that might be responsible for most inhibition on TMV. The supernatents from ultracentrifugation were dialysed against distilled water using cellulose dialysis tubing. Dialysate samples were taken at 12 hours intervals up to 48 hours. 48 hours retentate and pellets were also tested on six half leaves of host cell. As a result it showed most inhibitory compounds were in pellets, a large molecular substance which has over 99% inhibitory effect on TMV. This large molecule might have acted on the initial stages of the inhibition process, which blocked the receptive sites of the host cell or the protein coat of the virus itself. Therefore extract treated TMV particles could not start the replication. The samples that had the least inhibitory effect on TMV were the 48 hour dialysate a small molecule substances plus the addition of distilled water in 12 hours interval. These small molecules from dialysate of supernatent must have acted on the later stages of the inhibition process, since they are

capable of entering into the leaf host and may reach the sites at which virus multiplies in the cell. At the same time the small molecules of the inhibitors might also alter metabolism of the host cell where the virus could only replicate to a limited degree.

The data of inhibitory effect on TMV from both Allium cepa and Allium sativum extracts were statistically analyzed by obtaining the means and standard deviation (Appendix A and B).

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APPENDIX A

ANALYSIS OF STATISTICS

Statistical analysis of the local lesion produced by the Tobacco Mosaic Virus treated with Allium cepa and Allium sativum leaf and bulb extracts (dilution 10^{-1} to 10^{-5}). Mixture of TMV and diluted extract were incubated from 0 to 60 min.

Extracts of Allium cepa Leaves			Extracts of Allium sativum Leaves		
Incubation Time (Min.)	Mean	Standard Deviation	Incubation Time (Min.)	Mean	Standard Deviation
0	0.566	0.464	0	0.234	0.434
15	1.234	0.767	15	0.500	1.026
30	0.434	0.402	30	1.336	1.345
45	0.734	0.732	45	0.132	0.181
60	0.500	0.866	60	0.300	0.580
Extracts of Allium cepa Bulbs			Extracts of Allium sativum Bulbs		
Incubation Time (Min.)	Mean	Standard Deviation	Incubation Time (Min.)	Mean	Standard Deviation
0	0.232	0.223	0	1.568	1.121
15	0.232	0.326	15	0.334	0.747
30	0.000	0.000	30	0.300	0.318
45	0.668	0.798	45	0.634	0.719
60	0.034	0.076	60	0.534	0.925

Ex. 0 Incubation Time of leaf extracts of Allium cepa of 5 dilutions (10^{-1} to 10^{-5})

$$\text{Mean} = \bar{x} = \frac{\sum_{i=1}^{N} xi}{N} = \frac{2.83}{5} = 0.566$$

$$\text{Stand. Dev.} = S = \frac{\sum_{i=1}^{N} (xi-\bar{x})^2}{N - 1}$$

$$= \frac{(.33-.566)^2 + (1.33-5.66)^2 + (.17-.566)^2 + (.33-.566)^2 + (.67 + .566)^2}{4}$$

$$= 0.464$$

APPENDIX B

ANALYSIS OF STATISTICS

Statistical analysis of the local lesion produced by the Tobacco Mosaic Virus treated with Allium cepa and Allium sativum leaf and bulb extracts (dilution 10^{-1} to 10^{-5}). Mixture of TMV and diluted extract were incubated from 1 to 5 hours.

Extracts of Allium cepa Leaves			Extracts of Allium sativum Leaves		
Incubation Time (Hr.)	Mean	Standard Deviation	Incubation Time (Hr.)	Mean	Standard Deviation
1	0.500	0.866	1	0.634	0.569
2	1.266	0.571	2	0.300	0.415
3	0.134	0.300	3	2.066	1.730
4	0.166	0.165	4	0.565	0.722
5	0.336	0.313	5	3.132	2.766
Extracts of Allium cepa Bulbs			Extracts of Allium sativum Bulbs		
Incubation Time (Hr.)	Mean	Standard Deviation	Incuation Time (Hr.)	Mean	Standard Deviation
1	1.036	1.671	1	1.200	1.310
2	0.598	0.712	2	0.900	0.491
3	1.302	1.513	3	1.832	0.708
4	0.232	0.223	4	1.002	0.736
5	0.464	0.248	5	2.634	1.584

Ex. 1 Hr. incubation time of leaf extracts of Allium cepa of 5 dilutions (10^{-1} to 10^{-5})

$$\text{Mean} = \bar{x} = \frac{\sum_{i=1}^{N} xi}{N} = \frac{2.5}{5} = 0.5$$

$$\text{Stand. Dev.} = S = \frac{\sum_{i=1}^{N} (xi-x)^2}{N-1}$$

$$= \frac{(.00-0.5)^2 + (.00-0.5)^2 + (.50-.5)^2 + (.00-.5)^2 + (2.00-.5)^2}{4}$$

$$= 0.866$$

APPROVAL SHEET

The thesis submitted by Min Lan Chang has been read and approved by the following committee:

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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

April 20, 1981
Date

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Director's Signature