CHARPTER 5

Discussions

A survey of field-grown cucurbits e.g. zucchini, Japanese pumpkin and Japanese cucumber in the Royal Project Development Center's area (RPDC's area) during 2008-2009 was observed. Common symptoms observed on the disease samples were mottling, mosaic, vein banding, and deformed leaf which were agreed with symptoms of the CMV, PRSV, SqMV, WMV-2 and ZYMV in cucurbit reported in other countries (Abou-Jawdah *et al*., 2000; Yuki *et al*., 2000; Cradock *et al*., 2001; Dukic *et al*., 2002; Zitikaite, 2002; Revill *et al*., 2004; Dikova, 2005; Ozaslan *et al*., 2006; Guner and Wehner, 2008; Malik *et al*., 2010). In Thailand, there have been reports on various symptoms in cucurbits including mottling, mosaic, mid mosaic, vein-clearing, vein-banding, interveinal chlorosis, malformation and distortion (Noda *et al*., 1993; Nontajak *et al*., 2012 and 2013; Plapung and Smitamana, 2014). Most of the viruses collected during the surveys shown similar symptoms on zucchini, pumpkin, and cucumber because the same virus produced a wide range of symptoms on different cultivars (Jossey and Babadoost, 2008). Mixed symptoms were normally observed in cucurbit plantations. The ranges of symptom resulting from mixed viruses make it difficult to identify viruses by symptoms alone. ELISA was used to evaluate the incidence of viruses from these diseased samples. The ELISA method is outinely used worldwide for detection of viruses in field surveys because of its sensitivity and it enables the handling large number of samples (Yuki *et al*., 2000; Varveri *et al*., 2002 and Hossain *et al*., 2007). ELISA can be the preferable method for the detection of plant viruses than PCR because of it does not requiring extensive training and is more economical (Gumus and Paylan, 2013). Therefore, double antibody sandwich ELISA (DAS-ELISA) is a common diagnostic technique used to detect cucurbit viruses (Moradi and Jafarpour, 2011; Shim *et al*., 2006; Siriyan *et al*., 2006; and Yoon *et al*., 2008). The results of ELISA tests showed that cucurbit plants were infected with either single or multiple virus infection in cucurbit growing areas. More than 30 different viruses were reported to infect cucurbit crops worldwide (Mazereeuw *et al*., 2010; Ko *et al*., 2007; Yuki *et al.*, 2000). Viral diseases are one of the most serious problems for cucurbit productions considering the fact that there are many reports of plant viruses affecting the crops in Thailand such as CMV, ZYMV, MNSV, PRSV-W, SqMV, WMV-2, CGMMV, geminivirus and tospoviruses i.e. TSWV, watermelon silver mottle virus (WSMoV) and melon yellow spot virus (MYSV) (Tantiwanich *et al*., 1999; Coohapitagtam and Hongprayoon, 2004; Seetou *et al*., 2004; Chiemsombat, *et al*., 2006; Siriyan *et al*., 2006; Thonmo and Thummabenjapone, 2008; Nontajak *et al*., 2012 and 2013; Plapung and Smitamana, 2014). Samples collected were detected with single virus infection e.g. ZYMV, CMV, SqMV, PRSV-W, and WMV-2 in the early through the end of growing stage. Mixed viruses were predominantly detected at flowering stage through the end of the growing season in cucurbit growing areas. Most of viruses detected in cucurbits in the RPF's area were the same as many reported worldwide and the presence of ZYMV, CMV, PRSV-W and WMV-2 has been reported from Australia (Coutts and Jones, 2005); Europe: Spain (Luis – Arteaga, *et al*., 1998), Yugoslavia (Dukic *et al*., 2002), Bulgaria (Dikova, 2005), Turkey (Koklu and Yilmaz, 2006) ; USA (Walter *et al*., 2003), Brazil (Yuki et al., 2000); Mediterranean region (Lecoq and Desbiez, 2012), South Africa (Cradock *et al*., 2001), and Asia: Taiwan (Chang *et al*., 1987), Lebanon Abound-Jawdah *et al*. (2000), Iran (Gholamalizaden *et al*., 2008). The results showed the highest incidence for ZYMV, followed by PRSV-W, CMV, WMV-2 and SqMV. ZYMV was the predominantly in all detected samples from early stage to the end of the growing season. Similarly result as reported by Ozaslan *et al*. (2000) who reported that ZYMV was the general virus of cucurbit growing in Turkey, Brazil (Yuki *et al*., 2000; Koklu and Yilmaz, 2005), South Africa (Cradock, 2002) while, PRSV was the highest incidence in the cucurbit crops in Oklahoma (Ali *et al*., 2012), Veitnam (Revill *et al*., 2004) and Bulgaria (Dikova, 2005). WMV was the most prevalent virus in cucurbit production in Southern Illinois (Walter *et al*., 2003). In 2007, Kassem *et al*. have reported cucurbit aphid-borne yellows virus (CABYV) was prevalent in field-grown cucurbit crops of southeastern Spain while Abou-Jawdah *et al*. (2000) reported that ZYMV and CABVY were the most common viruses of field-grown cucurbits in Lebanon while Bonilha *et al*. (2009) reported PRSV-W and ZYMV were the most common viruses in cucurbit crops in Brazil (2009). Moreover, Plapung and Smitamana (2014) reported that CMV I was detected at the highest incidence in cucumber in northern Thailand during 2007-2008. This variation in the incidence of viruses in different locations could be due to the initial source of inocula, hosts, locations and transmission vectors early in the season.

Zucchini: common symptoms found on the disease samples were consisted of varying degrees of mottling and mosaic with leaf blistering and leaf deformation or stunting. The common symptoms on fruits were developing knobby areas and deformed fruit. It was agreed with symptoms of these viruses in zucchini reported in other countries (Dukic *et al*., 2002; Yamidaci and Korkmaz, 2004; Cutts *et al*., 2011). The results showed the highest incidence for ZYMV, followed by PRSV-W, SqMV, CMV and WMV-2. ZYMV was the predominantly in all detected samples from early stage to the end of the growing season, similarly result as reported by Dukic *et al*. (2002) and Yardimci and Korkmaz (2008) based on surveys ZYMV infection was determined in the squash field. Moreover, Walters *et al*. (2003) and Jossey and Babadoost (2008) have reported SqMV and WMV, respectively was the most prevalent virus in squash in Illinois. Ali *et al*. (2012) reported that the highest incidence for PRSV followed by WMV-2 and ZYMV among the collected cucurbit samples in Oklahoma. Mixed viruses were detected at flowering stage through the end of the growing season. The ZYMV, CMV, PRSV, SqMV, PRSV, and WMV diseases were reported to induce systemic mottling with leaf malformation in zucchini squash (Bananej and Vahdat, 2008; El-Shamy, 2010; Nontajak *et al*., 2013). The combination viruses showed highest incidence for ZYMV+PRSV-W, followed ZYMV+ SqMV, ZYMV+WMV-2, ZYMV+CMV, PRSV-W+CMV and PRSV-W+WMV-2. Dual infection of ZYMV+PRSV-W was the common combination of viruses found in infected zucchini samples, 16.85% which was coincided with Coutts and Jones (2005) who reported the most prevalent viruses, ZYMV and PRSV, infected cucurbits especially squash and zucchini incidences (1-60%) in the Northern Territory and Western Australia. PRSV and ZYMV were reported to cause various types of symptoms e.g. mosaic, stunting, and malformation of foliage such as blistering and shoestring (Fletch *et al*., 2000). Furthermore, Fattouh (2003) has reported ZYMV and CMV infecting zucchini squash. WMV and SqMV were found to be the most prevalent mixed infection detected in squash (Jossey and Babadoost, 2008). Triple infections of ZYMV+PRSV-W+SqMV and ZYMV+WMV-2+SqMV were the combination of viruses found in infected zucchini samples, 5.34 and 4.78% respectively. Combinations of such viruses leading synergy including the potyviruses and other viruses belonging to different families were described on cucurbits (Wang *et al*, 2002; Malik et al., 2010). These results revealed that zucchini grown in commercial fields of Royal Project areas were infected with viruses. All viruses detected in the current study were spread efficiently by aphids and mechanical inoculation (Sevik and Arli-Sokmen, 2003).

Pumpkins: common symptoms found on the disease samples were mottling, mosaic, chlorotic spot, blistering, curling, vein banding, several leaves deformation and developing knobby areas and deformed fruits. It was agreed with symptoms of these viruses in zucchini reported in other countries (Revill *et al*., 2004; Jossey and Babadoost, 2008). The results showed the highest incidence for ZYMV, followed by PRSV-W, WMV-2, CMV and SqMV. ZYMV was the predominantly in all detected samples from early stage to the end of the growing season, similarity of Muller *et al*. (2006) showed that ZYMV was predominated in collected pumpkin samples from Germany. Not agreed with reported by Yuki *et al*. (2000) who reported PRSV-W was the most frequently found in pumpkin plantations in Brazil while Revill *et al*. (2004) have shown squash leaf curl virus-Vietnam (SLCV-Vn) predominantly infecting pumpkin in Vietnam. Jossey and Babadoost (2008) were reported WMV was the most prevalent virus in squash in Illinois. Furthermore, the yellow leaf curl disease was reported as serious losses to pumpkins in Thailand (Ito *et al*. 2008). The combination viruses showed highest incidence for ZYMV+PRSV-W, followed ZYMV+CMV, PRSV-W+CMV, ZYMV+ SqMV, PRSV-W+WMV-2 and ZYMV+WMV-2. Dual infection of ZYMV+PRSV-W was the common combination of viruses found in infected pumpkin samples, 11.54% which was agreed with Yuki et al. (2000) who reported PRSV-W and ZYMV were the most common viruses infecting *C. moschata* collected from Brazil. Unlinked with Revell *et al.* (2004) who reported the most prevalent viruses, 10% infected with PRSV and CMV in cucurbit samples especially pumpkins and cucumbers incidences collected from Vietnam. Moreover, WMV and SqMV were found to be the most prevalent mixed infection detected in pumpkins (Jossey and Babadoost, 2008). Triple infections of ZYMV+WMV-2+SqMV and ZYMV+PRSV-W+SqMV were the combination of viruses found in infected pumpkin samples, 9.61 and 4.81% respectively. Combinations of such viruses leading synergy including the potyviruses and other viruses belonging to different families were described on cucurbits (Wang *et al*, 2002; Malik *et al*., 2010). These results revealed that pumpkin grown in RPF's areas were infected with viruses.

Cucumbers: common symptoms found on the disease samples were mottling, mosaic, internal chlorosis, yellowing, crinkle, blister, curling, vein banding, and leaf deformation chlorotic spot, blistering, curling, vein banding, several leaves deformation and deformed fruits. It was agreed with symptoms of these viruses in cucumber reported in other countries (Zitikaite, 2002; Revill *et al*., 2004; Muller *et al*., 2006) except cucumber diseased samples were collected from Mae Tha Neua where the field-grown showed silver patch or interveinal silver patch on leaves. Cucumber symptoms were collected from Mae Sa Mai where the greenhouse at summer season were showed severe yellowing and interveinal chlorosis on cucumber leaves more than Mae Phae, followed Huai Luk and Mae Tha Neua. Similarity to Abou-Jawdah *et al*. (2000) were that in greenhouse, the yellowing symptoms predominated on cucumber reached 100% during summer and early fin all in some locations in Lebanon. In greenhouse with insect proof, the situation differed symptoms were various yellowing and interveinal chlorosis types. In summer season, there was due to either high temperature inside greenhouse or due to nutritional disorders. The results showed the highest incidence for ZYMV, followed by PRSV-W, CMV, WMV-2 and SqMV. ZYMV was the predominantly in all detected samples from early stage to the end of the growing season similarity of showed that ZYMV was predominated in collected cucumber samples from Germany. Not agreed with reported by Revill *et al*. (2000) were reported CMV was the most frequently found in cucumber cultivations in Vietnam and Lithuania (Zitikaite, 2002). Plapung and Smitamana (2014) were reported that CMV I was detected at the highest incidence in cucumber in Northern Thailand during 2007-2008. In addition CGMMV was common detected in cucumber samples from Korea (Choi *et al*., 2001; Yoon *et al*., 2008) and Iran (Moradi and Jaforpour, 2011). Abou-Jawdah *et al*. (2000) have reported that CABVY was the most common viruses of field-grown cucumbers in Lebanon. The combination viruses showed highest incidence for ZYMV+CMV, followed PRSV-W+CMV, ZYMV+WMV-2, ZYMV+PRSV-W, PRSV-W+WMV-2 and ZYMV+SqMV. Dual infection of ZYMV+CMV was the common combination of viruses found in infected cucumber samples, was agreed with Muller *et* *al*. (2006) who reported mixed infection with ZYMV and CMV resulted in synergism effects, increasing the severity of damage. While Yuki *et al*. (2000) reported PRSV and ZYMV were high incidence in collected cucumber samples from Brazil. ZYMV and CABYV were the most common viruses causing severe symptoms and yield loss in cucumber plantation in Lebanon (Abou-Jawdah *et al*., 2000). Plapung and Smitamana (2014) have reported that double infection with CMV I and PRSV was detected at 4.4% in cucumber in Northern Thailand during 2007-2008. Triple infections of ZYMV+PRSV-W+CMV and ZYMV+PRSV-W+WMV-2 were the combination of viruses found in infected pumpkin samples, 5.20 and 2.02% respectively. Combinations of such viruses leading synergy including the potyviruses and other viruses belonging to different families were described on cucurbits (Wang et al, 2002; Malik *et al*., 2010). These results revealed that cucumber grown in RPF's areas were infected with viruses.

The results were detected with single virus infection in the early stage of cucurbit plantation and mixed viruses with one or two viruses were predominantly detected at flowering stage through the end of the growing season in cucurbit growing areas. Jossey and Babadoost (2008) have reported that single virus infection was detected earlier in the growing season while the mixed infections were more common from the second week until the end of growing season of pumpkin and squash fields. The 177 samples as 21.96% that showed virus-like symptoms did not react with both antisera used in serological test. The absence of positive reactions may be due to any other agents such as CGMMV, MNSV, CABYV and WSMoV or due to abiotic agents causing virus-like symptoms, as reported by Yuki *et al*. (2000). The results showed the highest incidence for ZYMV, followed by PRSV-W, CMV, WMV-2 and SqMV. ZYMV was the predominantly in all detected samples from early stage to the end of the growing season.

Mixed infections of the three potyviruses i.e. ZYMV, PRSV-W and WMV-2 were not a new finding. Similar mixed infections were observed in cucurbits worldwide (Lecoq et al., 2001; Ali *et al*., 2012). Most of viruses detected in cucurbits in the RPDC's area were synergistic interaction between potyviruses and other virus such as CMV. However, the accumulation of the other viruses increased while the accumulation of potyviruses decreased. This mixed infection causes considerable damage worldwide in severe epidemics in cucurbit plantations. Dual infection with ZYMV and CMV on zucchini resulted in a synergistic effect where symptoms in doubly infected plants were more enhanced than plants singly infected by either virus (Fattoh, 2003; Zeng *et al*., 2006). In addition, Wang *et al*. (2002) reported strong synergistic mixed infection with ZYMV and CMV in zucchini. This was attributed to increase in the level of accumulation of CMV positive strand RNV level. Example of other synergistic combination between potyvirus and viruses belonging to other genera was ZYMV and CABYV in muskmelon (Bourdin and Lacoq, 1994); CVYV and cucurbit yellow stunting disorder virus in cucumber (Gil-Salas et al., 2011); WMV and CGMMV in cucurbits (Kim et al., 2000; Moradi and Jafarpour, 2010).

This study provides information on the overall incidence of viruses in zucchini, pumpkin, and cucumber in RPDCs' areas, which could help to develop effective strategies for management of viral diseases in cucurbit fields. From the result shown numbers of vectors especially aphids were increased follow growth stage of plant and viral diseases from field were found increasing. However, ZYMV, PRSV-W, WMV-2 and CMV cause a significant economic loss in cucurbits spreads the most by aphid but non-persistent efficiently worldwide (Desbiez and Lecoq, 1997). ZYMV and PRSV were commonly found in the disease samples. Potyviruses and CMV transmitted non-persistently by aphid species (Garzo *et al*., 2004; Gildow *et al*., 2008; Pinto *et al.*, 2008; Mantri et al., 2004; Lima *et al*., 2012). *Aphis gossypii*, *A. craccivora* and *Myzus percicae* were commonly found high efficiency of potyvirus transmission. At least 26 aphid species reported enable to transmit ZYMV to cucurbit crops (Katis *et al*., 2006; Ozaslan *et al*., 2006; Simmons *et al*., 2011). Aphids spread viruses in a non-persistent manner. This means that the virus can be picked up by a feeding aphid on its stylet or mouth parts in only a few seconds, and then injected into the next healthy plant it feed in a matter of only a few seconds. The virus was retained in the aphid for only a short time, i.e. a matter of minutes to hours. As aphid transmission was non-persistent, spread generally occurs from nearby inoculums sources. Winged aphids were responsible for the bulk of the spread of the viruses up to several kilometers if wind assisted (Andret-Link and Fuchs, 2005). Moreover, from the result showed single aphid shown virus symptoms at 15 days after aphids' inoculation. So, ZYMV was transmitted by only one aphid. These reasons are rapidly spread of virus diseases in cucurbit fields.

Tospovirus was only found in cucumber. It had been reported tospoviruses cause severe damage on various economically important crops, such as pepper, tomato, peanut, watermelon, cantaloupe and cucumber in Thailand. Members of four tospovirus species including tomato necrotic ring spot virus (TNRV), Capsicum chlorosis virus (CaCV), watermelon silver mottle virus (WSMoV) and melon yellow spot virus (MYSV) have been reported in Thailand (Seetou *et al*., 2004 and 2005; Chiemsombat *et al.*, 2005; Ito *et al*., 2008). MYSV was the major tospovirus detected in cucumber (96%) and watermelon (55%), while *Thrips pallmi* Karmy were the major thrips species found in cucurbit fields. Mixed infection of tospoviruses was also found in cucurbit. Tospoviruses are naturally transmitted by at least eleven species of thrips (Thysanoptera: Thripidae) in a persistent propagative manner (Seepiban *et al*., 2013). Additionally this study showed that alternate hosts collected from natural and commercial field were detected many viruses such as ZYMV, PRSV-W and CMV were detected in *Benincasa hispida, Cucurbita moschata, Luffa acutangula, L. cylindrica, Sechium edule* and *Solanum lycopersicum. Sechium edule* or chayote was a popular crop grown in almost the high land, being frequently found near other cultivated cucurbit crops. Several chayote plantings were located near cucurbits from which collected samples tested positive for many viruses, it was suggested that high incidence of cucurbit viruses in chayote associated with virus infection might be due to epidemic of viruses in the field. This way was one reason which virus diseases spread from infected plant to plant.

The relationship between the CGMMV transmitted seed and proper detection stages of the infected cucumber plants. Japanese cucumber is one of the most popular cucurbits in the highland of Northern Thailand. Viral diseases are one of the most serious problems for cucumber production considering the fact that there are many reports of plant viruses affecting the crop in Thailand such as CMV, SqMV, MNSV, PRSV-W, and Tospovirus (Coohapitagtam and Hongprayoon, 2004; Siriyan *et al*., 2006; Thonmo and Thummabenjapone, 2008; and Nontajak et al., 2012). CGMMV is a newly emerging virus affecting cucumber production in the country. Accurate identification and detection of CGMMV are the first steps in successful management of the disease. CGMMV was studied because this virus can be seed–borne, survive in the soil, be transmitted mechanically during agricultural practice and through water and have the potential of wide distribution throughout the world. There are some reports that showed seed, pollen, and soil transmissions are important factors as primary sources in epidemic development of tobamovirus diseases (Liu, *et al*., 2013). CGMMV is physically very stable and accumulates in high concentrations in the infected tissue of host crops and is easily transmitted by plant sap. Plant viral diseases are diagnosed by applying a combination of methods, and relying only on symptoms may be misleading because different viruses such as ZYMV and SqMV can cause similar or identical symptoms for example leaf mosaic, distortion and stunting (Zhao *et al*., 2003). Our observations of the disease symptoms were based on samples collected from the main cucumber growing areas and from a location in which cucumber had never been grown before. Common symptoms observed included mottle, mosaic, and deformed leaves which were in agreement with symptoms of CGMMV in cucurbits reported in other countries (Antignus *et al*., 2001; Choi *et al*., 2002; Kim *et al*., 2003; Jafarpour, 2011 and Nematollahi *et al*., 2013). DAS-ELISA result, CMV, ZYMV, SqMV and CGMMV were not detected in the commercial seeds of zucchini (*C. pepo*) cultivar SENATOR (hybrid squash, lot No.978883); Japanese pumpkin (*C. moschata*) cultivar DELICA (hybrid squash, lot No.08021); and Japanese cucumber (*C. sativas*) cultivar PRETTY SWALLOW 279 (cucumber F1 hybrid, lot No. EA25061). The virus was not found in the ground cucumber seeds, endosperm, seed coated, cotyledons and true leaves (10 dap.) saps Coutts *et al*.(2011) were reported that Low level as 0.7% seed transmission of ZYMV was found in seedlings grown from seed collected from zucchini (*C. pepo*) fruit infected with isolate Cvn-1. Simmons *et al*., 2011 reported 1.6% seed transmission in *C. pepo* subsp. texana (wild gourd), with infected seedlings beings symptomless. CGMMV was not detected by DAS-ELISA in the sap of ground whole cucumber seeds, endosperm without peel, seed coats, cotyledons or leaves (10 dap.) in the seed lot tested. In 2006, Shim *et al*. reported that CGMMV seed transmission rate for cucumber were usually 8% up to one month after seed harvesting; this rate further decrease to 0.1% in 5 months. The infection rates for the seeds could be much lower than was detectable by our methods. The rate of CGMMV contamination for seed from the mechanically inoculated plants was similar to a previous study of watermelon (100%) and melon (93.85%), which used ELISA to detect the presence of CGMMV in single seeds (Wu *et al.*, 2011). Seed transmission of CGMMV in cucumber was detected at low rates

(3.0%) despite very high rates of seed contamination as 17% (Liu, *et al*., 2013). In current study, CGMMV was detected in30% of seedlings from Huai Luek where the crop was grown in the greenhouse. Liu *et al*. (2013) reported that the rate of transmission from seed to seedling in cucumber was up to 76.7%. In our study, CGMMV was detected in disease samples that were collected from the main cucumber growing area at flowering through the end of growing stage. Thonmo and Thummabenjapone found CGMMV in 25% of cucurbits samples collected from cucurbit seed production fields in Northeast Thailand. CGMMV causes one of the most common diseases in commercial cucumber production throughout the world (Slovokhotova *et al*., 2007; Yoon *et al*., 2008). The relatively low percentage of positive results for CGMMV in the symptomatic samples might have been due to the fact that the cucumber plants were infected with different viruses such as CMV, ZYMV, PRSV-W, WMV-2, and SqMV as well as by CGMMV (Noda *et al*., 1993; Notajak *et al*., 2012; Siriyan *et al*., 2006; Shabanian *et al*., 2007). Mixed infection of cucumber plants by CGMMV and other viruses and abiotic causes (nutrient deficiencies, herbicide, etc.) of the symptoms also cannot be ruled out. Yield of cucumber from the main cucumber growing areas was lower than from the area where cucumber was a new crop. CGMMV is a worldwide problem in cucumber production areas like the Europe, United stated and Asia (Varveri *et al*., 2002; Moradi and Jafarpour, 2011; Yoon *et al*., 2008; Zhou *et al*., 2008). The cucumber fruit infected with this virus appear to be distorted and reduced in size, resulting in poor quality and marketability. Many of the fruit in this study exhibited symptoms of CGMMV infection, being dark green, with a blistered appearance (Liu *et al*., 2013). Yield losses caused by CGMMV may be 15% (Mazereeuw, 2010; Rashni, 2005; Varveri *et al*., 2002). So, control of CGMMV and other virus diseases in the cucumber production involves preventive measures designed to reduce sources of infection from inside and outside the crop and to minimize the effect of infection on yield. Use of virus-free seed for cucumber production and avoidance of infecting transplants during production is essential to ensure that only uninfected transplants are set in the field. During the growing seasons, control measures include rouging and sanitation (Budzanivska *et al*., 2006; Choi, 2001).

A study of correlation between viral combination and symptom on zucchini was observed. Zucchini seedlings were inoculated with single, double, triple and multiple infections with ZYMV, CMV, PRSV, WMV-2 and CGMMV. Symptom development for the virus infected plants with either single or multiple viruses in zucchini were found that all viruses produced similar symptoms during the early stages of infection. Common symptoms observed on the zucchini at 7 dai were mottle and mosaic on young leaves. According to the paper reported by Choi *et al*., (2002) that ZYMV produced similar symptoms in zucchini at 7-10 dai including severe yellow-green mosaic and leaf malformation. In 2003, Fattouh *et al*. reported that infection with PRSV alone caused severe mosaic symptoms on all zucchini plants, while plants infected with ZYMV mild strain alone presented, as expected, milder leaf symptoms, and WMV caused vein clearing, mosaic, and blistering of zucchini leaves at 7-10 dai (Moradi and Jafarpour, 2010). Inoculation of zucchini with ZYMV, PRSV-W and CGMMV alone at seedling stage also reduced plant growth. Our findings showed that the strains of CGMMV, PRSV-W and ZYMV used in this study were highly virulent and caused severe mosaic, deformed apical leaves and plant death, whereas the less virulent strains of CMV or WMV-2 produced mosaic, mottling and vein clearing. In general, the interactions between these two groups were identified as antagonistic effect. In nature, plants are commonly infected by two or more viruses (Hull, 2002), therefore different interactions among viruses have been observed, such as synergism, helper-dependence, cross-protection, replacement, mutual suppression, and a mixture of antagonistic and synergistic interactions (Zeng *et al.*, 2007). Combination of CMV and/or WMV-2 with other virus produced milder symptoms from which CMV or WMV-2 showing the capability to lessen symptoms caused by ZYMV, PRSV-W and CGMMV at 14, 21, 28 and 35 dai. Antagonistic effects, shown by the attenuation of symptoms including prevention of plant death during the experiment, were first observed at 14 dai with the mixed infection of the CMV and/or WMV-2 with the other viruses or virus combinations. These results do not agree with previous reports that indicated that mixed infections of viruses from the family Potyviridae and those from other genera typically produce synergism in plants (Gil-Salas *et al*., 2011). Zucchini plants doubly infected with ZYMV and CMV have been shown to exhibit a severe synergistic pathological response and showed a strong increase in the level of accumulation of CMV, with no increase in the accumulation of ZYMV (Kosaka, 1997; Zeng *et. al*., 2001; Choi *et al*., 2002; Wang *et al*., 2002; Fattouh, 2003; Bonilha *et al*., 2000).

Nevertheless, the interactions did not always induce synergistic symptoms (Wang *et al*., 2004; Untiveros *et al*., 2007) and the outcome depended on the particular combination of virus species (Kokkinos and Clark, 2006). In another case, peanut plants inoculated with the tomato spotted wilt virus and peanut mottle virus did not result in an intensification of symptom severity compared with either virus alone (Hoffmann *et al*., 1998). The interactions between plant viruses in mixed infections are generally categorized as synergistic or antagonistic, creating usually unpredictable biological and epidemiological consequences were likely occurred in plants (Zhang *et al*., 2001; Syller, 2012). The clear mechanisms of these effects are still unknown. Our research identified more antagonistic than synergistic effects of the mixed plant virus infections, but more data is still needed in order to elucidate more a concrete explanation of this effect.

The presence of cultivated cucurbit crops, alternate hosts and favorable weather for vector populations, made virus diseases problem for the growers. During the growing seasons, control measures include rouging and sanitation (Budzanivska *et al*., 2006; Choi, 2001). Thus, a better understanding of virus and insect vectors reservoirs, vector population behavior, and vector transmission efficiency were necessary for the development of effective control measurements. The most important strategy for control of virus diseases on cucurbit crops in the high land of the northern Thailand would be the elimination of the common practice of starting new crops near old infected cultivations. Old crops should be destroyed before starting new plantings. Control of alternate hosts in the neighborhood of cucurbit crops would be reducing virus source. Chemical spraying used to control insect vector populations.