IDENTIFICATION OF VIRUS DISEASES AFFECTING SOME VEGETABLE CROPS IN WEST MALAYSIA AND THE SOUTHERN PART OF CHINA

Ichiro Fujisawa*, Saharan Haji Anang**, Yansong Shen*** and Anjing Zhou****

ABSTRACT

Many horticultural crops belonging to the Solanaceae and Cucurbitaceae were seriously affected by virus diseases both in West Malaysia and the southern part of China. Five kinds of viruses, chilli veinal mottle virus (CVMV), tomato spotted wilt virus (TSWV), cucumber mosaic virus (CMV), tobacco mosaic virus (TMV) and tobacco leaf curl virus were isolated from chilli plants in West Malaysia, and six kinds of viruses, CVMV, a pepper strain of TMV, CMV, broad bean wilt virus, alfalfa mosaic virus and a new potyvirus were isolated from sweet pepper in China. Most of the tomato plants were also found to be infected with viruses such as TSWV, CMV and TMV both in West Malaysia and China. Three kinds of viruses from cucurbitaceous crops, cucumber, pumpkin and wax gourd, have been isolated, and zucchini yellow mosaic virus was found to cause the most serious damage to the production of the crops.

The effect of agricultural materials used for the cultural control of chilli viruses was tested in West Malaysia. When the seedlings grown under net cover were transplanted into the field, the incidence of virus diseases and damage to chilli were very low, compared with the seedlings grown under open conditions.

Introduction

Most of the vegetables commonly grown both in West Malaysia and the southern part of China belong to the family of Solanaceae, Cucurbitaceae, Cruciferae and Leguminosae. These vegetable crops have been seriously affected by virus diseases, and within the last few years the problem has escalated especially for the solanaceous and cucurbitaceous crops. Recently, the author has had the opportunity to observe the occurrence and distribution of virus diseases on vegetables in both countries, and to examine the cultural control of virus diseases affecting chilli under the project "Promotion of Vegetable Production in the Tropics" (Fujisawa *et al.*, 1988). In the present paper, the properties of the viruses isolated from some vegetables in West Malaysia and the southern part of China, and effect of agricultural materials used for the control of chilli viruses in West Malaysia will be described.

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Identification of virus diseases of some vegetable crops

I Malaysia

1 Chilli (Hot pepper)

Chilli which is a lowland crop in West Malaysia was cultivated over a surface covering about 1,115 ha in 1983. The occurrence of virus diseases has become a major constraint on production. In our survey, more than 80% of the cultivated crops were found to be infected with mosaic disease in the field (Fujisawa *et al.*, 1986). The viruses reported earlier include cucumber mosaic (CMV), tobacco mosaic (TMV), chilli veinal mottle (CVMV) (Ong *et al.*, 1977), tomato spotted wilt (TSWV) and potato Y (PVY). Recently, the presence of alfalfa mosaic virus (AMV) and potato X virus (PVX) has also been reported (Abu Kassim, 1986). In the present study, four kinds of viruses, CMV, TMV, CVMV and TSWV were isolated from infected chilli plants (Fujisawa *et al.*, 1986) and the occurrence of a strain of tobacco leaf curl virus (TLCV) was also observed.

1) Chilli veinal mottle virus : CVMV infected 13 species from 2 families by sap inoculation, out of 39 species from 10 families tested, and induced clear mosaic symptoms and sometimes leaf distortion on *Capsicum annuum* (Fig. 1). The virus was transmitted by *Myzus persicae* and *Aphis gossypii* in a non-persistent manner. Filamentous virus particles were readily observed in leaf dip-preparations from chilli, and their length ranged from 700 to 750 nm (Fig. 2). In SDS-agar gel double diffusion tests, the crude sap

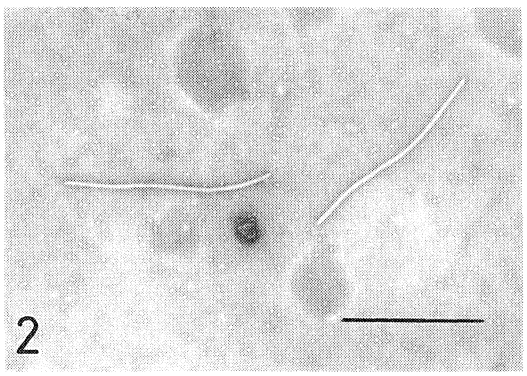


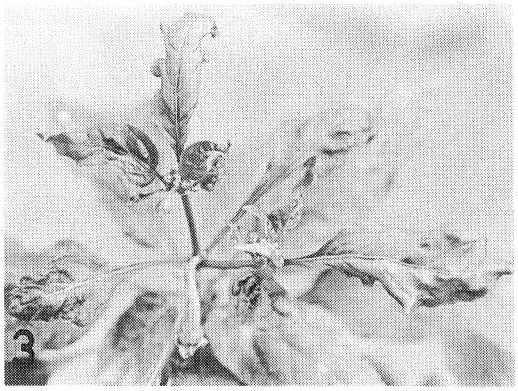
Fig. 1 Severe mosaic symptoms and leaf distortion in hot pepper caused by infection with chilli veinal mottle virus

Fig. 2 Electron micrograph of chilli veinal mottle virus particles (Bar represents 500 nm)

of infected leaves did not react with antisera against PVY and pepper veinal mottle virus (PVMV) (Brunt and Kenten, 1971). However, the antiserum against CVMV reacted weakly with PVY.

2) Tomato spotted wilt virus : TSWV infected 19 species from 7 families by sap inoculation, out of 22 species from 9 families tested. The symptoms observed consisted of pronounced necrosis in many of the susceptible plants, especially the plants belonging to Solanaceae. The virus induced vein-clearing and then necrotic streaks on leaves, stems or fruit on *C. annuum* (Fig, 3). Leaf-dip preparations from diseased plants contained large spherical particles, 70-100 nm in diameter (Fig. 4). Tomato varieties, 'Kewalo' and 'PI126944' that were immune to the infection of Japanese isolates of TSWV developed mosaic symptoms when they were infected with the Malaysian isolate. Furthermore, the antiserum against the nucleocapsid fraction of TSWV did not react with those from Japanese isolates of TSWV. Based on these properties, the virus should be considered as a different strain from the Japanese isolates of TSWV.

3) Tobacco mosaic virus : TMV isolated from chilli infected tomato and tobacco 'Samsun' systemically. However, the virus caused local necrotic lesions without subsequent systemic infection on *Petunia hybrida*, *Nicotiana sylvestris* and tobacco 'Bright Yellow'. The field-infected chilli plants generally exhibited mild mosaic symptoms and did not show any necrotic symptoms. Electron micrographs of the sap from infected



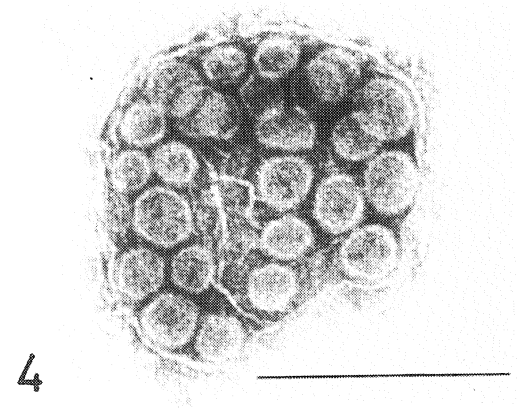
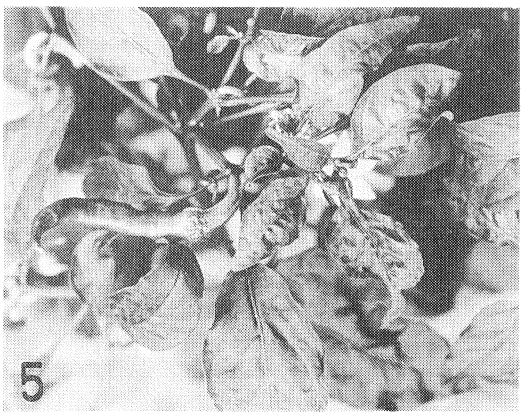


Fig. 3 Severe mosaic and partial necrotic symptoms caused by infection with tomato spotted wilt virus
Fig. 4 Electron micrograph of tomato spotted wilt virus particles inside the membrane (Bar represents 500 nm)

plants revealed the presence of rod-shaped particles with a modal length of 300×18 nm.

4) Cucumber mosaic virus : Based on the present study, two strains of CMV, ordinary strain (O) and chilli strain (C), were found to infect chilli plants. CMV-O had a wide host range and induced ordinary mosaic symptoms on *C. annuum*. On the other hand, CMV-C induced clear mosaic symptoms and sometimes chlorotic vein banding on *C. annuum* (Fig. 5). The host range of the CMV-C resembled that of CMV-O. On *Cucurbita pepo and C. sativus*, however, the virus induced characteristic necrotic local lesions on the inoculated leaves without subsequent systemic infection. Antiserum against CMV-C was prepared using the same procedure as that described previously. This antiserum reacted positively both with CMV-Y or pepper strain of CMV (CMV-P) isolated in Japan, and spurs developed between precipitin lines to the virus and CMV-Y or CMV-P (Fig. 6). Based on these properties, the virus may be distinguished from the two Japanese strains of CMV.

5) A strain of tobacco leaf curl virus : The field-infected chilli plants displayed upward or downward curling of young and old leaves, and stunting in most cases. The



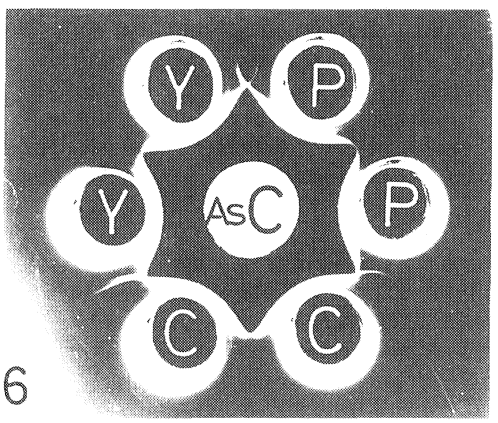


Fig. 5 Mosaic symptoms in hot pepper caused by infection with the chilli strain of cucumber mosaic virus

Fig. 6 Agar gel serological test with cucumber mosaic virus chilli strain. Center well contained antiserum against chilli strain of cucumber mosaic virus. Wells Y and P contained Y strain of cucumber mosaic virus from tobacco in Japan and P strain of cucumber mosaic virus from sweet pepper in Japan, respectively

virus could not be transmitted by sap-inoculation. Fifteen to twenty days after whitefly, *Bemisia tabaci*, transmission, leaf curl symptoms appeared on chilli, tomato, tobacco and *Ageratum conyzoides*. Therefore, the virus appeared to be a strain of tobacco leaf curl virus.

2 Sweet pepper

Since most sweet pepper plants were cultivated under a plastic rain shelter, the incidence of virus diseases was slightly lower than that in chilli. In some areas, however, a high incidence of virus diseases was observed, Although several kinds of symptoms were observed as in the case of chilli plants, mosaic symptoms were very common. Based on the results of the identification tests of the causal virus, 3 samples out of 4 were found to contain CVMV alone while the other was doubly infected with CVMV and TSWV.

3 Tomato

Tomato plants grown in lowland areas and showing yellow leaf curl symptoms associated with tomato yellow leaf curl virus (TYLCV) infection were observed in the fields where whiteflies often occurred. The virus caused curling of young leaves which remained small. Lower leaves were curled, twisted, puckered and became yellow. The plants became stunted and bushy. The virus is a geminivirus transmitted by the whitefly, *B. tabaci* in a persistent manner, but not by inoculation with sap. Some tomato plants in Cameron Highlands showed mosaic symptoms, and were found to be infected with TMV tomato strain alone. Tomato plants showing leaf roll or purple vein symptoms were found to be infected with TSWV.

4 Cucumber and pumpkin

Many pumpkin plants showed mosaic symptoms in the field and the incidence of virus diseases was almost 100%. Some cucumber plants also showed mosaic symptoms, and they generally produced deformed fruit. Zucchini yellow mosaic virus (ZYMV) was isolated from these diseased pumpkin and cucumber plants. CMV was also isolated from cucumber plants showing mild mosaic symptoms.

Zucchini yellow mosaic virus: ZYMV infected 4 species from Cucurbitaceae systemically, and induced clear mosaic symptoms and sometimes leaf distortion. However, *Chenopodium quinoa*, *C. amaranticolor* and *Gomphrena globosa* showed necrotic local lesions on the inoculated leaves ten days after inoculation. The virus was transmitted by *M. persicae* and *A. gossypii* in a non-persistent manner. In SDS-agar gel double diffusion tests, the crude sap of infected leaves reacted positively with antiserum against ZYMV, but not with watermelon mosaic virus (WMV) antiserum.

5 Cruciferous crops

Main cruciferous crops in Peninsular Malaysia which consist of cabbage, radish, mustard leaves and kangkong, are mainly cultivated in Cameron Highlands. Virus diseases of these crops in this area were not observed. TuMV, however, was isolated from Pack-Choy in the leafy vegetable production area of Johor Baharu. The plants showed clear mosaic symptoms, developed deformed leaves, and became stunted.

Turnip mosaic virus : TuMV infected systemically 19 species from 7 families by sap inoculation, out of 32 species from 10 families tested, and induced clear mosaic symptoms and sometimes leaf distortion on turnip, radish and Chinese cabbage. Local lesion hosts were *C. amaranticolor*, *G. globosa*, and *N. tabacum*'Xanthi nc' reacted with local chlorotic spots. Filamentous virus particles were readily observed in leaf dip-preparations from Pack-Choy, and their length ranged from 700 to 750 nm. In SDS-agar gel double diffusion tests, the sap of infected leaves reacted positively with the antiserum against TuMV.

6 Other crops

A survey on virus diseases of eggplant was performed in 2 fields, and CMV-O was detected from samples showing severe mosaic symptoms with necrotic ring spot.

Spinach plants in the area near chilli fields showed severe mosaic symptoms and malformation of leaves caused by infection with TSWV.

II China

1 Sweet pepper

In our survey, more than 80% of the cultivated crops were found to be infected with mosaic disease in the fields of Shanghai and Guangdong. The plants showed severe mosaic symptoms with yellowing and stunting. So far, the occurrence of four kinds of viruses, TMV, CMV, PVX and PVY has been reported in China. Based on the present study, however, six kinds of viruses, namely CMV, pepper strain of TMV (TMV-P), CVMV, broad bean wilt virus (BBWV), AMV and an unknown potyvirus were isolated from infected pepper plants. TMV-P and CVMV were found to be the most prevalent viruses in the Shanghai area and in Guandong, respectively.

1) A pepper strain of tobacco mosaic virus : The field-infected sweet pepper plants showed clear mosaic symptoms and became often stunted. Mosaic symptoms also appeared on fruits of infected sweet pepper. The virus infected many kinds of solanaceous plants, and induced clear mosaic symptoms on *P. hybrida*, *N. tabacum* 'Samsun' and *N. clevelandii*. However, the virus could not infect *Lycopersicon esculentum*. Seeds collected from infected sweet pepper were germinated in the greenhouse, and the seedlings were assayed. Of the 74 seedlings tested, 4 were infected with TMV-P. The extracts of systemically infected tobacco leaves reacted positively with TMV-P antiserum from Japan in the immunodiffusion test and the precipitin lines to the virus and TMV-P from Japan fused with each other.

2) Broad bean wilt virus : The field-grown infected plants showed faint mosaic symptoms. The virus which has a relatively wide host range infected systemically many species from Leguminosae and Solanaceae. Many spherical particles, 25 nm in diameter were observed in leaf dip-preparations from infected plants and the virus was transmitted easily by *M. persicae* in a non-persistent manner. The virus reacted positively with antiserum against BBWV isolated from carrot in Japan.

3) Alfalfa mosaic virus : When inoculated with AMV, sweet pepper plants developed clear mosaic symptoms (bright yellow : calico), while the virus induced characteristic necrotic lesions on inoculated leaves of both *Phaseolus vulgaris* and *Vigna sesquipedalis*. The virus with bacilliform particles of different lengths, the longest being 60 nm reacted positively with antiserum against AMV isolated from soybean in Japan.

4) New potyvirus : Symptoms on the field-grown sweet pepper plants consisted of systemic mild mosaic and dwarfing of plant. A virus was isolated from necrotic local lesions on the inoculated leaves of *N. tabacum* (White Burley), and induced clear mosaic symptoms on *C. frutescens*. The host range of the virus and morphology of the virus particles resembled those of CVMV described above. On *C. amaranticolor* and *C. quinoa*, however, the virus induced characterisitic necrotic local lesions on the inoculated leaves without subsequent systemic infection. Furthermore, *Datura stramonium* and *D. metel* developed systemic mosaic symptoms after inoculation with the virus, but were not infected with CVMV. In SDS-agar gel double diffusion tests, the virus reacted weakly with antiserum against CVMV, and a clear spur developed between the precipitin lines to the virus and CVMV. However, the virus did not react with antisera against PVY, PVMV, pepper mottle virus and tobacco etch virus. Since the properties of the virus differed from those of several kinds of potyvirus described above, the virus may be a distinct new potyvirus of sweet pepper.

2 Tomato

TMV, CMV and PVX have been reported to commonly occur on tomato in China (Xinshun *et al.*, 1986). In our survey, two viruses, CMV and TMV were isolated. Two strains of CMV were found to infect tomato plants. The first caused fern leaf disease and systemic mosaic, narrowed leaves and stunting of plants. Yield of infected plants decreased and fruits were small. The other induced lethal necrotic symptoms on tomato. When seedling of tomato were infected with this strain of CMV, plants developed top necrosis symptoms and necrotic streaks on stem, and then died. TMV isolated from tomato was found to be a tomato strain of TMV, since the virus could not infect systemically tobacco (Bright Yellow, White Burley), *N. sylvestris* and *P. hybrida*.

3 Cucumber, pumpkin and wax gourd

Although two kinds of viruses, CMV and ZYMV, were isolated from cucurbit species, they often occurred together in nature. Many of the cucurbit species seem to be resistant or tolerant to CMV, and infection with CMV alone usually caused minimum loss of yield in each cucurbit crop. In those plants, however, ZYMV caused prominent mosaic symptoms, leaf reduction, cupping, rugosity, and plant stunting. Fruits also displayed distortion, discoloration and deformation.

4 Watermelon

1) Watermelon mosaic virus : A potyvirus with filamentous rods about 750 nm in length was isolated from watermelon showing mild mosaic symptoms in Shanghai. The virus could infect many cucurbit species and some leguminous plants, and could be easily transmitted by aphids in a non-persistent manner. Generally, the symptoms caused by the virus were less severe than those caused by ZYMV. Fruits were not distorted, but the color changed. In SDS-agar gel double diffusion tests, the sap of infected leaves reacted positively with the antiserum against WMV but not with ZYMV.

5 Cruciferous crops

Three kinds of viruses, TuMV, CMV and TMV have been reported in China. In our survey, TuMV and TMV were isolated from Pak-Choy in Guangdong.

1) A crucifer strain of tobacco mosaic virus : TMV from Pak-Choy, characterized by straight rods 300 nm in length, infected many species of Cruciferae, *Brassica rapa*, *B. rapa* var. Komatsuna and *B. chinensis*, and the plants showed clear mosaic symptoms. The sap of infected leaves reacted positively with antiserum against a cruciferous strain of TMV from spinach in Japan.

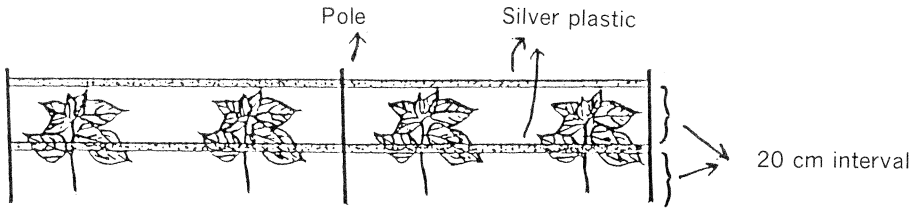
6 Other crops

A survey on virus diseases of eggplant was performed in 2 fields, and CMV was detected from samples showing mosaic symptoms.

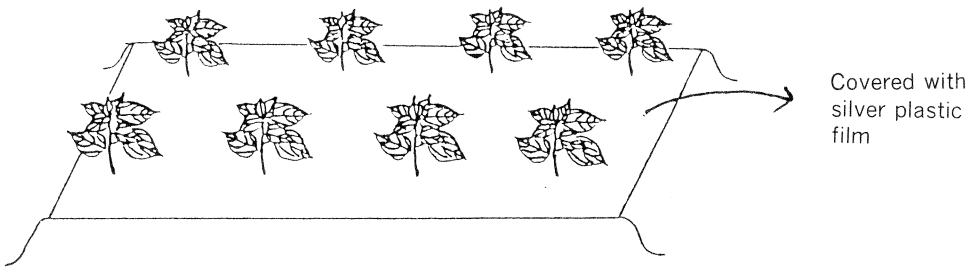
Cultural practices for the control of virus diseases of chilli

Among the viruses isolated in West Malaysia, CVMV and CMV can be transmitted by aphids. TSWV and TLCV have been reported to be transmitted by thrips and whiteflies, respectively, and the most common kinds of viruses in West Malaysia included CVMV, TSWV and CMV. Therefore, the control of aphids and thrips by chemicals or by mechanical protection of plants from the insect vectors is essential for the control of chilli virus diseases. In the present experiment, the effect of various kinds of agricultural materials such as plastic film or cheesecloth was tested for the purpose of cultural control of chilli virus under tropical Malaysian conditions (Fig. 7). A chilli variety 'Local' was sown into 15 cm plastic pots. Two treatments were applied at the nursery stage. Half of the nursery plants were kept under net (cheesecloth) cover and the other half exposed

1. Control (Open)
2. Silver plastic tape (5 cm width)



3. Mulching with silver plastic film (0.023 mm in thickness)



4. Spraying insecticides once a week (Elsan 50 EC)
5. Mulching with black and silver stripe plastic film and overhanging silver plastic tape

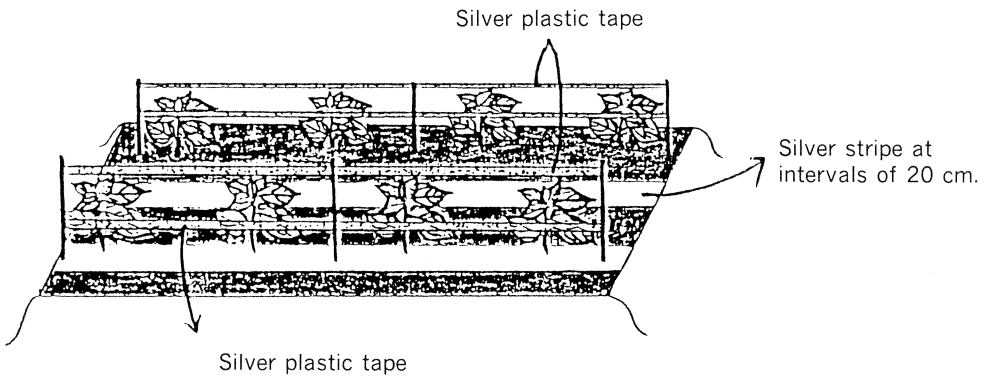
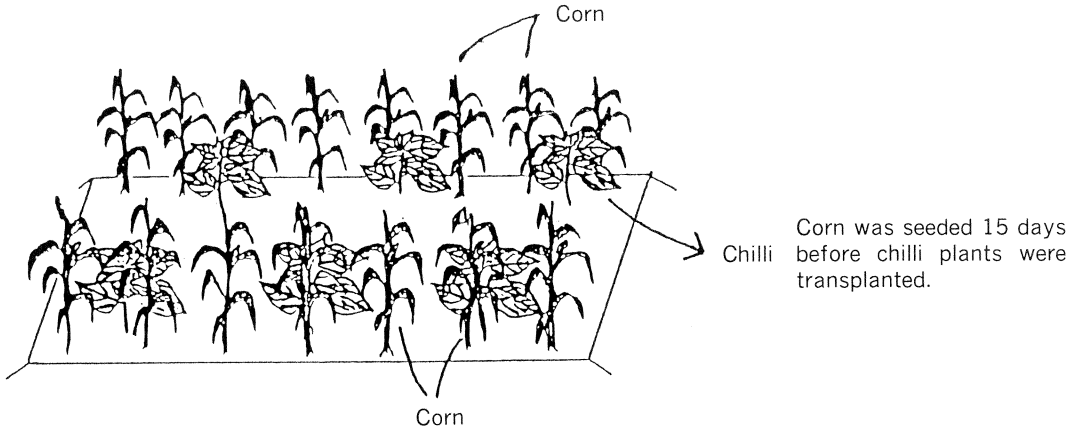
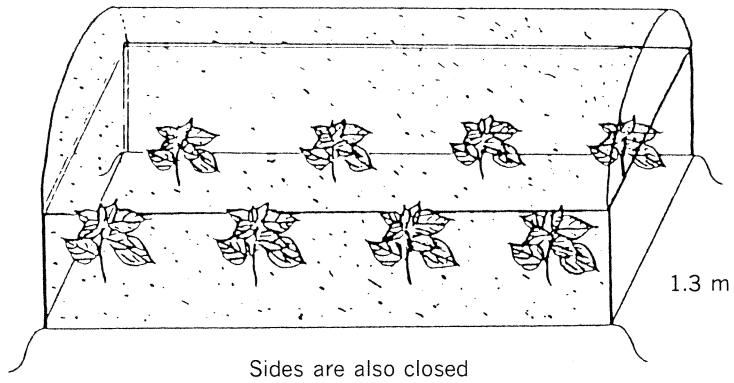


Fig. 7 Experimental design for cultural control of chilli virus

6. Intercropping with barrier crop (corn)



7. and 8. Sheltering with silver net or ultraviolet ray-free plastic film (0.1 mm thickness)



9. Mulching with silver plastic film, top sheltering with ultraviolet ray-free plastic film and side sheltering with silver net.

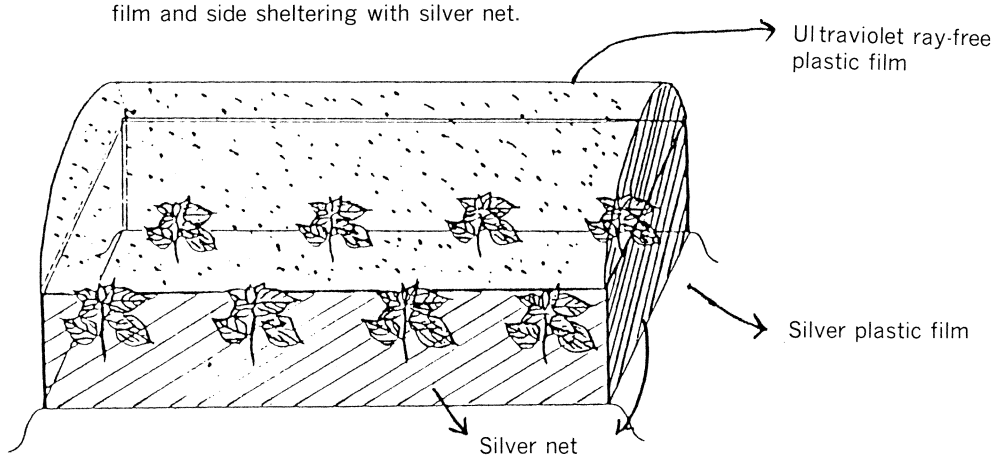


Fig. 7 (continued) Experimental design for cultural control of chilli virus

to open conditions. No insecticides were used for both groups of seedlings until transplantation to the fields. Among the seedlings grown under open conditions, the parasitic rates of aphids and thrips were 10% and 1%, respectively and 4% of the seedlings developed mosaic symptoms caused by virus infection. However, none of the seedlings grown under net cover conditions were parasitised by both aphids and thrips, and showed any virus symptoms. Fifty days after sowing, the seedlings were transplanted to the field except for those showing virus symptoms. On the 12 th, 34 th and 46 th day after transplanting, the population of insect vectors such as aphids, thrips and whiteflies, and the incidence of virus diseases on chilli were investigated. Although, no insects were observed on chilli plants at transplanting time, some plants grown under open conditions at the seedling stage showed mosaic symptoms associated with CVMV or CMV infection on the 12 th day of examination. On the other hand, none of the plants grown under net cover conditions at the seedling stage showed any symptoms. It is considered that the plants showing symptoms were already infected by viruses before transplanting time.

Thirty-four days after transplanting, a second investigation on the population of insect vectors and the incidence of virus diseases on chilli plants was carried out. The population of aphids was higher in each of these treatments, i. e. silver tape, intercropping and stripe mulch plus silver tape as compared with that of the control. However, the use of UV ray-free film, net cover or silver mulch reduced the population of aphids. Thus it was confirmed that these treatments prevented the parasitism of aphids to a large extent. The parasitism of thrips was commonly observed in many plots though the rate was low. The treatment with UV ray-free film, however, completely protected the chilli plants from parasitism of thrips. As for whiteflies, it was not worthwhile to record exact data due to their scarcity. In several treatments, the incidence of virus diseases was found to have increased compared to that observed at the nursery stage for seedlings without net cover. Some of the plants in the treatments with silver tape, silver mulch, intercropping and insecticides developed virus symptoms at the field stage even though they had been grown under net cover conditions at the seedling stage. The third stage of investigation on the incidence of virus diseases took place at fourty-six days after transplanting. In comparison with the data from the second investigation, the number of virus-infected plants increased slightly due to the spread by secondary transmission from infected plants. However, symptoms associated with virus infection could not be observed on plants in some treatments, such as UV ray-free film, net cover, and silver mulch plus net cover plus UV ray-free film, when the plants at the seedling stage had been grown under net cover conditions. On the 100 th day after transplanting, a final investigation on the incidence of virus diseases was carried out. Based on the results (Fig. 8), sheltering with UV ray-free film or net cover was found to be most effective in reducing the incidence of virus diseases. The treatment with silver tape or mulching with black and silver stripe film also protected the plants though the effects on the control of virus diseases were less conspicuous than in the case of treatment with UV ray-free film or silver net. Though silver mulch also reduced the incidence of the diseases, treated plants could not grow normally because the treatment had increased the soil temperature. It is considered that plastic mulch is unsuitable for tropical Malaysian conditions. Treatment with insecticides could not decrease the incidence of virus diseases due to continuous rain during the experimental period.

Chilli plants are generally cultivated for a long time, four to five months. In addition, they can be cultivated all the year round under the tropical climatic conditions. Furthermore, there are many kinds of viruses in the tropics, and the populations of vector insects are also high. As a result the incidence of virus diseases is always very high. Based on our experiments, virus-free chilli seedlings were obtained, when they were grown under net cover conditions for about 60 days until the first flowers started to bloom. When these virus-free seedlings were transplanted into the field, the occurrence of virus infection was delayed and treatment with net cover decreased the final incidence of virus diseases. It

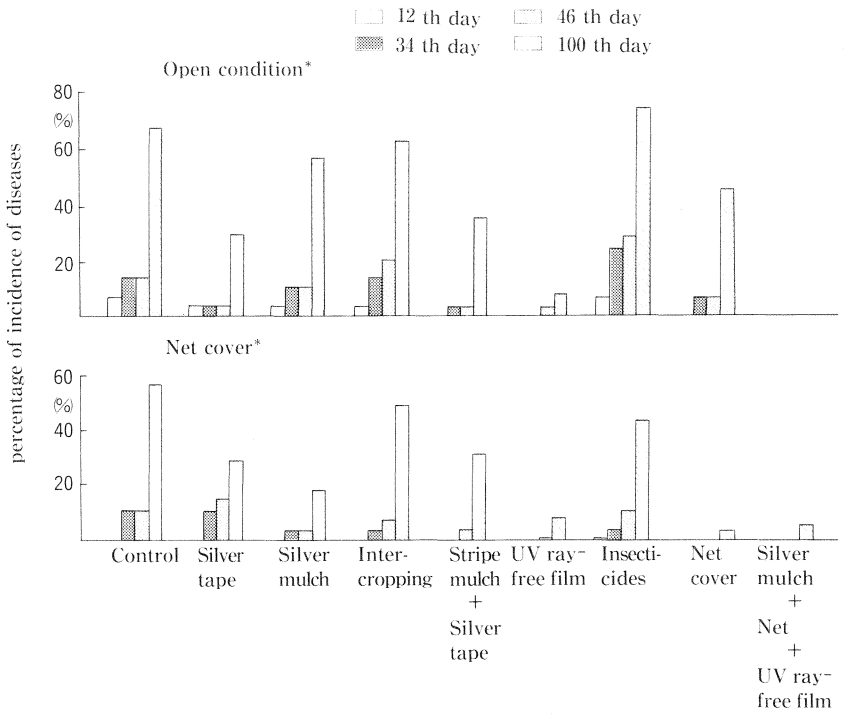


Fig. 8 Effect of agricultural materials on the incidence of virus diseases on chilli (*Growth condition at seedling stage)

is thus considered that the treatment at the nursery stage may result in a reduction of yield loss in chilli.

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