Occurrence and Distribution of Viruses Causing Diseases in Pumpkin and Evaluation of Selected Management Practices for CMV

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Occurrence and Distribution of Viruses Causing Diseases in Pumpkin and Evaluation of Selected Management Practices for CMV

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DEDICATED
TO
MY BELOVED
PARENTS, BROTHER
AND
HUSBAND

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CERTIFICATE

This is to certify that the thesis entitled " ***Occurrence and Distribution of Viruses Causing Diseases in Pumpkin and Evaluation of Selected Management Practices for CMV"*** *submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in PLANT PATHOLOGY,*** *embodies the result of a piece of bona fide research work carried out by* ***TOMANINA BINTE RAHMATULLAH,*** *Registration No.* ***11-04657*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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Abstract

Pumpkin (*Cucurbita moschata*) belongs to the family Cucurbitaceae, is an important crop in the tropical and subtropical regions of the world. Due to high content of vitamin A, it is very nutritious and can play a vital role in meeting the vegetable shortage and nutritional problems. Diseases caused by viruses have a negative effect on the yield of pumpkin and other cucurbit crops. A survey was conducted to collect virus infected leaf samples of pumpkin to find the occurrence and distribution of viral diseases of pumpkin from three districts of Bangladesh. A field experiment was also conducted to determine specific symptom (s) associated with *Cucumber mosaic virus* CMV of pumpkin to aid visual diagnosis and serological detection and to find suitable management strategies for pumpkin infecting CMV diseases. The experiment was conducted during October'2017 to April'2018. The experiment was laid out in RCBD with three replications in the field. The seedlings with two cotyledons were inoculated with CMV and then transplanted in main field for management this virus. During survey, ten (10) characteristics symptoms were recorded as indicator of virus infection through visual observation. Among these symptoms, four symptoms showed positive to serological test by using CMV antiserum. By observing color of ELISA test, it was concluded that mosaic, yellow mosaic, chlorosis and hardy leaves symptoms showed positive to CMV. In field management experiment, CMV incidence and severity both showed the lowest in treatments T_1 (Inter crop coriander) which was 21.10% and 11.11%, respectively whereas disease incidence (%) and disease severity (%) both were maximum in T_6 (Control) and which were 70.84(%) and 26.67(%) respectively. In case of growth and yield attributes, there were significant variations found in all attributes. Thus, in this study the effective management was intercropping by coriander. A negative relation between CMV disease severity (%) and yield (in kg) per treatment indicated that with the increase of disease severity (%), yield of pumpkin decreased. On the contrary, positive relation between CMV disease severity (%) with aphid population (no.) which indicated that with the increase of aphid population (no.), infection rate is increased. Inoculated CMV was identified in pumpkin leaves by visual observation and six (6) major categories of viruses symptoms were found in field viz. mosaic, yellow mosaic, fern leaf, chlorotic spot, leaf distortion and hardy leaves by visual observation. Among them, in serological test, barrier crop maize, yellow trap, chemical Malathion 57 EC and control treatments of pumpkin were infected with CMV which symptoms categories were mosaic, yellow mosaic, leaf hardening, curling and chlorosis shown positive during serological test by using CMV antiserum.

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LIST OF ACRONYMS

BBS	**= Bangladesh Bureau of Statistics**
BARI	**= Bangladesh Agricultural Research Institute**
RCBD	**= Completely Randomized Block Design**
LSD	**= Least significant difference**
Cm	**= Centimeter**
CV (%)	**= Percentage of coefficient of variance**
et al.	**= And others**
Kg	**= Kilogram**
Ha	**= Hectare**
SRDI	**= Soil Resources Development Institute**
ELISA	**= Enzyme Linked Immunosorbent Assay**

CHAPTER 1

INTRODUCTION

Pumpkin (*Cucurbita moschata*) belongs to the family Cucurbitaceae. It is an important and popular vegetable crop grown in the tropics and subtropics (Lovisolo, 1981). It is seed propagated, day neutral, monoecious (bearing male and female flowers in the same plant); vine, insect pollinated an annual crop having a climbing or trailing habit (Katyal and Chadha, 2000). Some scientists believe that Central America and Northern South America (Whitaker and Davis, 1962) are the origin of pumpkin.

Bose and Som (1986) mentioned that pumpkin fruits are good source of vitamins, especially high carotenoid pigments and minerals. It is very nutritious due to high content of vitamin A and can play a vital role in meeting the vegetable shortage and nutritional problem (Begum *et al.*, 2016). The nutrient per 100 g edible portions of fruit is 90 ml water, 8 g carbohydrate, 1 g protein, 0.5 g fibers, 20 mg calcium, 0.8 mg iron, 21 µg β-carotene, 0.05 mg thiamine, 0.05 mg riboflavin, 0.5 mg niacin and 15 mg ascorbic acid (Tindall, 1987) (Appendix-IV).

In Bangladesh, the total area under cultivation of pumpkin is 11,359.526 ha with an annual production of 1, 04,723 M ton in Kharif season and 17,254.177 ha and production 1, 86, 112 in Rabi season (BBS, 2016).

The production of pumpkin is declining due to attack by several diseases, such as fungal, bacterial and viral diseases. More than 50 different viruses have been found to infect cucurbits including pumpkin (Lovisolo, 1981). The most common viruses infecting cucurbits are from the CMV, ZYMV, WMV, PRSV, CGMMV and ZGMMV. These viruses occur in complex or which may cause sole infection (Provvidentii, 1996).

Identification of pumpkin viral diseases by farmers and their advisors is difficult because the diseases cannot be identified reliably by their symptoms. CMV, PRSV-W, WMV and ZYMV may exhibit different symptoms at times, and at other times have overlapping symptoms. In addition, different isolates of a virus may result in different symptoms (Davis and Mizuki, 1987). Among these viruses *Zucchini yellow* *mosaic virus (*ZYMV), an economically important virus belonging to the family Potyviridae, genus *Potyvirus* (Regenmortel *et al*., 1982).

Cucumber mosaic virus (CMV) is an important pathogen, which belongs to genus *Cucumovirus* in the family Bromoviridae. It has the broadest host range known for any plant virus with approximately 1000 susceptible plant species, including monocots and dicots, herbaceous plants, shrubs, and trees (Roossinck, 2001). It is also reported (Douine *et al*., 1979) to cause severe leaf mosaic and deformed, stunted, or mottled fruits. PRSV-W is characterized by drastic reduction of vegetative growth and loss of fruit yield upto 100% (Pereira *et al.,* 2007; Freitas and Rezende, 2008; Rahman *et al*., 2010).

Pumpkin viruses are transmitted in a non persistent manner by 24 species (15 genera) of aphids. Virus diseases caused by non-persistently transmitted viruses which are difficult to prevent by insecticide application (Raccah, 1986) which is the most frequent measure use by growers.

Control of virus in Bangladesh is difficult due to unavailability of virus resistant cultivar, presence of virus and their vectors round the year and growing of crops in numerous small plots over a large area with little isolation (Gonsalves, 1989).

Control of virus spread is required but since once infected, there is no way to cure of greenhouse or field grown plants of viruses. Accurate diagnosis of the viruses present in a region is required for developing appropriate integrated management of these diseases (Ali *et al*., 2012). But so far, basic information and research on the existence and distribution of viruses causing diseases of cucurbits in the region is not yet available.

Begum *et al.*, 2016 conducted host range test on fourteen indicator plants which were mechanically inoculated with four detected viruses (PRSV-W, WMV2, CMV and ZYMV).

Insecticide sprays against the aphid vectors are not effective in reducing virus disease because aphids transmit virus before the insecticides act to kill them (Jayasena and Randles, 1985; Maelzer, 1986; Simmons, 1957; Webb and Linda, 1993).

Several management practices for the control of virus diseases of cucurbits have been reported including the use of different types of plastic mulch (Brown *et al*., 1993; Summers *et al*., 1995), mineral oil (Simons and Zitter, 1980).

The occurrence of aphid-borne virus diseases was significantly reduced with both mulches as opposed to bare soil, and reflective plastic performed better than wheat straw. Plants grown over straw mulch produced higher overall yields, including large-size melons, than those grown over bare soil (Summers, *et al.*, 2005).

Virus diseases of plants are best managed by an integrated approach that includes planting healthy seed, plant resistance, isolation, sanitation, elimination of plant reservoirs of viruses such as weeds or volunteer plants, cross protection, crop rotation, virus or vector avoidance by alternating planting or harvesting time, host free periods, control of insect virus vectors through pesticides, yellow traps, sticky traps, netting, trap or border crops, or reflective mulches, and rouging (Ali *et al*., 2012).

The presence of the virus is confirmed in the sampled plant parts after symptom expression by ELISA. For serological detection of viruses by dot-immunosorbent assay leaf samples of ribbed gourd having symptoms of virus diseases such as fern leaf, chlorotic spot, mosaic, inter veinal chlorosis, vein-clearing and leaf curl were used (Kader *et al.,* 1997). Detection of CMV, PRSV, SLCV, SqMV, WMV, and ZYMV in cucurbits has been achieved by utilizing alkaline phosphatase enzyme-linked immunosorbent assay (ELISA) kits (Provvidentii, 1996, Walters *et al.*, 2003).

This research aimed to find out the presence and distribution of viruses infecting pumpkins in the different regions of Bangladesh and developing effective management strategies against them. Considering the above circumstances, the present study was undertaken with the following objectives:

1) To find out occurrence and distribution of viruses causing pumpkin diseases in Bangladesh using serology;

2) To determine specific symptom (s) of virus associated with pumpkin in field condition, aid to detect by visual diagnosis and serology; and

3) To find suitable management strategies for CMV diseases of pumpkin in field.

CHAPTER 2

REVIEW OF LITERATURE

Among cucurbit crops pumpkin is one of the most important vegetables as its different parts like fruits, flowers, vines are used in various purposes. Its nutritional value is also high. But the viral diseases cause severe losses of pumpkin production. This chapter is representing the available literature on various aspects of pumpkin viral diseases so far.

2.1. Origin and distribution

Pumpkin (*Cucurbita moschata*) which is from the family cucurbitaceae is an important and popular vegetable crop grown in the tropics and subtropics (Lovisolo, 1981 and Annon., 1990). Though the origin of pumpkin is not known definitely, but they are thought to have originated in North America. Pumpkin-related seeds dating between 7000 and 5500 BC, were found in Mexico is the oldest evidence (Credo Reference, 2008). Moreover, some scientists believe that Central America and Northern South America (Whitaker and Davis, 1962) are the origin of pumpkin. The cultivation of pumpkin was started from Southern part of USA and continues up to Peru of South America. Now a days it is grown throughout the entire tropical and subtropical regions of the world and also in the milder areas of the temperate zones of hemispheres, in many countries of the world; In India, China, Malaysia, Taiwan and Bangladesh are the countries where it is widely cultivated. It is distributed widely such as Southeast Asia, tropical Africa, South and Central America (Peru and Mexico), the Caribbean and most parts of the tropics. According to Tindall, 1987 *C. moschata* is probably the most widely grown species of Cucurbits (Tindall, 1987).

2.2. Nutritional value of pumpkin

Bos and Som (1986) mentioned that pumpkin fruits are good source of vitamins, especially high carotenoid pigments and mineral**s.** Tindall (1987) stated that the nutrient per 100 g edible portions of fruit is 90 ml water, 8 g carbohydrate, 1 g protein, 0.5 g fibers, 20 mg calcium, 0.8 mg iron, 21 µg β-carotene, 0.05 mg thiamine, 0.05 mg riboflavin, 0.5 mg niacin and 15 mg ascorbic acid. Shanmugavelu (1989) stated that the young leaves, male flowers and mature or immature fruit of pumpkin are used as vegetable and also cattle feed in Bangladesh.

2.3. Survey on Cucurbit Viral Diseases

Coutts and Jones (2005) surveyed which was done to determine the incidence and distribution of virus diseases infecting cucurbit crops growing in the field. The survey carried on in Australia. Overall 43 cucurbit-growing farms and 172 crops of susceptible cultivars were sampled in this survey. The result showed that, 56% of sampled crops and 72% of farms were virus-infected.

Dukić *et al,* (2006) surveyed on cucurbit diseases, in the Vojvodina region of Serbia where severe symptoms resembling those caused by viruses were observed on bottle gourd (*Lagenaria siceraria* (Molina) Standl.). In this survey the symptoms which were taken in consideration were stunting, mosaic, green vein banding, blistering, yellowing, chlorotic spots, leaf deformation and fruit distortion.

Köklü and Yilmaz (2006) showed a result through covering 17 melon fields and 19 watermelon fields in the Tekirdag, Edirne and Kırklareli provinces by the survey of June and July, 2005 of Turkish. The survey was carried for the detection of C*ucumber mosaic virus* (CMV), *Papaya ring spot virus*-W (PRSV-W), *Squash mosaic virus, Squash Melon necrotic spot virus* (MNSV), *Cucumber green mottle mosaic virus* (CGMMV), *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus*-2 (WMV-2).

Jossey and Babadoost (2008) identified the viruses infecting pumpkin and squash in Illinois. *Cucumber mosaic virus* (CMV), *Papaya ring spot virus* (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), and unknown poty viruses were detected in pumpkin, squash, and gourd fields during the survey using enzyme-linked immunosorbent assay (ELISA). Overall, 86, 11, 75, and 79% of jack-o-lantern pumpkin, processing pumpkin, squash, and gourds, respectively, were tested positive for virus infection during the survey. WMV was detected in 47, 46, and 52% of the samples in 2004, 2005, and 2006, respectively which was the most prevalent virus throughout the state.

Kaveh Bananez and Asian Vahdat (2008), surveyed on cucurbit viruses in the major cucurbit-growing areas of 17 provinces in Iran was conducted in 2005 and 2006.

Screening for 11 cucurbit viruses by double-antibody sandwich ELISA (DAS-ELISA) or RT-PCR, found that 71% of the samples were infected by at least one virus, of which *Cucurbit aphid-borne yellows virus* (CABYV) was the most common. The most frequent double infections were WMV+CABYV and ZYMV+CABYV in melon, squash and cucumber, followed by WMV+ZYMV. In watermelon, the most frequent double infection was WMV+ZYMV, followed by WMV+CABYV.

Ali *et al*., (2012) surveyed and found the following viruses: Eighteen plant viruses were detected, including, *Cucumber mosaic virus*, *Garlic common latent virus*, *Iris yellow spot virus*, *Onion yellow dwarf virus*, *Melon necrotic spot virus*, *Papaya ring spot virus*, *Pepino mosaic virus*, *Pepper mild mottle virus*, *Potato moptop virus*, *Potato virus M, Potato virus X, Potato virus Y, Squash mosaic virus*, *Tomato mosaic virus*, *Tomato spotted wilt virus*, *Tomato yellow leaf curl virus*, *Watermelon mosaic virus*, and *Zucchini yellow mosaic virus*. Virus incidence was close to 100% on some crops, including cucurbit and onions where double or triple infections were common.

2.4. Viruses of cucurbits

Squash leaf curl virus (SLCV; genus *Begomovirus*, family Geminiviridae) has been reported on cucurbits from Arizona, California, and Texas (Cohen *et al.*, 1983; Nameth *et al*., 1986).

Papaya ring spot virus (PRSV; Davis, and Muzuki, 1987, Nameth *et al*., 1986, Sammons *et al*., 1989, Ullman *et al.*, 1991), *Watermelon mosaic virus* (WMV; Davis, R. F, and Muzuki, 1987; McLean, and Meyer 1961; Nameth *et al.*,1986; Sammons *et al*., 1989), and *Zucchini yellow mosaic virus* (ZYMV) (Davis and Muzuki, 1987; McLeod *et al*., 1986; Nameth *et al*., 1986; Provvidenti *et al*., 1984; Ullman *et al*. 1991) of the genus Potyvirus (family Potyviridae) also have been reported in squash, pumpkin, and other cucurbit crops from many regions in the United States.

Squash mosaic virus (SqMV; genus *Comovirus*, family Comoviridae) has been detected in South Carolina and Texas (McLean and Meyer, 1961; Sammons *et al.*, 1989).

Viruses are the most important pathogens of cucurbits (cucumber, watermelon, melon and pumpkins) belonging to the Cucurbitaceae family. More than 30 infectious viruses causing destructive symptoms and considerable economic losses were reported on these plants (Zitter *et al*., 1996).

Diseases caused by viruses are among the serious threats to cucurbit production in Illinois. About 32 different viruses have been reported to be economically important on cucurbits in the world (Provvidentii, 1996).

Tobacco ring spot virus (TRSV) and *Tomato ring spot virus* (ToRSV), in the genus *Nepovirus* of the family Comoviridae, have been reported in cucurbits. TRSV has been reported from South Carolina, Texas, and Wisconsin (McLean, and Meyer 1961, Sammons and Barnett, 1987, Sammons *et al.*, 1989 Sinclair, and Walker 1956) and ToRSV has been reported from the northeastern United States (Provvidentii, 1996).

In a study conducted in southern Illinois, WMV was reported to be the most prevalent cucurbit virus (Walters *et al.,* 2003). In addition, CMV, PRSV, SqMV, and ZYMV were detected to cause mixed infections with WMV late in the season in southern Illinois. Pumpkin (*Cucurbita moschata*), an important Cucurbitaceae vegetable, is cultivated throughout tropical and subtropical regions of the world.

Pumpkin yellow vein mosaic disease (PYVMD) is a major constraint for the cultivation of pumpkin in India (Muniyappa *et al*., 2003; Jayashree *et al*., 1999).

2.5. Cucumber Mosaic Virus

Cucumber mosaic virus (CMV) is the type species in the genus *Cucumovirus*, family Bromoviridae (Roossinck, *et al*., 1999) CMV has the broadest host range of any known virus, infecting more than 1,000 species of plants, including monocots and dicots, herbaceous plants, shrubs, and trees. In the 85 years since its discovery (Doolittle; 1916; Jagger, 1916), CMV has been found in all parts of the world, and numerous strains have been characterized.

Cucumber mosaic virus (CMV; genus *Cucumovirus*, family Bromoviridae), has been reported from all over the United States in squash and watermelon (Davis, and Muzuki 1987, McLean, and Meyer 1961, McLeod *et al*. 1986, Sammons *et al*. 1989, Ullman *et al.*, 1991).

LMV or CMV consists of stunting, chlorosis, mosaic and improper heading of infected plants (Cock, 1968; Bruckart and Lorbeer, 1975). The virus is readily transmitted in a non-persistent manner by more than 75 species of aphids (Palukaitis *et al*., 1992).

Virus diseases are a major constraint in commercial cucurbit production (Lovisolo, 1980, Provvidentii, 1996.), causing sporadic epidemics. More than 39 different viruses have been reported to cause cucurbit diseases and many are responsible for economic losses in the quality and quantity of cucurbit crops (Lecoq, 2003., Lecoq, 1998, Provvidentii, 1996).

Cucumber mosaic, first described in 1916 (Doolittle, 1916), was one of the earliest plant diseases attributed to a virus (Jagger, 1916). As many as 40 different plant diseases were later shown to be caused by *Cucumber mosaic virus* (CMV) (Kaper and Waterworth, 1981).

A number of extensive reviews have been published on CMV which detailed the biology of the virus (Edwardson and Christie, 1991; Kaper and Waterworth, 1981; Palukaitis *et al*., 1992; Roossinck *et al*., 1999).

CMV infects over 1000 species of hosts, including members of 85 plant families, making it the broadest host range virus known. The virus is transmitted from host to host by aphid vectors, in a non persistent manner. The virus particles are about 29 nm in diameter, and are composed of 180 subunits. (Roossinck, 2001)

A large number of CMV strains have been described, and the sequence databases contain about 60 different coat protein sequences, as well as 15 complete viral genome sequences. The species includes three subgroups, IA, IB and II, with as much as 25% nucleotide sequence divergence between them (Roossinck *et al*., 1999). Thus, CMV has proved itself as a highly adaptable virus, with an unusual capacity for evolutionary change, making it both a menace to agriculture worldwide, and an ideal model for studying RNA virus evolution. (Roossinck, 2011)

2.6. Occurrence, distribution and Identification of pumpkin viruses

The occurrence, spreading, intensity of infection and destructiveness of viruses depend on complex interrelations between the virus, its host plant, the vectors and the environment.

Zucchini yellow mosaic virus (ZYMV) was detected in squash grown in greenhouses on the Mediterranean coast of the country (Davis, 1986).

Davis and Mizuki (1987) reported that it is difficult to identify the viral diseases of pumpkin by the farmers and advisors because the diseases can not be identified reliably by their symptoms. CMV, PRSV-W, WMV and ZYMV may exhibit different symptoms at times, and at other times have overlapping symptoms.

Three other viruses recorded in Turkey are *Watermelon mosaic virus*-1 (renamed *Papaya ring spot virus*; [PRSV]; (Erdiller, G., and Ertunc, F. 1988), *Cucumber vein yellowing virus* (Yilmaz, *et al*., 1995), and *Melon mosaic virus* (Yilmaz, *et al*., 1991).

In 1992, *Tomato ring spot virus* and *Tomato black ring virus* were detected only in cucumber (Fidan, 1995).

Krstiã *et al.,* (2002) identified the major viruses of Serbia infecting pumpkins (*Cucurbita pepo*). Plants showed different symptoms which are virus infected. Only by visual examination, the causal viruses could not be fully and precisely determined due to the great variability of the symptoms.

Singh *et al.,* (2003) recently identified that, potyvirus causing severe economic damage to zucchini squash. From leaves and fruits of zucchini (*Cucurbita pepo* L.) the virus was isolated which were collected from commercial fields near Pune.

Farhangi *et al*., (2004) reported that viral diseases are the main threat of cucurbits. They determined the distribution of *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), and *Watermelon mosaic virus* (WMV). 466 samples were collected from squash field in Tehran province. Through DAS-ELISA distribution of CMV, ZYMV and WMV were determined. The percentage of ZYMV, WMV and CMV were 35.6, 26.1 and 25.1% respectively. Triple infection (ZYMV + CMV +WMV) were found in 6.4% of samples. ZYMV were found the most frequently the viruses. This is the first report of WMV on squash in Tehran province.

Zitikaitė *et al.,* (2011) collected plant leaves in Ukraine and examined. They found that *Cucumber mosaic virus* (CMV) causing viral diseases of many important plants worldwide which have been isolated from pumpkin (*Cucurbita pepo* L.). The determination of causal agent has been based on host range, symptom expression in the test plant species and morphological properties of the virus particles using transmission electron microscopy (EM), and using specific oligo nucleotide primers in reverse transcription-polymerase chain reaction (RT-PCR).

Pumpkin (*Cucurbita moschata*) samples showing yellow vein mosaic disease in Varanasi region were identified with *Begomovirus* infection using PCR amplification. (Namrata Jaiswal *et al*., 2012)

2.7. Incidence of viral diseases

According to Yuki *et al.,* (2000) in Brazil PRSV-W and ZYMV were the most common viruses infecting pumpkin and other cucurbitaceous crops. They observed that PRSV-W and ZYMV were accounting 49.1 and 24.8% of incidence; on the other hand CMV and WMV-2 were accounting 6.0 and 4.5% incidence respectively.

Incidence of the disease can go up to 100 % under mono-cropping (Maruthi *et al*., 2003).

Coutts and Jones (2005) found that, the growing areas with Darwin and Carnarvon were the highest incidences of virus infection, and lowest incidences found in the area Katherine and Perth. Virus infection found in overall 78% of farms and 56% of crops for WA, and in the NT 55% of farms and 54% of crops were virus infected. ZYMV and PRSV were most prevalent viruses, each being detected, respectively and In 5 and 4 of 6 cucurbit-growing areas, the most prevalent viruses were found, with infected crop incidences of <1–100%. SqMV was detected in 2 cucurbit-growing areas, sometimes reaching high incidences (<1–60%). In 3 and 4 of 6 cucurbit-growing areas WMV and CMV were found, respectively, but generally at low incidences in infected crops (<1–8%).

Köklü and Yilmaz (2006) showed a result where the tested viruses on watermelon were found in the following rates of incidences: ZYMV (45.5%), WMV-2 (34.2%), CMV (19.9%), PRSV-W (2.1%), SqMV (1.8%) and MNSV (0.4%), while the rates of incidence on melon were ZYMV (40.3%), WMV-2 (31.2%), CMV (7.2%), PRSV-W (2.3%), SqMV (0.5%) and MNSV (1.8%)., The WMV- 2+ZYMV mixed infection type was the most widespread both on melon and on watermelon samples and which was 16.7% and 11.4%, respectively.

Kaveh Bananez and Asian Vahdat (2008) found that 71% of the samples were infected by at least one virus, of which *Cucurbit aphid-borne yellows virus* (CABYV) was the most common overall, occurring in 49, 47, 40, and 33% of cucumber, squash, melon, and watermelon samples respectively. The second most common virus on melon and watermelon was *Watermelon mosaic virus* (WMV) (incidence 30–33%); on cucumber, *Cucumber mosaic virus* (CMV)(33%); and on squash, *Zucchini yellow mosaic virus* (ZYMV) (38%).Mixed infections occurred in 49% of symptomatic samples.

Bananej and Vahdat (2008) **s**creened for 11 cucurbit viruses where 71% were *Cucurbit aphid-borne yellows virus* (CABYV)The second most common virus on melon and watermelon was *Watermelon mosaic virus* (WMV) (incidence 30–33%); on cucumber, *Cucumber mosaic virus* (CMV) (33%); and on squash, *Zucchini yellow mosaic virus* (ZYMV) (38%).

Kone *et al.*, (2017) found that the rate of infection of various cucurbit crops by the three viruses (CMV, ZYMV and PRSV) varied from one cucurbit species to the other at various planting dates. For instance, in the dry season, CMV had 100% infection of *lagenaria*, followed by zucchini (42.7%), cucumber (30%) and pumpkin (25%) whereas ZYMV was more prevalent in pumpkin (75%), and followed by cucumber (63%) and zucchini (42.4%).

2.8. Symptoms of pumpkin viruses

Many scientists (Bos, 1978; Holmes, 1964; Matthews, 1981 and Smith, 1972) emphasized that symptoms in all cases may not identify the causal virus but its use in preliminary diagnosis of many plant viruses have been well established.

In many cases each individual virus produces symptoms on the host which is unique or particular for the certain virus (Bos, 1978; Holmes, 1964).

CMV, PRSV, SqMV, TRSV, and WMV have been reported to induce systemic mottling with leaf malformation in squash (Webb 1971).

According to (Yeh *et al.*,1984) the symptoms for example, produced by *Papaya ring spot virus* (PRSV) to papaya and other host are characterized by ring spot development on the infected foliar parts including fruits, *Cucumber mosaic virus*

(CMV) induces fern leaf in tomato, *Tomato ring spot virus* produce circular ring on leaves and fruits, *Tomato spotted wilt virus* produce lunate necrotic spot on the leaves and fruits of tomato etc. are the diagnostic characteristics of those viruses or can be said identical for those viruses.

It was reported by the scientists (Hollings and Burnt, 1981; Akanda, 1991; Smith 1972; Lovisolo, 1980; Purcifull *et al.,* 1984) that symptoms produced by PRSV-W may be as mottling, mosaic, vein clearing, and according to (Gonsalves, 1998 and Lecoq, 2001) chlorosis, distortion and leaf deformation.

PRSV and ZYMV have been reported to cause mosaic, plant stunting, and malformation of foliage such as blistering and shoestring symptom (Davis *et al*., 1987).

Virus symptoms on cucurbit vary from mild mosaic or vein banding to severe systemic mosaic and malformation of leaves, color change and deformation of fruit, and plant stunting (Davis *et al*., 1987, Mclean *et al*., 1961, Sammons *et al*., 1989).

In squash and pumpkin, SLCV induced leaf curl and mosaic symptoms (Cohen *et al.* 1983).

Choi *et al*., 1990; Bilgrami and Dub, 1996) stated that, at early stage of infection Pumpkin leaf showed mosaic symptom. But leaves showed yellow mosaic with vein banding and leaf distortion at later stage of infection.

Somowiyarjo, 1993; showed that, at later stage of infection when pumpkin plant was infected by ZYMV specially fern leaf and shoestring type leaf distortion was appeared. A mild mosaic symptom was recorded in field-collected samples of *Cucurbita maxima* which were confirmed as PRSV using DIBA.

Dahal *et al.,* (1997) also found severe mosaic, leaf distortions, blisters and shoestring on squash, on the other hand mosaic or yellow mosaic, leaf distortion and blisters were recorded on other cucurbits which were infected by PRSV.

Brunt *et al.,* (1997) noted that the symptoms of PRSV-W which were mosaic, systemic chlorotic mottling, green blistering or spotting, leaves and fruit malformation etc. are shown by different cucurbitaceous crops.

Kader *et al.,* (1997) showed that the samples which showed positive reaction to PRSV-P antiserum exhibited mosaic, vein clearing and leaf curl and samples which were positive to PRSV-W showed chlorotic spot and inter veinal chlorosis while the other type of symptoms observed were necrotic severe mottle, severe mottle and mild mottle along with deformation of leaves in PRSV infected cucurbits.

The most common symptoms in infected plants are leaf mosaics and distortions, reduction in fruit size, and abnormal fruit color and shape (Sevik and Arli-Sokmen, 2003).

Pumpkin yellow vein mosaic disease (PYVMD) is a major constraint for the cultivation of pumpkin in India (Jayashree *et al*., 1999; Muniyappa *et al.,* 2003).

Incidence of the disease can go up to 100 % under mono-cropping (Maruthi *et al*., 2003). Infected plants are exhibit yellowing of veins in young leaves and intensive mosaic patches at later stages. The affected plants become stunted and exhibit premature flower drop.

Singh *et al.,* (2003) stated that symptoms of PRSV differed in some of the cucurbits. Chlorotic spots and mottling in *Luffa acutangula*, mottling, mosaic, puckering along with vein clearing in *Cucumis sativus* and *Cucumis pepo,* chlorotic and necrotic spots on *Cucumis melo* var. *utillissium* were observed in a study of host range of PRSV.

Singh *et al.,* (2003) reported that mosaic, vein banding and blotching on leaves and produced mottled, irregularly shaped blisters and filiform leaves were shown by the infected zucchini plants. The virus was readily transmitted by mechanical sap inoculation.

Farhangi *et al*., (2004) reported the symptoms showed by the infected plants which were: mosaic, yellowing, deformation, shoe string of leaves and fruit deformation and yield reduction.

And it was stated that, cucurbit growing is affected negatively due to diseases caused by cucurbit viruses. Cucurbit virus was identified by serologically in order to prevent this damage. Occasionally symptoms are curling, wrinkling, spot mosaics, yellowing, shape deformation on leaves, smaller leaves than normal, buff-colored mosaics, observed on younger leaves of cucurbits and stunting, distortion and fruit deformation on the plants but due to this study, it is usually difficult to give definitive diagnosis based on symptoms.

According to Jossey and Babadoost (2008), Dual infection of WMV and SqMV was the most prevalent mixed virus infection detected in Illinois. Most viruses infecting pumpkin and squash showed similar symptoms. The most common symptoms observed in the commercial fields and in the greenhouse studies were light- and dark-green mosaic, vein banding, vein clearing, puckering, and deformation of leaves of pumpkin, squash, and gourds. Severe symptoms included fern leaf and shoe string on leaves and color breaking and deformation of fruit.

Zitikaitė *et al.,* (2011) found the symptoms like: light green mottled foliage. Leaves were smaller, yellow mottled and crinkled.

Begum *et al*., (2016) recorded the symptoms of the viral infection of pumpkin include fern leaf, mosaic, leaf curling, chlorosis, leaf distortion, and smaller leaflets of plants.

2.9. Management

Several management practices for the control of virus diseases of cucurbits have been reported including the use of different types of plastic mulch (Summers *et al*., 1995), mineral oil (Simons and Zitter, 1980), floating row covers with fine mesh placed directly over the plants (Perring *et al*., 1989) and cross-protection using mild strains of the predominant virus or viruses (Lecoq *et al*., 1991; Walkey *et al.*, 1992; Rezende and Pacheco, 1998).

The elimination of primary sources of virus inoculums by crop isolation in time and space, and by elimination of alternate hosts, are potential management strategies that have been successful for some virus diseases (Sylvester, 1989).

2.9.1 Intercrop

Damicone and Edelson (2007) conducted five field trials over 3 years, control of aphid-transmitted, non-persistent virus diseases on pumpkin, caused mostly by the potyviruses *Watermelon mosaic virus* (WMV) and *Papaya ring spot virus* type-W (PRSV-W) and achieved by intercropping with grain sorghum, as opposed to clean tillage. Reductions in disease incidence ranged from 43 to 96% ($P \leq 0.05$).

Damicone and Edelson (2007) reported that, intercropping soybean and peanut with pumpkin reduced disease incidence by 27 to 60% ($P \leq 0.05$), but disease control generally was less than for grain sorghum.

According to Pitan and Filani, (2014), the effectiveness of maize (*Zea mays* L.) planted at different times in a maize–cucumber intercrop to reduce the density of cucumber insect pests was investigated in 2007 and 2008. Irrespective of the cucumber variety, there was a significant reduction (over 50%) in the density of insect pests in the cucumber–maize intercrop compared with cucumber alone. Fruit damage was significantly lower (about 50%) in the intercropped cucumber. Therefore, a significant control of cucumber insect pests and a higher cucumber yield were obtained when cucumber and maize were planted on the same day.

2.9.2 Border crop

Damicone and Edelson (2007) reported that, surrounding pumpkin plots with borders of peanut, soybean, or corn was not effective. Borders of grain sorghum were effective, but disease control was generally less than for the intercrop treatment.

According to Nderitu *et al.,* (2008) some border crops have potential use in aphid management in okra crop and can be used in combination with border spraying in an integrated pest management strategy to maintain the pest below economic damage.The four crops used as border crops; maize (*Zea mays* L.), Sorghum (*Sorghum bicolour* L.) Moench) pigeon peas (*Cajanus cajan* L. Milisp.) and millet (*Pennissetum glaucum* L.) gave the following results: The plots bordered by pigeon peas and maize had lowest and highest mean aphid population among the border crops respectively. However, maize bordered plots recorded the highest number of parasitized aphids in both seasons. In all the treatments, there was no significant difference ($p>0.05$) in the yield of okra.

2.9.3 Yellow trap

Abdel-Megeed *et al.,* (1998) demonstrated that for control purposes, yellow sticky traps can significantly reduce the density of *B. tabaci* in field. But all these mentioned studies about the effect of traps on whitefly were conducted during only part of a crop’s growing period.

Yellow sticky traps are a commonly used method for population monitoring of many pests. In recent decades, studies of these traps mainly focused on how to use them to monitor populations of pest species such as whiteflies, leaf miners, and aphids (Byrne *et al.,* 1991; Shen and Ren 2003; Zhou *et al*.,2003; Qiu *et al*., 2006; Gu *et al*., 2008).

In recent years, yellow sticky traps have also been used as a method for the control of some pests, especially for the control of whitefly. The combination of yellow sticky traps and parasitoids has proven to be an effective method for the control of *B. tabaci* in a greenhouse (Shen and Ren, 2003; Gu *et. al.*, 2008).

Lu *et al*., 2012, in the greenhouse, yellow sticky traps significantly suppressed the population increase of adult and immature whiteflies. The whitefly densities in the greenhouse with traps were significantly lower than the greenhouse without traps. In the field, traps did not have a significant impact on the population dynamics of adult and immature whiteflies. The densities in fields with traps were very similar to fields without traps. These results suggest that yellow sticky traps can be used as an effective method for the control of whiteflies in the greenhouse, but not in the field. This information will prove useful for the effective management of whiteflies in greenhouses.

2.9.4 Chemical control

Insecticide sprays against the aphid vectors are not effective in reducing virus disease because aphids transmit virus before the insecticides act to kill them (Jayasena and Randles1985; Maelzer 1986; Simmons 1957; Webb and Linda, 1993).
There are many different insecticides available for managing whiteflies; however, imidacloprid and spiromesifen have been shown to be highly effective in managing all the developmental stages of the whitefly (Topanta *et al*., 2008; Palumbo 2009; Palumbo *et al.,* 2001; Nyoike and Liburd 2010; Stansly *et al*., 1998)
According to Webb *et al*., 2011, several insecticides available for managing whiteflies that encompasses various modes of action as defined by the Insecticide Resistance Action Committee (IRAC).

Success of the insecticide treatments in reducing WVD can likely be attributed to the efficacy of whitefly suppression by the two insecticides used in the study combined with the semi-persistent nature of SqVYV transmission (Webb *et al*., 2012).

Kousik *et al*., 2015, found that the insecticide-treated plots had significantly fewer fruits with WVD symptoms compared to the non-treated plots regardless of the mulch treatment in each of the three years. In all three experiments, the insecticide-treated plots had significantly fewer symptoms of WVD on both the foliar and vine tissues and fruit suggesting that application of insecticides to manage whitefly populations can help mitigate the effects of SqVYV.

2.9.5 Uses of Field mulches

Reflective film mulches of white or silver color have been effective in providing partial disease control by delaying the onset of virus epidemics (Conway *et al.*, 1989; Green 1991). A limitation of reflective films in cucurbits has been that plant growth rapidly covers the mulch and thereby lessens reflectivity. The application of row covers to summer squash until flowering was not effective in reducing virus disease, and caused some yield reduction (Conway *et al.*, 1989)

Stapleton and Summers (2002) tested and compared the effectiveness of reflective polyethylene and biodegradable, synthetic latex spray mulches for management of aphids and aphid-borne virus diseases of late-season cantaloupe (*Cucumis melo* L. var. *cantalupensis* cv. Primo) in the San Joaquin Valley. Beneficial responses were obtained from the reflective mulches, under conditions of high aphid populations and virus inoculums potential, during each of the experiments. Aphid numbers on leaves of plants growing over mulches were consistently lower than on those growing over bare soil. Partial bed coverage with spray mulch, and alternate row applications of polyethylene film mulches, were less effective than complete coverage of every planted row.

According to Summer *et al*., 2004 Plastic UV reflective mulch (metalized mulch) and wheat straw mulch delayed colonization by *Bemisia argentifolii* Bellows & Perring and the incidence of aphid-borne viruses in zucchini squash.. In 2000, yield of marketable fruit in the plastic and straw mulched plots was approximately twice that from the imidacloprid plot. In 2001, yield from the straw mulch plots was twice that of the imidacloprid and plastic mulch plots.

Summers *et al*, 2005 compared reflective plastic and wheat straw mulches with conventional bare soil for managing aphid-borne virus diseases and silver leaf whitefly in cantaloupe. The occurrence of aphid-borne virus diseases was significantly reduced with both mulches as opposed to bare soil, and reflective plastic performed better than wheat straw.

Filho *et al.*, 2014 experimented on organic mulches, like peel and rice-straw, besides other materials affect the UV and temperature, which cause a reduction in the aphid arrival. The aim was to evaluate the effect of covering the soil with straw on the populations of the green peach aphid. The temperature increased in the mulched plots to a maximum of 21–36°C and to 18–32°C in the plots with or without soil covering, respectively. The first experiment evaluated the direct effect of the rice-straw mulch and the second its indirect effect on aphid immigration, testing the plant characteristics that could lead to the landing preference of this insect. The third experiment evaluated the direct effect of the mulch on the aphid population. This was partially due to temperatures close to 30°C in these plots and changes in the plant physiology. The soil mulching with rice-straw decreased the aphid, *M. persicae* landing, increased the plot temperatures and improved the vegetative growth.

2.10. Serology for identification of pumpkin virus

Serodiagnosis has been highly evaluated as effective and quick method. In plant virus research several serological methods have been developed and applied. Clark and Adams (1977) developed enzyme-linked immune sorbent assay (ELISA).

Yeh *et al*., (1984) carried out research on the serological comparison of *Papaya ring spot virus* (WMV-1). Difference between PRSV and WMV-1 was that former infected papaya but the later did not. By Agar gel immune diffusion test with antiserum to PRSV and WMV-1All the isolates of PRSV and WMV-1 were serologically tested which were indistinguishable as determined. The conclusion was that PRSV isolates have similar biological and serological properties irrespective of geographic region.

Richter *et al*., (1989) conducted an experiment in serial detection of *Cucumber mosaic virus* by direct double antibody sandwich ELISA (DAS-ELISA) and the results led to the development and testing of indirect ELISA using test plants and that were unsatisfactory results. Only in the absence of other cucumoviruses the indirect ELISA could detect CMV from the samples and therefore, recommended for serial detection of CMV in crude leaf extracts of different cucurbits.

According to Akanda *et al*., 1991, the ELISA has been extensively used since its introduction to plant virology for rapid diagnosis of viruses from field sample. However, in the recent years many scientists has been recommended DIBA for diagnosing viruses from field samples due to several merits like high sensitivity, rapidness, reliable, economic etc. over ELISA.

Akanda *et al.*, (1991) observed that samples of various cucurbitaceous crops showing virus disease-like symptoms reacted positively against antiserum of CMV, PRSV, WMV-2 and SqMV, respectively in different region of Bangladesh. None of the samples reacted with antiserum of ZYMV or CGMMV.

Kader *et al.* ,(1997) reported that for serological detection of viruses by dot-immunobinding assay leaf samples of ribbed gourd having symptoms of virus diseases such as fern leaf, chlorotic spot, mosaic, interveinal chlorosis, vein-clearing and leaf curl were used. Out of the six different samples fern leaf, mosaic, vein-clearing and leaf curl were found to be positive against antiserum of PRSV-P. Chlorotic spot and inter veinal chlorosis were found positive against the antiserum of PRSV-W and WMV-2 respectively.

Yilmaz and Sherwood (2000) detected that, formats of protein-A ELISA (PASELISA), antigen-coated plate ELISA (ACP-ELISA),and indirect ELISA kit were examined and compared for their usefulness in detection of Cucumber mosaic virus (CMV), *Papaya ring spot virus* type W (PRSV-W), *Squash mosaic virus* (SqMV),*Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV). Though results indicated that CMV can be detected by all three assays but indirect ELISA kit is recommended for CMV. In PAS- ELISA, SqMV specifically and strongly reacted against SqMV antiserum, but not in ACP-ELISA and indirect ELISA formats. The three potyviruses, PRSVW, WMV and ZYMV reacted with antiserum of these viruses and cross reacted with all the three antiserum in the three ELISA formats. Results revealed that indirect ELISA kit was suitable for the detection of CMV, PRSV-W, WMV and ZYMV, while PAS-ELISA was useful for the detection of SqMV.

Krstiã *et al*., (2002) by the biotest tested infected samples, as well as by two serological methods, ELISA and EBIA. Against *Cucumber mosaic cucumovirus* (CMV), *Zucchini yellow mosaic potyvirus* (ZYMV), *Watermelon mosaic* potyvirus 1

(WMV-1), *Watermelon mosaic potyvirus* 2 (WMV-2) and Squash *mosaic comovirus* (SqMV) Polyclonal antibodies were raised and used. One or two viruses were detected in each of the 50 collected samples. ZYMV (62%) and CMV (58%) were most prevalent viruses infecting pumpkins. WMV-2 was extremely rare.

Sevik and Arli-Sokmen (2003) reported that, the presence of the virus is confirmed in the inoculated plants after symptom expression by ELISA. The objective of this study was to determine the incidence of viral diseases in pumpkin and squash in Illinois for the goal of developing effective strategies for their management. The most common symptoms in infected plants are leaf mosaics and distortions, reduction in fruit size, and abnormal fruit color and shape.

Detection of CMV, PRSV, SLCV, SqMV, WMV, and ZYMV in cucurbits has been achieved by utilizing alkaline phosphatase enzyme-linked immunosorbent assay (ELISA) kits (Provvidentii 1996, Walters *et al.*, 2003).

Papayiannis *et al*., (2005) done a survey to determine the identity and prevalence of viruses affecting cucurbit crops in Cyprus, 2993 samples of cucumber, zucchini, melon and watermelon were collected from the five major cucurbit-growing areas in Cyprus. The detection of *Zucchini yellow mosaic virus* (ZYMV), *Papaya ring spot virus* type W (PRSV-W), *Watermelon mosaic virus* (WMV), *Cucurbit aphid-borne yellows virus* (CABYV), *Cucumber mosaic virus* (CMV) and *Squash mosaic virus* (SqMV) were done by enzyme-linked immunosorbent assay (ELISA), and by reverse transcription polymerase chain reaction (RT–PCR) *Cucurbit yellow stunting disorder virus* (CYSDV), *Beet pseudo-yellows virus* (BPYV) and *Cucumber vein yellowing virus* (CVYV) ZYMV were detected which were the most prevalent virus of cucurbits in Cyprus with an overall incidence of 45%. PRSV-W, CABYV and WMV were detected in 20.8%, 20.8% and 7.8% of the samples tested, respectively.

Coutts and Jones (2009) reported that, to determine the incidence and distribution of virus diseases infecting cucurbit crops growing in the field at Australia a survey was done. In this regard, as a whole, 43 cucurbit-growing farms and 172 crops of susceptible cultivars were sampled. Enzyme-linked immune sorbent assay (ELISA) was performed in case of every samples using antibodies to *Cucumber mosaic virus* (CMV), *Papaya ring spot virus-cucurbit strain* (PRSV), *Squash mosaic virus*

(SqMV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV).

Dukić *et al.,* (2006) collected leaf samples from 25 symptomatic plants. For virus identification samples were collected from two localities using mechanical transmission and serological testing. Using double-antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA). Field-collected bottle gourd and inoculated plants were tested. On collected and inoculated plants with polyclonal antiserum (Loewe Biochemica, Sauerlach, Germany), Positive reactions were obtained to *Zucchini yellow mosaic virus* (ZYMV) in 23 samples, with antiserum to *Watermelon mosaic virus* (WMV) in eight samples, and with antiserum to *Cucumber mosaic virus* (CMV) in seven samples. Each of the three viruses was detected in single as well as in mixed infections with the other two viruses.

Köklü and Yilmaz (2006) tested 502 melon and watermelon samples for the confirmation of the presence of seven viruses with ELISA tests using polyclonal antiserum. For the investigated viruses overall, 333 out of 502 samples tested positive: 167 out of 235 plant samples in Tekirdag, 103 out of 187 samples in Edirne, and 63 out of 80 samples in Kırklareli were positive. Serological tests showed that in the Thrace region of Turkey, six out of the seven tested viruses were present.

Banane and Vahdat (2008) **s**creened for 11 cucurbit viruses by double-antibody sandwich ELISA (DAS-ELISA) or RT-PCR, found that 71% of the samples were infected by at least one virus, of which *Cucurbit aphid-borne yellows virus* (CABYV) was the most common overall, occurring in 49, 47, 40, and 33% of cucumber, squash, melon, and watermelon samples respectively.

Begum *et al.,* (2016) conducted ELIZA against 26 pumpkin breeding lines. Among the lines, seven (Pk13-1-1, Pk20-2-1, Pk02-2-1, Pk19-4-1, Pk54- 4-12, Pk01-10-9-4 and Pk106) did not react to any of the four antiserum tested. Of the rest line, five (Pk55-2-2, Pk05-1-2, BARI mistikumra 1, BARI mistikumra 2 and Pk101) were positive to PRSV-W; five (Pk05-4-1, Pk05-8-2, Pk75-1, Pk07-4-7 and Pk102) ZYMV, two (Pk34-4-3 and Pk67-1-9) CMV, and only one (Pk105) WMV2. Six lines (Pk31-2-4, Pk37-1-4, Pk61-1-1, Pk04-7-12-3, Pk05-7-11-8 and Pk107) showed positive reaction to Potyvirus group while negative to four antiseras tested.

2.11. Works done in Bangladesh

Kader *et al*., (1997) reported that leaf samples of ribbed gourd were used for serological detection of viruses by dot immunobinding assay. The samples having symptoms of virus diseases such as fern leaf, chlorotic spot, mosaic and leaf curl. Against the antiseras of PRSV and WMV-2 fern leaf, chlorotic spot, mosaic and leaf curl were found to be positive respectively out of the 6 different samples.

Rahman *et al*., (2008) conducted studies on 1500 pumpkin plants, to find out the prevalence of Papaya ring spot viruses- *Watermelon strain (*PRSV-W). Symptoms, mechanical inoculation and DAS-ELISA were employed. About 75.8, 1.33, 1.00 and 0.13% plants had pure infection of *Papaya ring spot viruses Watermelon strain* (PRSV), *Watermelon mosaic virus-2* (WMV-2), respectively.

Begum *et al*., (2015) have done an experiment to elucidate resistant response of pumpkin from 26 breeding lines. The test lines ranged virus incidence and severity from 0.00 to 79.90 % and 0.00 to 83.3 % respectively. Detection of four viruses such as PRSV-W, ZYMV, CMV and WMV-2 were done. These viruses caused fern leaf, mosaic, chlorosis and vein banding and leaf distortion symptoms, respectively. ELISA results showed PRSV-W and ZYMV were the most prevalent virus followed by CMV and WMV2 related to number of infected lines.

Sadia 2017, worked on effect of different sowing time on viral disease incidence and severity of pumpkin collected from four districts of Bangladesh. And In two sowing date, the highest incidence (%) and disease severity were found in T_2 (Narshingdi) and the lowest incidence (%) and disease severity (%) were found in T_1 (Narayanganj). Serological test, only one type antiserum CMV was used to identify pumpkin viruses. By observing color of ELISA kit, it was concluded that mosaic, chlorosis and yellowing symptoms produced by CMV in treatment T_2 (Narayanganj).

CHAPTER 3

MATERIALS AND METHODS

To study the occurrence and distribution of viruses causing diseases in pumpkin and developing effective management strategies, a survey was conducted and different diseased samples were collected from three selected districts named Mymensingh, Gazipur and Pabna of Bangladesh.

The survey was done to collect virus infecting pumpkin samples from selected districts and management was undertaken in Sher-e-Bangla Agricultural University, Dhaka during 2017-2018. Biological properties like symptomology and serological test like DAS-ELISA was performed for identification and characterization of identified viruses. A field experiment was conducted to screen suitable CMV management strategies.

Experiment-1

3.1. Survey study

3.1.1. Area selected

The survey was done in three selected districts of Bangladesh. Selected districts are Mymensingh, Gazipur and Pabna.

3.1.2. Identification of viruses of pumpkin by visual observation in selected area

The recorded symptoms include mosaic, fern leaf, yellow mosaic, chlorosis, leaf distortive of hardy leaves of virus diseases were recorded. Photographs of the symptoms were taken and compared with standard literatures (Zitter *et al*., 1996).

3.1.3. Virus infected sample collection and preservation

Virus and virus like symptoms of pumpkin leaves were collected from selected locations. At the time of sample collection, characteristic symptoms of virus infection under natural conditions were carefully recorded from each infected plant according to the procedure of Bos (1978). The leaves of actively growing plants showing prominent symptoms were cut with a razor blade and put in polythene bags following standard procedures (Bos 1969**,** Noordam 1973, Gibbs and Harrison 1979). Immediately after collection, the samples were cut into small pieces and put on blotter paper placed on silica gel in petri dish. The petridish containing the samples were sealed with adhesive tape and stored at 4ºC as suggested by Bos (1969).

3.1.4. Identification of viruses using DAS-ELISA

The test was carried out in Plant Pathology lab of BARI. All collected samples from experimental field and eleven samples out of fifty collected samples of survey were tested against one major pumpkin infecting viruses viz. CMV in polystyrene microtiter plate through standard Double Antibody Sandwich (DAS) ELISA as described by Clark and Adams (1977) using antibodies (BIOREBA AG kit) and enzyme substrate. ELISA plates were coated with monoclonal Immunoglobulin (IgGts) 100µl/well (CMV) diluted at 1:500 in coating buffer and incubated for 3 hours at room temperature followed by washing through 1X phosphate buffer saline tween (PBST) three times at 3 minute intervals. Leaves were chopped into small pieces and ground in sterile pestle and mortal with extraction buffer and sap was filtered through double layer of muslin cloth. Each well loaded 100 µl antigen (sap of infected leaf tissue) with micropipette, buffer and healthy samples were also loaded for control and plate was incubated for 24 hours at 4°C. Followed by washing, (100 µl of enzyme alkaline phosphatase ALKP) conjugated IgG diluted at 1:500 in conjugate buffer was added each well and incubated at room temperature for 3 hours. After washing, substrate buffer (150 µl) containing p-nitro phenyl phosphate (1 mg/ml) was added to each well. Incubation was done at room temperature for 55 minutes in dark and the reaction was observed visually by yellow color. A sample is considered as virus infected when the absorbance of 405 nm when read in ELISA reader (EPSON LX300) shows at least thrice of that healthy control.

Experiment- 2

3.2. Field Experiment

3.2.1. Experimental site

The experiment was conducted in the research field of Sher-e-Bangla Agricultural University, Dhaka central farm under the department of Plant Pathology, Dhaka-1207 during the period from October'2017 to April '2018. The experimental plot no. was 10. The location of the experimental site was at 23◦46′ N latitude and 90◦24′ longitude with the elevation of 9 meters above the sea level (Appendix I). The experiment was conducted during October'2017 to April'2018

3.2.2. Climatic condition

The experimental site was under the sub-tropical monsoon climatic condition, which is characterized by heavy rainfall during Kharif season (May-September) and scanty or near zero rainfall in the Rabi season (October-March). There was very low or no rainfall during the month of December, January, February, March. The average maximum temperature during the period of investigation was 28.5°C and the average minimum temperature was 17.5°C. Details of the metrological data in respect of temperature, rainfall and relative humidity the period of experiment were collected from Bangladesh Meterological Department, Agargaon, Dhaka (Appendix III).

3.2.3. Soil type

The soil of the experimental site of the SAU central farm is actually a medium high land which is belonging to the Modhupur tract under the agro ecological zone (AEZ) 28. The soil texture of the farm was silty loam, non-calcarious, dark grey soil of Tejgaon soil series 22 with a pH of 6.7.

Soil samples of the experimental site were collected from a depth of a 0 to 30 cm before conducting the experiment and analyzed in the Soil Resources Development Institute (SRDI), Farmgate, in Dhaka (Appendix II).

3.2.4. Seed Collection and sowing

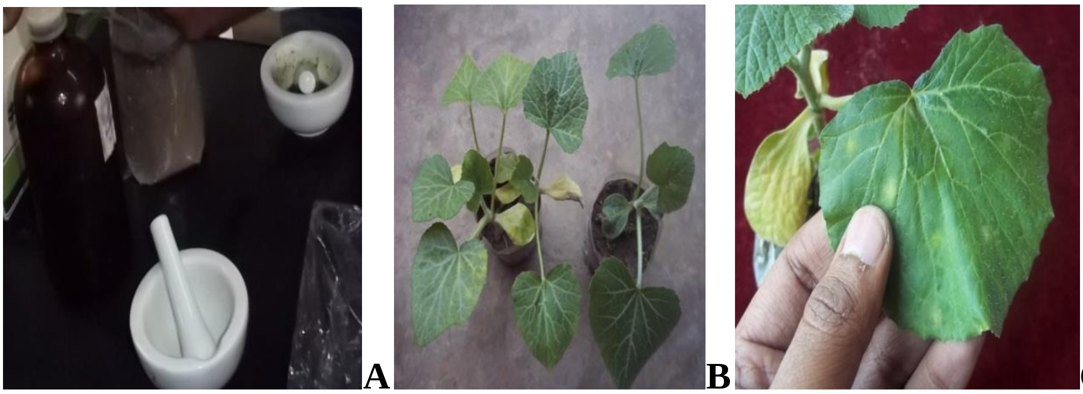
Seeds were (BARI mistikumra 1) was collected from vegetable division, HRC, BARI, Joydebpur, Gazipur. Pumpkin seeds (each poly bag contained two seeds) were sown in poly bag which were of diameter (15 x 10 cm). Each poly bag received 2 kg soil which was mixed with decomposed cow dung. Sowing date was 4th December 2017.

3.2.5. Raising of seedling

After sowing the seeds in the poly bags they were inspected everyday and watered after every second or third day. For three replications per treatment and 2 seeds were sown in each poly bag. Healthy seedlings were produced through inspection and intensive care.

3.2.6. Inoculation of CMV and transplanting of pumpkin seedlings

Leaf samples of pumpkin plants infected with only CMV were collected from the experiment-1. Plants infected with CMV were confirmed by serological test. Inoculum of CMV was prepared by grinding the infected 'pumpkin' leaves using mortar and pastle in 0.02M phosphate buffer, p^{H}7.0. Leaf to buffer ratio was 1:10 (1g infected leaf to 10 ml buffer). The sap obtained after passing through double ply cheese cloth was used as inoculum. For inoculation mechanical inoculation method using carborundum powder (800 meshes, Fisher Scientific, Fair Lawn, NJ) was followed (Daryono, 2006). Before development of true leaf, both cotyledons of pumpkin seedlings were rubbed with the carborundum to make minor injuries. The inoculums sap was soaked with cotton and rubbed on the injured areas of leaf for inoculation (Plate 1). After inoculation carborundum powder was rinsed off with water. All operations were done under sterile conditions. Inoculated pumpkin seedlings were kept in aphid-proof cages for 10 days. Ten days after inoculation, the seedlings were transplanted in the main field.



A B C

Plate 1: Inoculation CMV in pumpkin seedlings, A. CMV sap preparation, B: Inoculated seedlings C: Inoculated leaf showing symptom

3.2.7. Land preparation (for transplanting)

The experimental field was upland plot with drainage facility which was located at a high elevation. The preparation of the land had taken place through land ploughing and cross ploughing with power tiller followed by laddering to confirm good tilth. The land was cleaned such a way that all types of weeds and debris of previous crops were removed

3.2.8. Design and layout

The experiment was laid out in Randomized Complete Block Design (RCBD) with six treatments and tree applications. Seedlings were planted in three replications in prepared soil followed by irrigation. For better drainage raised beds were prepared carefully. The depth of the drain was 1m and drain was dug around the field. The measurement of the beds was as like: 20 cm height, 2.5 m width and 4m long beds.

3.2.9. Manure and fertilizers application

Recommended doses of fertilizers @ of 175kg Urea,175 kg TSP, 150kg MP, 100 kg Gypsum and 10 kg Borax, and 16000 kg cow dung per hectare were applied (Bhuyan, 2010). All of organic manure, phosphorus, potassium, sulphur, zinc and boron were applied in pit 5-7 days before planting and mixed thoroughly with the soil. Nitrogen were applied around the plant as side dressing at 15, 35, 55 and 75 days after planting under moist soil condition and mixed thoroughly with the soil as soon as possible for better utilization.

3.2.10. Pit preparation

The size of the pits was maintained as like: Pits were of 45 cm x 45 cm x 40 cm size. Row to row distance was 2.0 m and pit to pit spacing was of 2.0 m, respectively. Every plot contained three pits. Channels of irrigation and drainage were made and the diameter 0.5m was maintained. Before 9 days of transplanting the pits were prepared.

3.2.11. Seedling transplanting

After 10 days, inoculated seedlings were transplanted in the experimental field. The experiment was based on occurrence and distribution of virus causing diseases on pumpkin and determining the effective management procedures.

3.2.12. Treatments

Five different treatments with one control were used in this experiment which is shown in table 1.

Treatments	**Materials used**
T_1	Inter crop (Coriander)
T_2	Barrier crop (Maize)
T_3	Rice straw mulch
T_4	Yellow trap
T_5	Chemical (Malathion 57EC)
T_6	Control

Coriander seeds were sown as intercrop. The seeds were broadcasted in the plots in X shape. Seeds were broadcasted in the same day of seedling transplanting. As the barrier crop maize seeds were sown around the plots. Maize seeds were sown before 7 days of transplanting of pumpkin seedlings. After the growth of the pumpkin plants rice straw were applied in the plots. For yellow trapping three bowls of plastic were collected and colored with yellow color and dried. Then this bowls were filled with detergent water and then placed in the plots in a high region. As the part of chemical control Malathion 57 EC were applied in the plots. The dose maintained was 2 ml/1li and sprayed on the surface of the plants and drenched the leaves with chemical. The chemical was applied three times in 15 days interval each time. Control was kept to determine the differences of different treatments or managements in relation to control.

Every treatment was applied with three replications and each replication contained three plants.

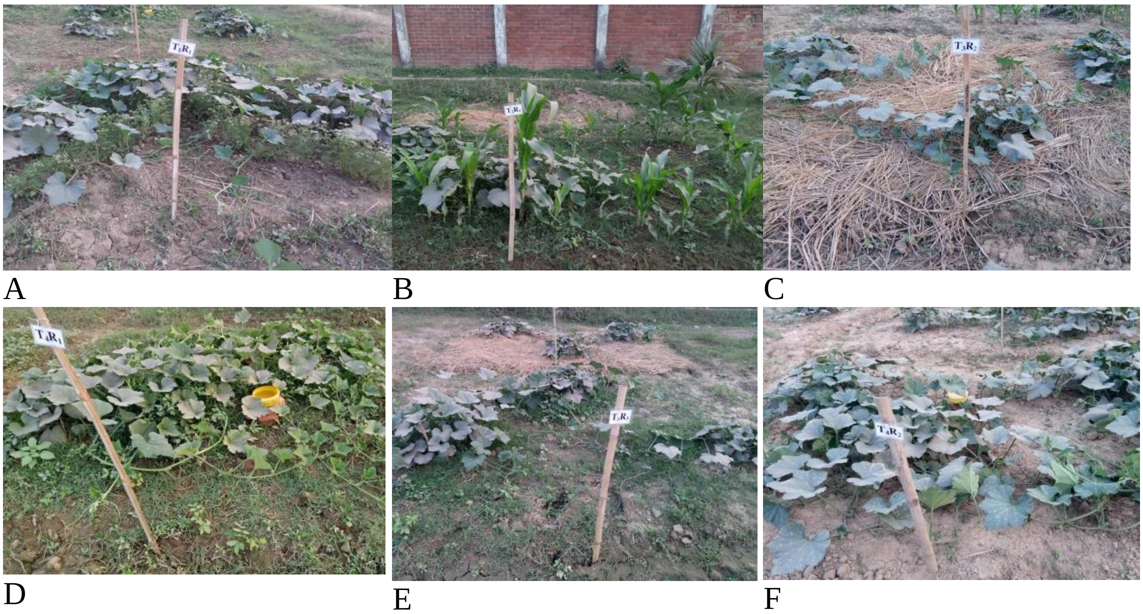


Plate 2: Overview of different treatments (A-Intercrop with coriander; B-Barrier crop with maize; C-Rice straw mulch; D-yellow trap; E- Chemical; F- Control)

3.2.13. Intercultural Operations

Weeding, top dressing of fertilizer, irrigation and other necessary intercultural operations were done throughout the cropping season for proper plant growth and development of flowers and fruits.

3.2.14.1. Thinning and gap filling

The seedlings were thinned out in such a way that the weak seedlings were eliminated. In case of not growing of the seedlings the gaps were filled after transplanting.

3.2.14.2. Irrigation

Irrigation was maintained through observation. According to the necessity the plot was irrigated.

3.2.14.3. Weeding

Weeding is very necessary for the production of crops. As weeds conserve moisture and nutrients from the soil and hampers the flowers and fruits production weeding is a must. In this experiment in total five weeding were done to keep the plot weed free.

3.2.14.4. Drainage

Stagnant water is not bearable in the field. That's why at the time of heavy rains, stagnant water was effectively drained out from the field.

3.2.15. Identification of viruses

Pumpkin plants were grown in the experimental field. After the 50 days of transplanting the symptoms of the plants were recorded gradually.

The symptoms which were recorded included mosaic, yellow mosaic, shoe string, deformation of leaf, chlorotic spot and hardy leaves of plants. Virus or viruses like symptoms of individual plants were recorded. Photographs of the symptoms were taken and compared with standard literatures (Zitter, *et al.*, 1996).

3.2.16. Harvesting

Fully ripen fruits was harvested and data on fruit yield, yield contributing characters, flesh thickness, placenta thickness were recorded.

3.2.17. Measured traits/Data Collection

Data collection on the basis of growth and yield contributing characters of infected plant or plant parts.

- No. of infected leaf/plant
- No. of healthy leaf/plant
- No. of female flower /plant
- Vine length (cm)
- Number of aphid association
- No. of fruits /plant (Infected and healthy)
- 1stNode no of female flower
- Fruit weight (Kg)
- Yield (Kg/treatment)
- Placenta thickness (cm)
- Flesh thickness (cm)

3.2.18. Collection of data

From the plants of the individual plot different measures are taken for data collection on different morphological parameters. Data were collected over the parameters in the following ways-

3.2.19. Number of infected leaves per plant

At 55, 65, 80, 95 and 110 days after sowing (DAS) number of infected leaves of selected infected plants from each treatments of three replications was recorded. Average number of infected leaves was calculated and the average number of healthy leaves was also recorded.

3.2.20. Number of flowers per plant

From each plot as per treatment combination, mean number of flower of selected plants was recorded.

3.2.21. Number of fruits per plant

Mean number of fruits of selected plants for each plot as per treatment combination was recorded.

3.2.22. Number of aphid association

Every plot contained three plants. From every plant mean average aphid population was recorded by selecting 5 leaves randomly and the insects of those 5 leaves were counted from the opposite side of the leaves. At last the means were calculated.

3.2.23. Number of aphids in yellow trap

Yellow traps were set after every two days and the number of dead aphids were collected through a sieve and then dried. Then the dried aphids were counted.

3.2.24. Disease incidence (%) calculation

At before and after flowering, incidences of viral diseases were recorded. For collection of data, every plant was observed from each plot and observation of the disease symptoms were done carefully. At an interval of 20 days, data on disease incidence were recorded commencing from first incidence and continued up to four times. Disease incidence, which measures the extent of propagation of a disease within a given field (Agrios 2005), was also estimated using the formula:

$$\text{Disease Incidence (\%)} = \frac{\text{Number of diseased plant (or parts)}}{\text{Number of total plants (or plants) observed}} \times 100$$

3.2.25. Disease severity (%) calculation

At before and after flowering severity of disease was recorded. For collection of data, every plant was observed from each plot and the observation of disease symptoms were done carefully. At an interval of 20 days, data on disease severity were recorded commencing from first severity and continued up to four times.

The severity of different virus diseases of pumpkin was indexed on a 0-5 indexing scale. Disease severity was expressed in percent disease index (PDI). The PDI was computed using a standard formula:

$$PDI = \frac{\sum \text{Disease grade} \times \text{number of plants in grade}}{\text{Total number of plants} \times \text{highest disease grade}} \times 100$$

According to Xu *et al*., (2004), the severity of virus disease of pumpkin was indexed on a 0-5 indexing scale, where 0 = no visible symptoms, 1 = slightly mosaic on leaves, 2 = mosaic patches and/or necrotic spots on leaves, 3= leaves near apical meristem deformed slightly, yellow, and reduced in size; 4= apical meristem with mosaic and deformation, and 5= extensive mosaic and serious deformation of leaves.

3.2.26. Yield (kg) and yield contributing parameters

3.2.26.1. Yield (kg)

Yield of the fruits were calculated by multiplying the mean fruit number and fruit weight as per treatment combination.

3.2.26.2. Fruit number

From each plot as per treatment combination mean number of fruits of every plant was recorded.

3.2.26.3. Fruit weight (kg)

From each plot as per treatment combination, mean fruit weight of every plant was recorded.

3.2.26.4. Flesh thickness (cm)

From each plot as per treatment combination, mean flesh thickness (cm) of every plant was recorded.

3.2.26.5. Placental thickness (cm)

From each plot as per treatment combination, mean placental thickness (cm) of every plant was recorded.

3.2.26.6. 1st Node no of female flower

The no of node where female flower were grown, were counted

3.2.26.7. Vine length

Vine length of the plants was measured.

3.2.27. Identification of viruses

Pumpkin plants grown in the experimental field was checked at 55 days after transplanting and gradual symptoms were recorded. Different viruses (CMV, PRSV-W, WMV2 and ZYMV) were identified studying visible symptoms followed by serological test by using CMV antiserum. ELISA test (Enzyme-Linked Immunosorbent-Assay) and host range test were performed for serological diagnosis of virus and transmission of virus infected plant.

3.2.27.1. Symptomology

Mosaic

Mosaic symptoms were observed in growing leaves. Vein clearing found from the edge of the leaf.

Yellow mosaic

The symptoms were yellow green spots with mottling. There were alternative yellow green patches on leaves, which enlarged rapidly and covered the entire leaf. With the aging of the plant, the infected leaves developed chlorosis, yellow patches and distortion.

Shoe string

The symptom appeared as the deformation of the leaf blades leading to the formation of fern leaf or shoe string like structure. In later stage of development totally deformed leaves with reduced size was observed. The older leaves were small and deformed fern leaf like appearance. The symptoms so far noted on pumpkin and named as fern leaf.

Leaf distortion

Pumpkin leaf showed mosaic symptom at early stage of infection. But at later stage of infection leaves showed yellow mosaic with vein banding and leaf distortion. Especially fern leaf and shoestring type leaf distortion was appeared at later stage of infection when pumpkin plant was infected by ZYMV.

Chlorotic spot

Different sized chlorotic spot appeared scatterly on the leaves in the initial symptom. In the next step chlorosis developed from the leaf margin followed by download curling. The main vein also showed chlorosis.

Hardy leaves

The hardy leaves symptom showed hard leaves in the young, growing leaves. The leaves were comparatively thick, hard and rough. These leaves did not appear large.

Fern leaves

The symptom appeared as the deformation of the leaf blades leading to the formation of fern leaf like structure. Infected leaves were severely enated. In acute stage of disease development totally deformed, reduced leaves were observed. The older leaves were small and deformed fern leaf like appearance.

Chlorosis

The symptoms appeared on younger leaves. Yellow green spots with mottling, alternative yellow green patches are the symptoms which enlarged rapidly and covered the entire leaf.

Vein banding

The first symptoms were yellow green spots with mottling which were appeared on the younger leaves. Then the yellow green patches on leaves enlarged rapidly and covered the entire leaf. The older leaves of aged plants the infected leaves developed vein banding, yellow patches and distortion.

Ring spot

The lives showed alternative yellow, green ring like spots which were spread to the entire leaves.

3.2.27.2. Identification of viruses using DAS-ELISA

The method was described before.

3.2.28. Statistical analysis

The collected data were subjected to analyses of variance (ANOVA) and the means were separated with the least significant difference (LSD) method at 5% level of significance. The statistical package MSTATC and STATISTICS 10 were used for this purpose.

CHAPTER 4

RESULTS

Experiment-1 (Survey study)

A survey was done for collecting different virus infecting pumpkin leaves sample from three selected areas of Bangladesh viz. Mymensingh, Gazipur and Pabna districts.

4.1. Identification of viral diseases of pumpkin in different location during survey by visual observation1

The survey was done from 10th November'2017 to 17th November'2017. From the selected areas about 50 samples were collected. Among these 50 samples, 10 characteristics symptoms were categorized by comparing with standard literature viz. mosaic, yellow mosaic, ring spot, shoe string, chlorosis, chlorotic spot, hardy leaves, fern leaves, leaf distortion and vein banding. Each symptom was thoroughly observed visually and compared with international literature for detecting viruses which infect pumpkin plants. The Symptoms of viral diseases of pumpkin during survey are presented in plate- 3.

Symptoms category

Mosaic

In initial stage, mosaic symptoms were observed in growing leaves. Vein clearing found in the initial stage from the edge of the leaf. Serological test confirmed the identification of the virus.

Yellow mosaic

The first symptoms were yellow green spots with mottling. There were alternative yellow green patches on leaves, which enlarged rapidly and covered the entire leaf. With the aging of the plant, the infected leaves developed chlorosis, yellow patches and distortion. The plants were stunted and became yellow.

Shoe string

The symptom appeared as the deformation of the leaf blades leading to the formation of fern leaf or shoe string like structure. In later stage of development totally deformed leaves with reduced size was observed. The older leaves were small and deformed fern leaf like appearance. The symptoms so far noted on pumpkin and named as fern leaf were identical with the symptoms produced by *Papaya ring spot virus* both watermelon strain or papaya strain (PRSV-W/P) in papaya according to literature.

Leaf distortion

Pumpkin leaf showed mosaic symptom at early stage of infection. But at later stage of infection leaves showed yellow mosaic with vein banding and leaf distortion. Especially fern leaf and shoestring type leaf distortion was appeared at later stage of infection when pumpkin plant was infected by ZYMV. However, identification of this virus by other methods is required to confirm the result.

Chlorotic Spot

Different sized chlorotic spot appeared scatterdly on the leaves in the initial symptom. In the next step chlorosis developed from the leaf margin followed by download curling. The main vein also showed chlorosis. This plant gave comparatively small and deformed fruits. The fruits yielded by the infected plants were deformed and usually small in size compared to healthy.

Hardy leaves

The hardy leaves symptom showed hard leaves in the young, growing leaves. The leaves were comparatively thick, hard and rough. These leaves did not appear large.

Fern leaves

The symptom appeared as the deformation of the leaf blades leading to the formation of fern leaf like structure. Infected leaves were severely enated. In acute stage of disease development totally deformed, reduced leaves were observed. The older leaves were small and deformed fern leaf like appearance.

Chlorosis

The symptoms appeared on younger leaves. Yellow green spots with mottling, alternative yellow green patches are the symptoms which enlarged rapidly and covered the entire leaf.

Vein banding

The first symptoms were yellow green spots with mottling which were appeared on the younger leaves. Then the yellow green pathches on leaves enlarged rapidly and covered the entire leaf. The older leaves of aged plants the infected leaves developed vein banding, yellow patches and distortion.

Ring spot

The lives showed alternative yellow, green ring like spots which were spread to the entire leaves.

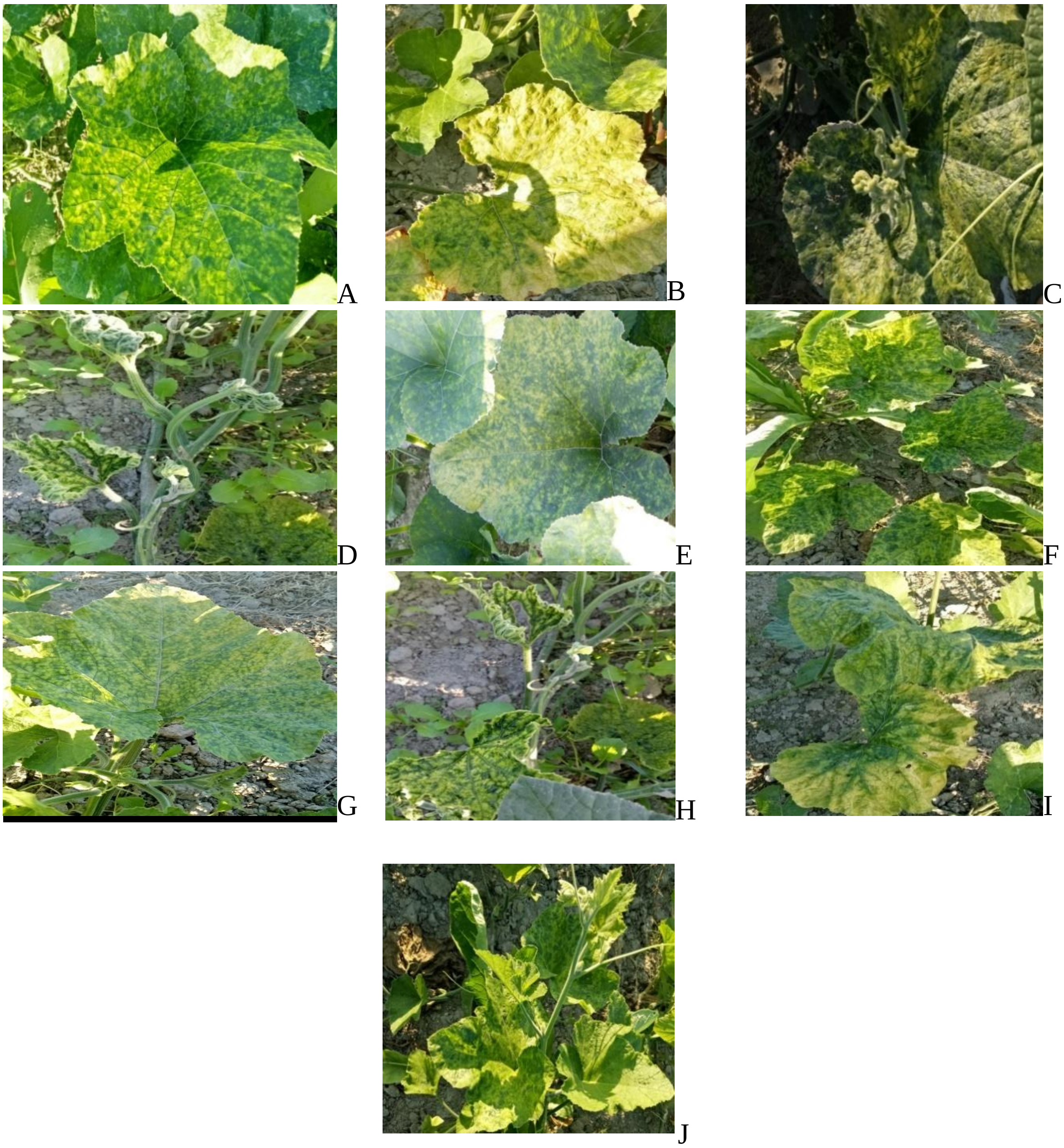


Plate 3: Symptoms of virus and virus like diseases of pumpkin (A-Mosaic; B- yellow mosaic; C-Ring spot; D-Shoe string; E- Chlorosis; F-Chlorotic spot; G-Hardy leaves; H-Fern leaves; I-Leaf distortion and J-Vein banding) in different locations

4.1.2 Identification of virus by Serological Test (DAS-ELISA)

In Serological test, only one antiserum *Cucumber mosaic virus* (CMV) was used to identify pumpkin infecting virus. By observing color of ELISA test, it was concluded that mosaic, yellow mosaic, chlorosis and hardy leaves symptoms showed positive to CMV. Based on results of DAS-ELISA in the present study four different types of symptoms were found to be associated with in the leaves infected with CMV and the symptoms categories were mosaic, yellow, chlorosis and hardy leaves which were collected from Mymensingh, Pabna and Gazipur respectively. The results of serological test are presented in table 2.

Table 2: Response of different symptoms of pumpkin against CMV by DAS-ELISA

Locations	Characteristics	Results of ELISA
Mymensingh	Mosaic	+
Mymensingh	Yellow mosaic	+
Pabna	Ring spot	-
Pabna	Shoe string	-
Pabna	Chlorosis	+
Gazipur	Chlorotic spot	-
Gazipur	Hardy leaves	+
Mymensingh	Fern leaves	-
Gazipur	Leaf distortion	-
Gazipur	Vein banding	-

'+' indicates presence of CMV, '-'indicates absence of CMV

Experiment-2 (Field experiment)

4.2.1. Effect of different treatments on disease incidence and severity of virus diseases in pumpkin

Significant variation was found in case of viral disease incidence and disease severity. In different treatments the results of disease incidence (%) and present disease index (PDI) are presented in table 3 and figure1.

4.2.1.1. Disease incidence (%)

The highest disease incidence (70.84%) was found in treatment T_6 (control) followed by treatment T_3 (Rice Straw mulch). On the other hand, the lowest incidence (21.10 %) was found in treatment T_1 (Inter crop) which were statistically similar to treatment T_5 (chemical, 21.35%) and T_4 (Yellow trap, 21.43%), respectively.

4.2.1.2. Present disease index (PDI)

The highest disease severity (26.67%) was found in treatment T_6 (control) followed by 24.44% in treatment T_3 (Rice Straw mulch). On the other hand, the lowest severity (11.11 %) was found in treatment T_1 (inter crop) proceeded by treatment T_2 (Barrier crop), T_4 (Yellow trap) and T_5 (Chemical).

Table 3: Effect of different treatments on viral disease incidence (%) and severity (%) of pumpkin at field condition

Treatments	(%) Disease Incidence	(%) Disease Severity
T_1	21.10 c	11.11 b
T_2	26.71 bc	13.33 b
T_3	31.32 b	24.44 a
T_4	21.43 c	13.33 b
T_5	21.35 c	13.33 b
T_6	70.84 a	26.67 a
LSD (5%)	7.883	6.883
CV (%)	13.49	22.21

T_1= Inter crop (Coriander) T_2= Barrier crop (Maize), T_3= Rice straw mulch, T_4= yellow trap, T_5= Chemical (Malathion 57 EC) ,T_6= Control

4.2.2. Effect of different treatments on growth parameters of pumpkin

Different growth contributing parameters such as vine length (cm), no of female flower, node of female flower were recorded during experiment. Significant differences were found among the treatments at growth attributes. Different growth contributing parameters among different treatments are presented in table 4.

4.2.2.1. Vine length

Maximum vine length (178.67 cm) was found in treatment T_3 (straw mulch. On the other hand, minimum vine length (147.67 cm) was found in T_2 (Barrier crop) treatment proceeded by treatment T_1 (Inter crop).

4.2.2.2. No of Female flower

There were significant differences found between the treatments. The highest no of female flowers (7.33) was found in T_5 which was statistically different with other treatments. On the other hand, the lowest no of female flowers (1.33) was found in treatment T_2 treatment.

4.2.2.3. 1st Node of Female flower

There were no significant differences in case of female flowers node no among the treatments. The highest node no (5.00) was found in treatment T_4 (yellow trap) whereas the lowest no of flowers node no (3.67) was found in treatment T_1 (inter crop) and T_6 (Control).

Table 4: Effect of different treatments on growth parameters of pumpkin

Treatment	Vine length(cm)	No of female flower /plant	Nodes of 1st female flower
T_1	156.67 c	5.00 b	3.67 a
T_2	147.67 d	1.33 e	4.00 a
T_3	178.67 a	3.00 b	4.33 a
T_4	168.00 b	4.67 bc	5.00 a
T_5	168.67 b	7.33 a	4.00 a
T_6	167.00 b	3.33 cd	3.67 a
LSD (5%)	0.9205	0.7992	2.122
CV (%)	17.50	17.69	16.18

T_1= Inter crop (Coriander), T_2= Barrier crop (Maize), T_3= Rice straw mulch, T_4= yellow trap, T_5= Chemical (Malathion 57 EC), T_6= Control

4.3. Effect of different treatments on yield parameters for controlling viral diseases of pumpkin

Different yield and yield contributing parameters such as number of fruit, fruit weight, yield (kg/treatment) were recorded. Significant differences were found among the treatments at yield parameters. Different yield and yield contributing parameters among different treatments are presented in Table-5.

4.3.1. Number of fruit

Maximum (2.33) number of fruits was found in treatment T_1 (Inter crop) and T_4 whereas minimum (1.00) number of fruits in T_6 (Control), T_3 (Straw mulch) and T_2 (Barrier crop) respectively.

4.3.2. Fruit weight (kg)

The highest fruit weight 2.463(kg) was found in treatment T_1 (Inter crop) whereas the lowest fruit weight 2.123 (kg) in T_6 followed by T_3, T_2, T_5, T_6.

4.3.3. Yield (kg)

The highest (5.727) kg yield was found in T_1 whereas the lowest (2.123) yield (kg) in T_6 (Control).

Table 5: Effect of different treatments on yield attributes of pumpkin

Treatment	No. of fruits/plant	Fruit weight (Kg)	Yield (Kg/treat.)	Yield (Kg/ha.)
T_1	2.333 a	2.463 a	5.727 a	5727.00 a
T_2	1.000 b	2.267 a	2.267 b	2267.00 b
T_3	1.000 a	2.140 b	2.140 b	2123.00 b
T_4	2.333 a	2.290 a	5.273 a	5273.00 a
T_5	1.333 b	2.243 a	3.027 b	3027. 00 b
T_6	1.000 b	2.123 a	2.123 b	2140.00 b
LSD (5%)	0.7435	0.6406	1.645	1645.00
CV (%)	27.22	12.91	26.40	26.39

T_1= Inter crop (Coriander), T_2= Barrier crop (Maize), T_3= Rice straw mulch, T_4= yellow trap, T_5= Chemical (Malathion 57 EC), T_6= Control

4.2.4. Effect of different treatments on quality of fruits among different treatments

Different yield and yield contributing parameters such as number of fruit, fruit weight, yield (kg/treatment) were recorded. Significant differences were found among the treatments at yield attributes. Different yield contributing parameters among different treatments are presented in table 6.

4.2.4.1. Flesh Thickness

There were significant differences found between the treatments. The highest (3.43) flesh thickness (cm) was found in T_3 (Rice straw mulch), whereas the lowest (2.93) fruit thickness (cm) was found in T_6 (Control).

4.2.4.2. Placenta thickness

No significant difference of placental thickness was found among the treatments. The highest (9.43) placental thickness (cm) was found in T_6 (Control) and the lowest (9.00) placental thickness (cm) was found in T_1 (Inter crop), T_2 (Barrier crop) and T_3 (Rice straw mulch).

Table 6: Effect of different treatments on quality attributes of pumpkin

Treatment	Flesh thickness	Placenta thickness
T_1	3.20 ab	9.00 a
T_2	3.00 ab	9.00 a
T_3	3.43 a	9.00 a
T_4	3.00 ab	9.33 a
T_5	3.17 ab	9.17 a
T_6	2.9333 b	9.43 a
LSD (5%)	0.4700	0.8036
CV (%)	5.32	3.10

T_1= Inter crop (Coriander), T_2= Barrier crop (Maize), T_3= Rice straw mulch, T_4= yellow trap, T_5= Chemical (Malathion 57 EC), T_6= Control

4.2.5. Detection of virus in field

4.2.5.1. Detection of pumpkin viruses by visual observation

Various types of symptoms developed on pumpkin variety due to infection with different viruses are shown in table 5. Virus symptoms showed only on young leaves of the plants. The observed symptoms were classified into 6 symptom categories. They were mosaic, yellow mosaic, shoe string, deformation of leaf, chlorotic spot and Hardy leaf. The symptoms recorded from the experiment were compared with symptoms presented in standard literature and based on visible symptoms the viruses were identified as CMV. Photographs of virus infected leaves showing typical symptoms were taken and are presented in Plate 4. Symptomology is not a reliable method for confirmation of viruses but it is an initial step to disease diagnosis because symptom development is due to many factors such as insect sucking, environmental conditions, nutrition deficiency, growth stage, time of infection, host genotype, virus strain, etc.

Table 7: Categories of symptoms identified from infected pumpkin in field condition

Symptoms category	Description of the symptoms
1	Mosaic
2	Yellow mosaic
3	Shoe string
4	Deformation of leaf
5	Cholorotic spot
6	Hardy leaf

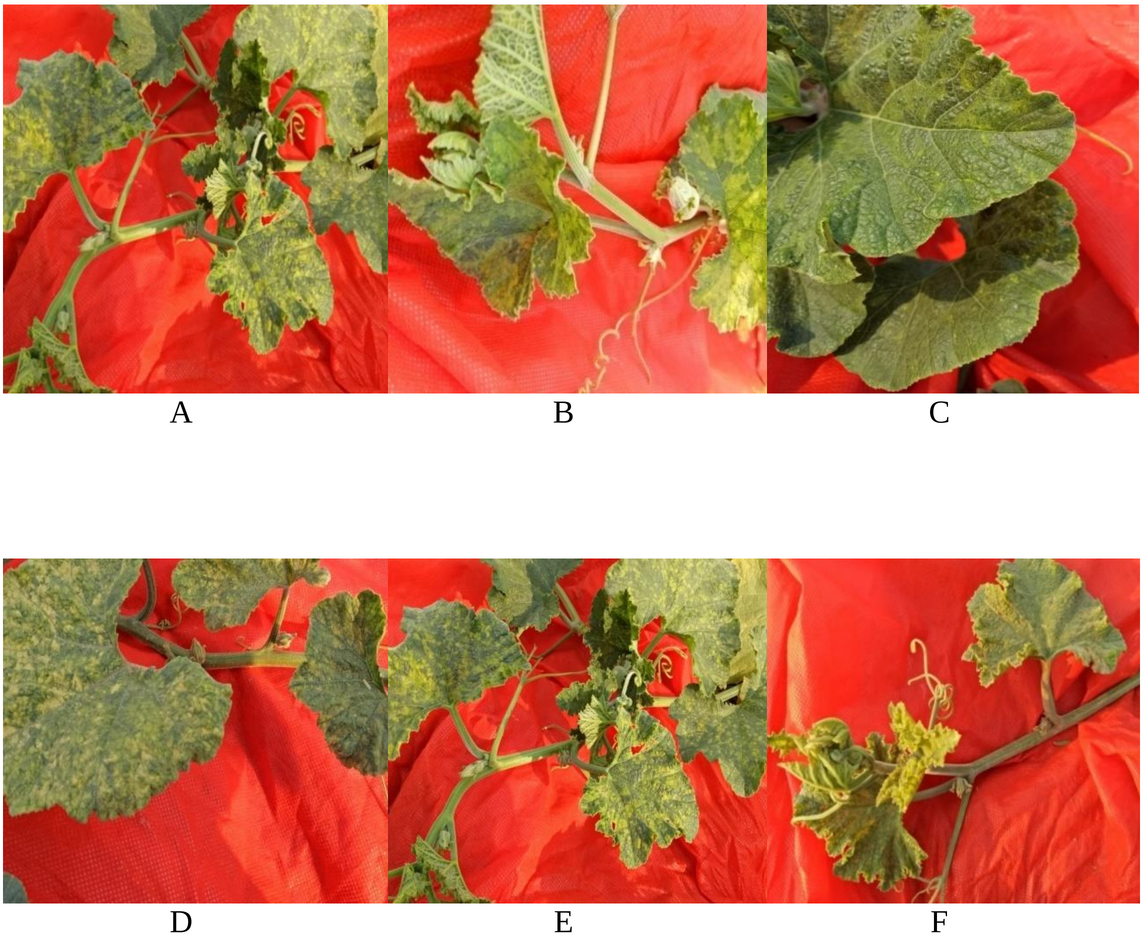


Plate 4. Different symptoms of viruses in experimental field (A- Mosaic; B- Yellow mosaic; C- Ring spot; D- Leaf distortion; E-Chlorotic spot and F- Hardy leaf)

4.2.5.2. Identification of pumpkin virus through Serological test

Serological test of healthy and diseased leaves of pumpkin leaves with six different categories of symptoms were performed using only CMV antiserum are shown in table 6. Among all treatments three categories of symptoms (mosaic, yellow mosaic and chlorosis) showed positive to serological test with CMV antiserum. Yellow color indicates that there was positive reaction with virus antigen using monoclonal antibodies of CMV. Others symptoms and treatments which showed symptoms in field condition but negative reaction against CMV antiserum, were detected as other potyvirus. Based on results of DAS-ELISA in the present study indicate that pumpkin plants were infected with CMV and other viruses.

Table 8: Response of different symptoms categories against CMV in DAS-ELISA

Sl. No.	Symptoms categories	Results
1	Mosaic	+
2	Yellow mosaic	+
3	Fern leaf	_
4	Chlorotic spot	+
5	Leaf distortion	_
6	Hardy leaf	+

4.2.6. Relationship between the disease severity (%) yields in kg per treatment

Relationship between the disease severity (%) and yield in kg per treatment is shown in figure 1. A negative relation exists between the disease severity (%) and yield in kg per treatment. It means that with the increase of disease severity (%), yield in kg per treatment is decreased significantly.

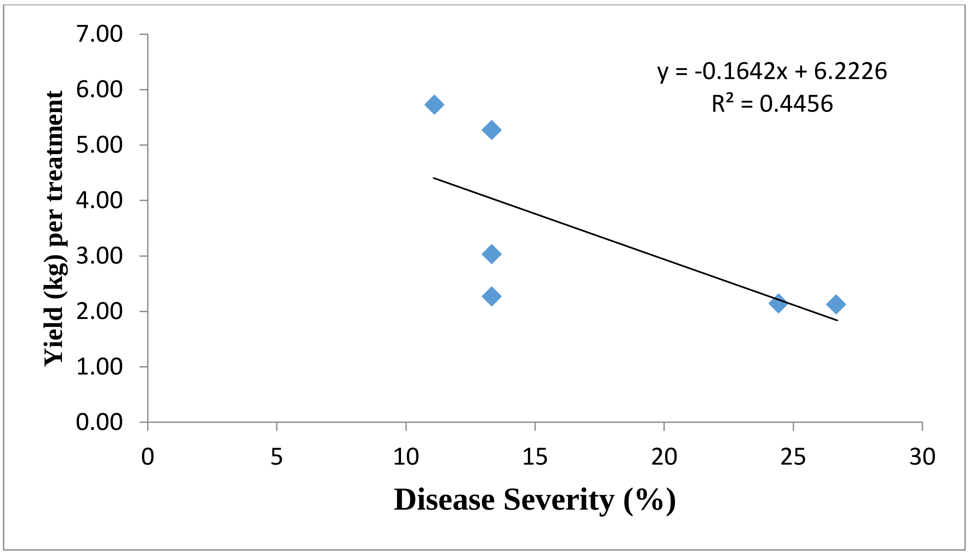


Figure 1: Relation between the disease severity (%) and yield (kg) /Treatment in CMV inoculated field

4.2.7. Relationship between the disease severity (%) and aphid population

Relationship between the disease severity (%) and aphid population in February of the field is shown in figure 2. A positive relation exists between the disease severity (%) and aphid population. It means that with the increase of aphid population, disease severity (%) can be increased.

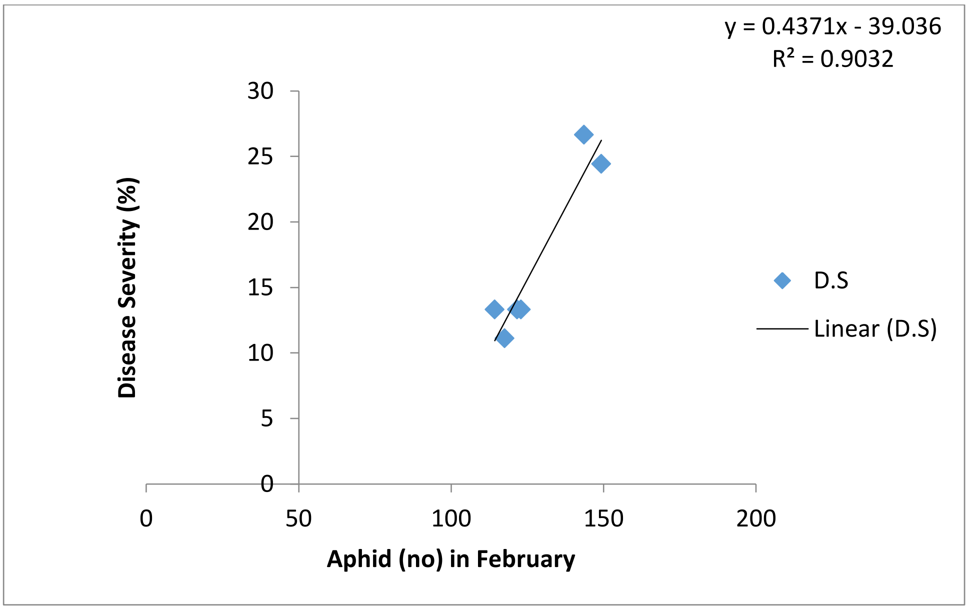


Figure: 2. Relationship between the disease severity (%) and aphid population

CHAPTER 5
DISCUSSION

Pumpkin (*Cucurbita moschata*) belongs to the family Cucurbitaceae. It is an important and popular vegetable crop grown in the tropics and subtropics (Lovisolo 1981). Of the total vegetable requirement about 14% vegetables come from pumpkin. In respect of vitamin A requirement, the people of Asia, particularly the vulnerable groups may certainly become able to improve nutritional status of them by the contribution of pumpkin. But production is declining due to attack by several diseases, such as fungal, bacterial and viral diseases. More than 50 different viruses have been found to infect cucurbits including pumpkin (Lovisolo, 1981). The most common viruses infecting cucurbits are from the CMV, ZYMV, WMV, PRSV, CGMMV and ZGMMV. These viruses occur in complex or which may cause sole infection (Provvidentii, 1996). Viral diseases have been reported to cause major losses of cucurbit crops worldwide and they represent one of the most important limiting factors for growers **(**Provvidentii, 1996). Among various pumpkin diseases, virus diseases (CMV, PRSV, ZYMV, WMV2 and other potyviruses) are significant ones **(**Lisa and Lecoq, 1984).

Therefore, to determine the presence and distribution of viruses infecting cucurbits in the different region of Bangladesh need to be identified. Then also picked up some samples of diseased crops representatively and brought them to the laboratory for detection of viruses using DAS ELISA. In serological test, only one antiserum (CMV) was used to identify pumpkin infecting virus. By observing color of ELISA kit, it was concluded that mosaic, yellow mosaic, chlorosis and hardy leaves symptoms showed positive to CMV. Based on results of DAS-ELISA in the present study four different types of symptoms were identified as CMV which symptoms categories were mosaic, yellow, chlorosis and hardy leaves. In the field experiment, then observation of the inoculated seedlings were took place to determine the effective management strategy as different management strategies were evaluated.

5.1. Survey and detection of viral diseases of pumpkin

A survey was done for collecting different virus infected pumpkin samples. The survey had taken place in three different areas of Bangladesh. From survey area 50 samples were collected, among these 10 characteristics symptoms were categorized by comparing with standard literature viz. mosaic, yellow mosaic, ring spot, shoe string, chlorosis, chlorotic spot, hardy leaves, fern leaves, leaf distortion and vein banding. In this perspective the literature which were compared with the survey sample were (Lovisolo, 1980; Purcifull, 1984; Begum *et al.,* 2016; Brunt, 1996; Purcifull *et al*., 1984).

5.2. Serological detection

Based on results of DAS-ELISA in the present study four different types of symptoms were identified as CMV which symptoms categories were mosaic, yellow, chlorosis and hardy leaves. Similar work also done by different scientists like Dukić *et al.,*(2006),Bananez and Vahdat 2008, Jossey and Babadoost (2008), Ali *et al.,*. 2012 also conducted such survey, collected samples of different symptoms and detected viruses.

Field Experiment

5.3. Disease incidence and severity

Significant variation was found in case of viral disease incidence and disease severity. The highest disease incidence (70.84%) was found in treatment T_6 (control) and the lowest incidence (21.10%) was found in treatment T_1 (Inter crop) which were statistically similar to treatment T_5 (21.35%) which are treated with chemical.

On the other hand, in case of disease severity, the highest disease severity (26.67%) was found in treatment T_6 (control) while the lowest severity (11.11 %) was found in treatment T_1 (Inter crop). Researcher like Coutts and Jones (2009); Köklü and Yilmaz (2006); Pitan and Filani, 2014; Kone *et al.,* (2017) also worked on different pumpkin viruses incidence at field condition and found similar results. Summers *et al.*, 2005 and Papayiannis *et al*., (2005); Damicone and Edelson, 2006 found that with the different treatments the disease incidence reduced.

5.4. Effect of Growth parameters due to virus infection

Different growth contributing parameters such as vine length (cm), female flower no, female flower node no were recorded during field condition. Significant differences were found among the treatments at growth attributes. Maximum (178.67 cm) vine length was found in treatment T_3 (straw mulch) and minimum (147.67 cm) vine length was found in T_2 (Barrier crop) treatment. The highest no of female flowers (7.33) was found in T_5 which was statistically different with other treatments. On the other hand, the lowest no of female flowers (1.33) was found in treatment T_2 treatment plot. There were no significant differences in female flowers node no among the treatments. The highest node no (5.00) was found in treatment T_4 (yellow trap) whereas the lowest no of flowers node no (3.67) was found in treatment T_1 (inter crop) and T_6 (Control).Similar works was also done by Begum *et al*. (2016 and 2015). They revealed that virus infection decreased different growth parameters which were similar to this finding.

5.5. Effect of Yield parameters due to virus infection

Different yield contributing parameters such as number of fruit, flesh thickness, placenta thickness, average weight (kg) of the fruit per treatment, yield (kg/treatment), yield (kg) per ha in every treatment were recorded. Significant differences were found among the treatments at yield parameters.

Maximum (2.33) number of fruits was found in treatment T_1 (Inter crop) and treatment (T_4) which was 2.33. On the other hand, minimum (1.00) number of fruits was found in T_6 (control) treatment.

There were no significant differences in average fruit weight found among the treatments. The highest fruit weight (2.463 kg) was found in treatment T_1 (Inter crop) whereas the lowest fruit weight 2.123 kg in Control (T_6).

In case of yield per treatment, there were significant differences found between the treatments. The highest yield (5.727 kg) was found in T_1 followed by T_4which was statistically different with other treatments. On the other hand, the lowest yield (2.123 kg) was found in treatment T_6 (Control) plot.

There found significant differences in yield (kg) per ha among the treatments. The highest yield (5727 kg) was found in T_1 (Inter crop). On the other hand, the lowest yield (2123 kg) was found in treatment T_6 (Control).

There were significant differences found between the treatments in case of flesh thickness. The highest (3.43) flesh thickness (cm) was found in T_3 (Rice straw mulch), whereas the lowest (2.93) fruit thickness (cm) was found in T_6 (Control).

No significant differences of placental thickness were found among the treatments. The highest (9.43) placental thickness (cm) was found in T_6 (Control) and the lowest (9.00) placental thickness (cm) was found in T_1 (Inter crop), T_2 (Barrier crop) and T_3 (Rice straw mulch).

Similar works was also done by Begum *et al*. (2016 and 2015). They revealed that virus infection decreased different growth parameters which were similar to this finding. Significant results found by different scientist who worked on effect of yield parameter due to virus infection. Damicone and Edelson (2007) on pumpkin**,** Pitan and Filani, 2014 on cucumber, Summer *et al*. 2004 on Zucchini squash worked on treatments and effect of them on production and found significant results in result.

5.6. Relationship between the CMV disease severity (%) with Yield (Kg/Treatment)

Significant relation was found in disease severity with yield (kg) per treatment. Relationship between the disease severity (%) and yield in kg per treatment was a negative relation. It means that with the increase of disease severity (%), yield in kg per treatment is decreased significantly.

Disease incidence and disease severity respectively affect negatively the pumpkin production. Similar research was done by Kader *et al*., (1997,Rahman *et al*., (2008);Begum *et al*.,(2015).

5.7. Identification of CMV disease in pumpkin in field by visual observation/ Symptomology

Virus symptoms showed only on young leaves of the plants. The observed symptoms were classified into 6 symptom categories. They were mosaic, yellow mosaic, Shoe string, deformation of leaf, cholorotic spot and Hardy leaf. The symptoms recorded from the inoculated pumpkin leaves were compared with symptoms presented in standard literature and based on visible symptoms the viruses were predicted as CMV or other poty viruses. Similar Symptomological study also done byBegum *et al.* (2016), Sadia (2017),Zitter *et al.,* (1996), Jossey and Babadost, 2008**;** Lecoq (2001**);**

Akanda (1991),Percifull *et al*., (1984)and Lovisolo (1980) which were similar to these symptoms.

5.8. Identification of CMV by serological test

In Serological test, only one type antiserum *CMV* was used to identify pumpkin viruses. By observing color of ELISA kit, it was concluded that mosaic, yellow mosaic, leaf hardening, curling and chlorosis symptoms produced by *CMV* in treatment $T_{2,}$ $T_{4,}$ T_5 and T_6. Others symptoms marked as poty virus group, which were not identified by ELISA. Based on results of DAS-ELISA in the present study indicate that $T_{2,}$ $T_{4,}$ T_5 and T_6 treatment of pumpkin were infected with CMV which symptoms categories were mosaic, yellow mosaic, leaf hardening, curling and chlorosis. Mosaic, yellowing and chlorosis symptoms also showed similar result which was found by Begum *et al*. (2016), Yilmaz and Sherwood (2000).

Dukić *et al.,* (2006); Köklü and Yilmaz (2006); Providenti, 1996**;** Walters *et al.,* 2003 conducted serological test to detect the viruses.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Pumpkin (*Cucurbita moschata)* belongs to the family Cucurbitaceae. It is an important tropical and subtropical vegetable for its high vitamin A content and nutritional value. Vegetables play a vital role in meeting the nutritional need and contribute to the economy of Bangladesh. Viral diseases are the main constraint to the production of cucurbit family crops. For viral diseases there is no curative measure. So the preventive measures need to be taken to control viral diseases. Therefore, the aim of the study was to determining the effective management strategies to control the viral infection of pumpkin. For that, accurate diagnosis of the viruses present in a region is required for developing appropriate integrated management of these diseases. But so far, basic information and research on the existence and distribution of viruses causing diseases of cucurbits in the different region of Bangladesh is not yet available. This research aimed to find out the presence and distribution of viruses infecting cucurbits in the different region of Bangladesh.

Therefore, the research conducted a survey in three different districts of Bangladesh. From these areas about 50 samples were collected. Among these 50 samples, 10 characteristics symptoms were categorized by comparing with standard literature viz. mosaic, yellow mosaic, ring spot, shoe string, chlorosis, chlorotic spot, hardy leaves, fern leaves, leaf distortion and vein banding. In serological test, only one antiserum (CMV) was used to identify pumpkin infecting virus. By observing color of ELISA kit, it was concluded that mosaic, yellow mosaic, chlorosis and hardy leaves symptoms showed positive to CMV. Based on results of DAS-ELISA in the present study four different types of symptoms were identified as CMV which symptoms categories were mosaic, yellow, chlorosis and hardy leaves.

A field experiment was also conducted to determine the effective management strategy of the inoculated seedlings by applying different management strategies viz. inter crop, barrier crop, rice straw mulch, yellow trap, chemical control, control treatments. Then also picked up some samples of diseased crops representatively and brought them to laboratory for the detection of viruses using DAS ELISA. For examining the effective management strategies to control the viral diseases of pumpkin the field experiment was conducted at Sher-e-Bangla Agricultural University (SAU) central farm under the Department of Plant Pathology, Dhaka-1207, during the period from October 2017 to April 2018. The experiment was laid out in Randomized Complete Block design with three replications. The experiment consists of six different management treatments. There were eighteen plots for six treatments and three replications for each treatment. Data were analyzed using MSTATC and Statistics 10. The mean differences among the treatments were compared by Least Significant Difference (LSD) at 5% level of significance.

The parameters which were taken in consideration in the field experiment (Disease incidence, disease severity, aphid population, growth parameters and yield parameters) gave significant variation.

Significant variation was found in case of viral disease incidence and disease severity. The highest disease incidence and severity was found in treatment T_6 (control) which were (70.84%) and (26.67%), respectively. On the other hand the lowest disease incidence (21.10%) and disease severity was found in treatment T_1 (Inter crop).

The results of aphid population predominantly show that yellow trap can control the number of aphid. Thus, the lowest number of aphid found in the treatment T_4 (yellow trap) in every data.

Significant differences were found among the treatments at growth attributes (vine length, female flower number and node number of female flower). Maximum (178.67 cm) vine length was found in treatment T_3 (straw mulch) and minimum (147.67 cm) vine length was found in T_2 (Barrier crop) treatment. The highest no of female flowers (7.33) was found in T_5 which was statistically different with other treatments. On the other hand, the lowest no of female flowers (1.33) was found in treatment T_2 (Barrier crop).

Different yield contributing parameters such as number of fruit, flesh thickness, placenta thickness, average weight (kg) of the fruit per treatment, yield (kg/treatment), yield (kg) per ha had significant variation. The variations were found due to the virus infection. Treatment T_1 gave the highest yield parameters viz. number of fruits, flesh thickness (cm), fruit weight (kg), yield per treatment (kg) and which were 2.33, 3.43, 2.463 and 5.727, respectively. There was no significant variation in placenta thickness and weight of fruits. T_6 gave the lowest result in average fruit weight (kg) and yield (kg) which were 2.123 in both cases.

Significant relation was found in disease incidence and severity with yield (kg) per treatment. The relationship found between the viral disease incidence (%) and severity (%) with yield in kg per treatment is a negative relation. It means that with the increase of disease incidence (%), severity (%) yield is decreased.

Virus infecting pumpkin leaves were used to identify the virus by ELISA test. By visual observation, six (6) major categories of viral symptoms were found in field viz. mosaic, yellow mosaic, fern leaf, chlorotic spot, leaf distortion and hardy leaves. In Serological test, only one antiserum (CMV) was used to identify pumpkin infecting virus. By observing color of ELISA kit, it was concluded that mosaic, yellow mosaic, chlorosis and hardy leaves symptoms showed positive to CMV. Based on results of DAS-ELISA in the present study four different types of symptoms were identified as CMV which symptoms categories were mosaic, yellow, chlorosis and hardy leaves. Field experiment was also conducted to determine specific symptom (s) associated with each virus to aid visual diagnosis and serological detection of pumpkin viral diseases and to find a suitable management strategies for pumpkin infecting virus diseases. The seedlings with two cotyledons were inoculated with CMV by using the sap and carborundum powder. In serological test, $T_{2,}$ $T_{4,}$ T_5 and T_6 treatments of pumpkin were infected with *CMV* which symptoms categories were mosaic, yellow mosaic, leaf hardening, curling and chlorosis shown positive during serological test by using CMV ELISA kit.

According to the different literature it was the prediction that, the viral symptoms may be due to the presence of the most common viruses which infect cucurbits and they are CMV, ZYMV, WMV, PRSV, CGMMV and ZGMMV. In Serological test, only one antiserum (CMV) was used to identify pumpkin infecting virus. By observing color of ELISA kit, it was concluded that mosaic, yellow mosaic, chlorosis and hardy leaves symptoms showed positive to CMV. Based on results of DAS-ELISA in the present study four different types of symptoms were infected with CMV which symptoms categories were mosaic, yellow, chlorosis and hardy leaves.

CONCLUSIONS

On the basis of the findings of the present investigation, the following conclusions may be made.

1. During survey, virus infected 50 leaves sample were collected from three districts of Bangladesh. Among them ten (10) characteristics symptoms were identified as virus diseases which were identified by visual observation. Among these symptoms, four symptoms showed positive to serological test by using only CMV antiserum.

2. In serological test, only one antiserum (CMV) was used to identify pumpkin infecting virus. By observing color of ELISA kit, it was concluded that mosaic, yellow mosaic, chlorosis and hardy leaves symptoms showed positive to CMV. Based on results of DAS-ELISA in the present study four different types of symptoms were identified as CMV which symptoms categories were mosaic, yellow, chlorosis and hardy leaves.

3. In field, management strategy, the lowest incidence and severity level in treatments T_1(Inter crop) was 21.10% and 11.11%, respectively whereas disease incidence (%) and disease severity (%) both were maximum in T_6 and which were 70.84(%) and 26.67(%) respectively.

4. Significant variation was found in different growth parameters and yield parameters during field experiment. Yield and yield attributes was found maximum in treatment T_1 (inter crop).

5. Significant relation was found in disease severity (%) with yield (kg) per treatment. There was negative relation between the disease severity (%) with yield in kg per treatment which indicated that with the increase of disease severity (%), yield of pumpkin decreased.

6. Significant relation was found in disease severity (%) with aphid population (no). There was positive relation between the viral disease severity (%) with aphid population (no) which indicated that with the increase of aphid population (no), infection is decreased.

7. Inoculated CMV was identified in pumpkin leaves by visual observation, ELISA test. Six (6) major categories of virus symptoms were found in field viz. mosaic,

yellow mosaic, fern leaf, chlorotic spot, leaf distortion and hardy leaves by visual observation.

8. In serological test, $T_{2,}$ T_4 and $T_{5,}$ T_6 treatments of pumpkin were infected with CMV which symptoms categories were mosaic, yellow mosaic, leaf hardening, curling and chlorosis shown positive during serological test by using CMV ELISA kit.

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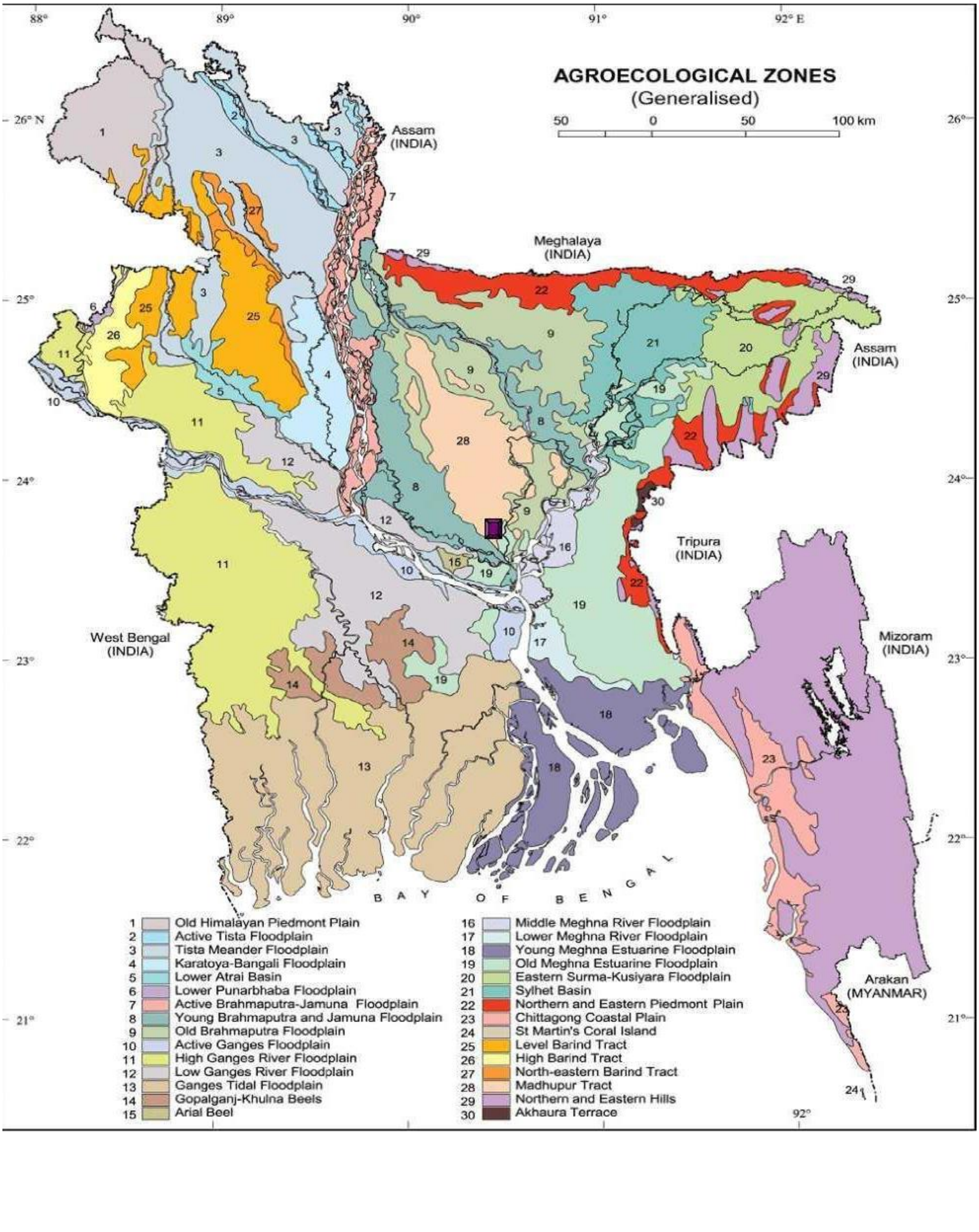
APPENDICES

Appendix I: Experimental site showing in the map under the present study



Appendix II: The mechanical and chemical characteristics of soil of the experimental site as observed prior to experimentation

Morphological characteristics of soil of the experimental plot

Morphological features	**Characteristics**
Location	Research farm, SAU, Dhaka
AEZ	Modhupur Tract (28)
General Soil Type	Shallow Red Brown Terrace Soil
Land Type	Medium high land
Soil Series	Tejgaon fairly leveled
Topography	Fairly level
Flood Level	Above flood level
Drainage	Well drained
Texture	Loamy

Chemical composition of the soil

Constituents	0-15 cm depth
P^H	5.45-5.61
Total N (%)	0.07
Available P (μ gm/gm)	18.49
Exchangeable K (μ gm/gm)	0.07
Available S (μ gm/gm)	20.82
Available Fe (μ gm/gm)	229
Available Zn (μ gm/gm)	4.48
Available Mg (μ gm/gm)	0.825
Available Na (μ gm/gm)	0.32
Available B (μ gm/gm)	0.94
Organic matter (%)	0.83

Source: Soil Resources Development Institute (SRDI), Farmgate, Dhaka.

Appendix III: Monthly records of meteorological observation at the period of experiment (October, 2017 to April, 2018)

Name of months	Temperature (0C)			Relative humidity (%)
	Maximum	Minimum	Mean	
October, 2017	32	23	27.5	79
November, 2017	30	17	23.5	65
December, 2017	25	13	24	74
January, 2018	24	11	17.5	68
February, 2018	28	14	21	57
March, 2018	32	20	26	57
April, 2018	34	23	28.5	66

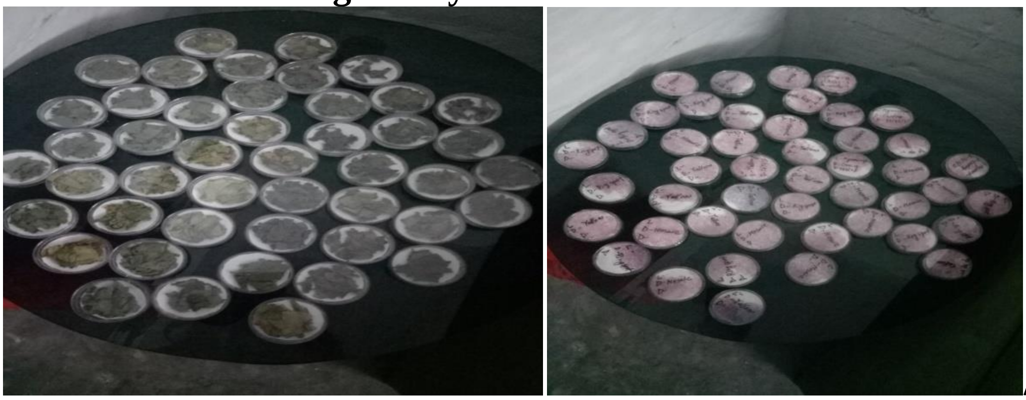
Source: www.holiday-weather.com/Bangladesh/Dhaka

Appendix IV: Nutrient content of Pumpkin (*Cucurbita moschata)* per 100 gm edible portion of fruit

Nutrient	Edible portion of fruit/100gm
Carbohydrate	8g
Protein	1g
Fibers	.5g
Calcium	20g
Iron	.8g
Beta-carotene	210µg
Thiamine	0.05mg
Riboflavin	0.05mg
Niacin	15mg
Water	90mg

Source: Tindall, 1987

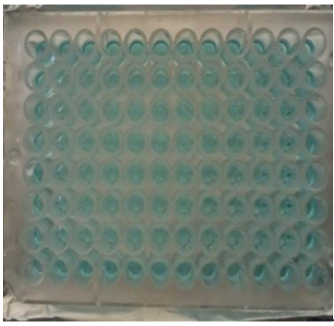
Appendix-V: Dry preservation of virus infecting leaf samples collected during survey



A.

Dry preservation of virus infecting leaf samples collected during survey

Appendix-VI: Steps of Serological Test


A

B


C


D

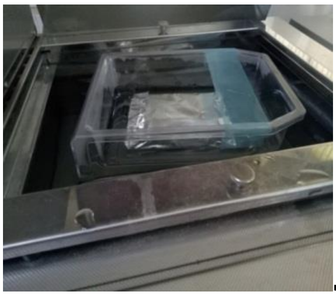

E


F

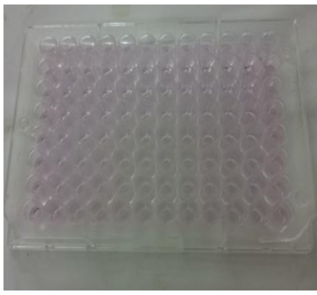

G


H


I

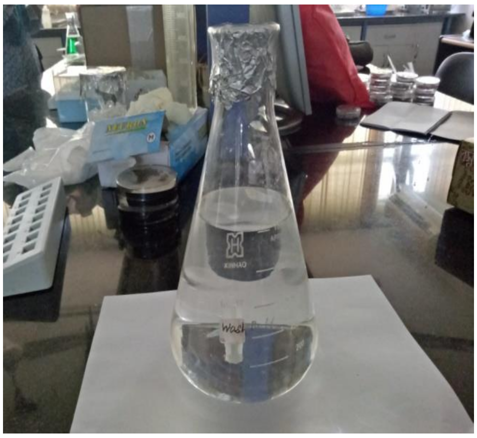

J

K


L

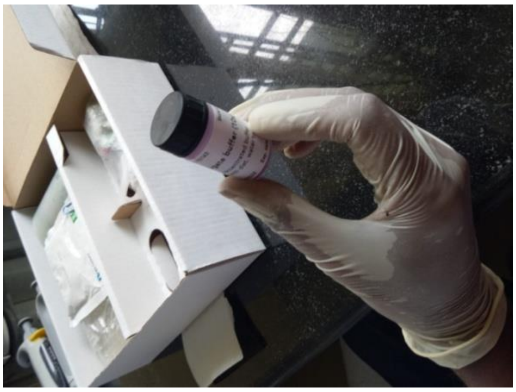
(A- Plate coated with monoclonal immunoglobulin (IgGts) 100ul/well; B- Incubation for three hours at room temperature; C- Sample collected from survey; D, E- Sample collected from survey with Buffer; F- Polystyrene microtitre plate with plant extract; G-Washing (100 µl of enzyme alkaline phosphate ALKP); H, I- Conjugate buffer J-Incubation for 55 minutes, K- Incubation temperature; L-: Color indicates the presence of CMV (serology test)

APPENDIX- VII: Different Buffers used in ELISA test



A. Coating Buffer **B. Washing Buffer**

C. Inoculation Buffer **D. Extraction Buffer**



E. Substrate Buffer **F. Congugate Buffer**

APPENDIX VIII

A. Seedlings grown in poly bag and transferring

APPENDIX IX: Different stages of field experiment

A. Plot view of field

B. Growth stage of plants

C.View of total field

D. Yellow trap

E. Female flower

F.A mature fruit

G. Harvested fruits

H. Cut fruit