EFFECT OF DIFFERENT SOWING TIME ON VIRAL DISEASE INCIDENCE AND SEVERITY OF PUMPKIN COLLECTED FROM DIFFERENT SOURCES OF BANGLADESH

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CERTIFICATE

This is to certify that the thesis entitled " ***EFFECT OF DIFFERENT SOWING TIME ON VIRAL DISEASE INCIDENCE AND SEVERITY OF PUMPKIN COLLECTED FROM DIFFERENT SOURCES OF BANGLADESH****" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS)*** *in* ***PLANT PATHOLOGY,*** *embodies the results of a piece of bona fide research work carried out by* ***SAIMA SADIA,*** *Registration No.* ***11-04427,*** *under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information as has been availed of during the course of this investigation has duly been acknowledged.

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TO

MY PARENTS

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EFFECT OF DIFFERENT SOWING TIME ON VIRAL DISEASE INCIDENCE AND SEVERITY OF PUMPKIN COLLECTED FROM DIFFERENT SOURCES OF BANGLADESH

ABSTRACT

A field experiment was conducted at the experimental farm of Sher-e-Bangla Agricultural University (SAU), Dhaka, during the period from November' 2016 to March' 2017. The study was done to evaluate the effect of sowing dates on pumpkin viral diseases and to detect different viruses of selected sources. In total of four type's pumpkin seeds were used in the experiment which are collected from four sources of Bangladesh, they are Narayanganj (S_1), Narshingdi (S_2), Dhaka (S_3), and Gazipur (S_4). These sources were tested in two sowing date that were 25th October'2016 and 5th November'2016 respectively. The experiment was laid out in a RCBD with three replications. At early sowing, the highest disease incidence and severity were found (92.41% and 97.33%, respectively) in S_2 (Narshingdi) and the lowest disease incidence and severity were found (63.33% and 81.61%, respectively) in S_1 (Narayanganj). Similarly in late sowing, the highest disease incidence and severity were found (94.14% and 97.32%, respectively) in S_2 (Narshingdi) and the lowest disease incidence and severity were found (57.49% and 82.26%, respectively) in S_1 (Narayanganj). Significant variations were found in both growth and yield contributing parameters. In both sowing times, the highest aphid population was found in month of February and the lowest in March. There were a positive correlation exists between disease incidence (%) and disease severity with aphid population both in early and late sowing. It revealed that disease incidence and severity increased with the increase of aphid population. During experiment, four different categories of symptoms were identified as viral symptoms in field such as fern leaf, mosaic, chlorosis and yellowing, and leaf distortion by visual observation. In Serological test, among four types of symptoms mosaic, chlorosis and yellowing confirmed as *Cucumber mosaic virus (CMV*) by using *CMV* antiserum through DAS-ELISA.

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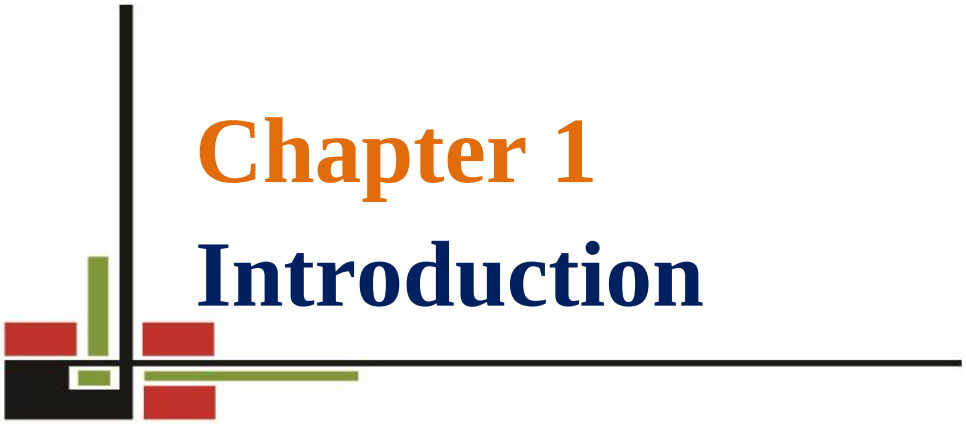
LIST OF ACRONYMS

AEZ	=	Agro-Ecological Zone
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
g	=	Gram
ha	=	Hectare
kg	=	Kilogram
mg	=	Milligram
SRDI	=	Soil Resources Development Institute



Chapter 1

Introduction

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 and Annon., 1990). The word pumpkin originates from the word pepon, which is Greek for "large melon". Pumpkin is locally known as 'Misti kumra' or 'Misti lau' or 'Misty kadu' and is considered to have originated from Central and North America (Whitaker and Davis, 1962) and distributed widely such as Southeast Asia, Tropical Africa, Central America (Peru and Mexico), the Caribbean and most parts of the tropics. It grows well throughout the entire tropical and sub-tropical regions of the world and milder areas of the temperate zones of hemispheres. It is widely cultivated in many countries of the world like India, China, Malaysia, Taiwan and Bangladesh. In Bangladesh, it grows in all the districts but plenty of pumpkins are produced in the region of Khulna, Sylhet, Jashore, Chuadanga, Noakhali, Kustia, Cumilla, Mymensingh, Chattogram, Tangail and Dhaka. In Bangladesh, the total area under cultivation of pumpkin is 11,359.526 ha with an annual production is 1, 04,723 M ton in kharif season and 17,254.177 ha and production 1, 86,112 in Rabi season (BBS, 2016).

The young leaves, male flowers and mature or immature fruit of pumpkin are used as vegetable and also cattle feed in Bangladesh (Shanmugavelu, 1989). Fruits of pumpkin are good and cheap sources of vitamins, especially high carotenoid pigments, fibre and minerals (Bose and Som, 1986). Moreover, it has the highest storability among all the vegetables; well-matured fruits can be stored for 2-4 months under ambient conditions (Yawalkar, 1985). 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).

Low yield and crop loss in pumpkin and other cucurbits is caused by several pathogens such as fungi, bacteria, viruses, nematodes and mycoplasmas etc. are known to cause disease. Among various factors responsible of pumpkin diseases, virus diseases *Cucumber mosaic virus* (*CMV*), *Papaya ring spot virus* - Water melon strain (*PRSV*-W), *Zucchini yellow mosaic virus* (*ZYMV*), *Watermelon mosaic virus -2(WMV2)* and other potyviruses are significant ones (Lisa and Lecoq, 1984). Due to virus infection both yield and nutritional levels are reduced in infected crops (Sreenivasulu *et al*., 1989, Muqit, 1995).

Identification of pumpkin virus diseases by farmers and its suggestion for management is difficult because the diseases cannot be identified reliably by their symptoms. *CMV, PRSV-W* 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).

Cucumber mosaic virus (*CMV*) is an important pathogen, which belongs to genus Cucumovirus in the family Bromoviridae. It is also reported (Douine *et al*., 1979) to cause severe leaf mosaic and deformed, stunted.

Papaya ring spot virus - Water melon strain (*PRSV*-W) is regarded as one of the most destructive pathogen infecting cucurbits causing significant reduction in yield (Rezende and Pacheco, 1998). Akanda (1991) reported that it may cause 70-100% yield reduction of cucurbits depending upon the stage of infection in Bangladesh. *PRSV*-W was known as watermelon mosaic virus-1 until it was shown that it was in fact a strain of *Papaya ring spot virus* (Provvidenti, 1993).

Zucchini yellow mosaic virus (*ZYMV*), an economically important virus belonging to the family Potyviridae, genus Potyvirus (Regenmortel *et al*., 2000). It can result in yellowing and stunting of the plant, as well as severe leaf and fruit deformities that can reduce yields up to 94% (Blua and Perring, 1989).

Control of virus in Bangladesh is difficult due to unavailability of virus resistant or tolerant 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). The use of resistant cultivars, when available, is the most cost-effective and reliable approach to protect crops (Kang *et al*., 2005).

Sowing has an important influence on aphid population growth and pumpkin diseases development. Mean prevalence and severity index of viral infection of cucurbits were significantly higher in the dry season than in the rainy season (Kone *et al.,* 2017). Aphid population is exterminated by high or low temperature prior to the growing period (Cadman and Chambers, 1960 and Beemster, 1961).

In Bangladesh, farmers are usually advised to spray chemicals to control the vector (aphid). But indiscriminate use of pesticide cause environmental pollution and also there is a risk of developing pesticide resistance. However, the most effective and environmentally safe method is the planting of tolerant varieties if available. Considering the above circumstances, the present study will be undertaken with the following objectives:

- To evaluate the effect of sowing times on pumpkin infecting viral disease incidence and severity under field condition
- To detect different viruses which infect pumpkin plants

Chapter 2

Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

Pumpkin is an important vegetable crop in Bangladesh. Its fruit, growing parts, flower etc. are used as vegetable. Viral diseases cause severe yield loss in pumpkin. Available literatures on various aspects of pumpkin viral diseases so far have been presented in this chapter

2.1. Origin and distribution of pumpkin

The origin of pumpkin is not definitively known, but they are thought to have originated in North America. The oldest evidence, pumpkin-related seeds dating between 7000 and 5500 BC, were found in Mexico (Credo Reference, 2008). Moreover, some scientists believe that pumpkin is originated in Central America and Northern South America (Whitaker and Davis, 1962). The cultivation of pumpkin was started from Southern part of USA and continues up to Peru of South America. It grows throughout the entire tropical and sub-tropical regions of the world and milder areas of the temperate zones of hemispheres, in many countries of the world; it is widely cultivated in India, China, Malaysia, Taiwan and Bangladesh. 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. *C. moschata* is probably the most widely grown species of *Cucurbita* (Tindall, 1987).

2.2. Nutritional value of pumpkin

Bose and Som (1986) mentioned that pumpkin fruits are good source of vitamins, especially high carotenoid pigments and minerals.

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. Viral disease problems in pumpkin

Virus diseases of crop plants are a common problem throughout the world. Almost all crops suffer from more than one virus disease causing serious loss of yield and quality of the crops (Harrison, 1970; Smith, 1972; Bos, 1983). Matthews (1981) reported that 650 different viruses belonging to 26 plant virus group have been found to infect cultivated species all over the world.

Sreenivasulu *et al.* (1989) and Muqit (1995) reported that virus diseases are responsible for huge economic losses of cultivated crops throughout the world. Due to virus infection both yield and nutritional levels are reduced in infected crops.

Beute (1970) and Mahgoub *et al.* (1997) reported that viral diseases have an important status because they not only cause direct damage to the host, but also predispose the plant to secondary invaders.

2.4. Cucurbits virus in General:

Webb and Scott (1965) divided *WMV* isolates from the two distinct groups, which were distinguished by host range and serological properties. Those isolates that failed to infect non- cucurbitaceous plants were designated as *Watermelon mosaic virus-1 (WMV*-1) whereas isolates that infected plants other than cucurbitaceae (e.g. certain species of the Chenopodiaceae, Leguminosae and Malvaceae) were designated as *Watermelon mosaic virus-2 (WMV-2*).

Lovisolo (1980) reviewed the virus diseases of cucurbitaceous crops and concluded that cucurbitaceous crops were highly susceptible to virus diseases and more than 25 viruses, including at least seven potyviruses, infect cucurbits naturally.

Lisa and Lecoq (1984) mentioned that low yield and crop loss in pumpkin is caused by several pathogens such as fungi, bacteria, viruses, nematodes and mycoplasmas etc., which are known to cause disease. Among various factors responsible of pumpkin diseases, virus diseases (*CMV, PRSV, ZYMV, WMV-2* and other poty viruses) are significant ones.

Lecoq *et al*. (2003) mentioned that viral diseases are the main problem in the production of cucurbit plants compared to diseases caused by other agents. Viruses causing significant yield losses to cucurbits worldwide are found within several families including Geminiviridae, Closteroviridae, Bromoviridae, Luteoviridae and Potyviridae.

Purcifull *et al.* (1984) described that three main potyviruses cause major diseases of cucurbits throughout the world: *PRSV-W, WMV-2* and *Zucchini yellow mosaic virus* (*ZYMV*). *PRSV*-W is commonly encountered in tropical or subtropical areas and occasionally in temperate areas and grouped into PRSV-P i.e., *Papaya ringspot virus*-watermelon strain and *PRSV*-P i.e., *Papaya ringspot virus*-papaya strain. *PRSV*-P infects papaya and cucurbits but PRSV-W infects cucurbits only, not papaya.

Gonasalves *et al.* (1998) published a paper on the inclusion of potyvirus infected plants. Cylindrical inclusion seemed to be the unique characteristics of all potyvirus including PRSV-W.

Quiot *et al.* (1986) stated that *WMV*-1 (*Watermelon mosaic virus*-1) spreads in the Middle East and the South and Central American region, Australia, China, France, Germany, India, Italy, Mexico, and the USA.

Hsu *et al.* (1987) found that in Taiwan *Zucchini yellow mosaic virus* (*ZYMV*) was most prevalent followed by *Watermelon mosaic virus* 2 (*WMV*-2).*Cucumber green mottle mosaic tobamovirus* (*CGMMV*) was rare in cucumber, *Luffa* spp., *B. hispida* and pumpkin. *ZYMV* was most prevalent, then *WMV* followed by *CMV*, but *CGMMV* was predominant in *L. siceraria. ZYMV* is thought to be the most important virus in cucurbit cultivation in Taiwan.

Akanda *et al.* (1991) reported that pumpkin was found to be affected by two different viruses namely, *PRSV* and *WMV*-2.

Dahal *et al.*(1997) made a survey of papaya and 10 cucurbitaceous vegetables (ash gourd, zucchini, watermelon, cucumber, pumpkin, bottle gourd, snake gourd, sponge gourd, bitter gourd and choyote) from more than 68 locations (both experimental plots and farmers' fields) in Nepal, observed that these crops were heavily affected with various virus disease-like symptoms. The most commonly observed symptoms were severe mosaic, leaf distortion, blisters and shoe stringing on Zucchini and mosaic or yellow mosaic, blisters, and leaf distortion on other cucurbits. Average incidence of plants with symptoms ranged from 75% to 100% on papaya, 85% to 100% on marrow, 4% to100% on cucumber, 4%to 100% on pumpkin and 10-100% on bottle gourd, choyote and watermelon .The virus isolated from papaya and zucchini was confirmed as PRSV-W.

Yuki *et al.* (2000) observed that in Brazil *PRSV*-W and *ZYMV* were the most common viruses infecting pumpkin and other cucurbitaceous crops.

Begum *et al.* (2016) detected *Papaya ringspot virus*-watermelon strain (*PRSV*-W), *Watermelon mosaic virus* 2 (*WMV*-2), *Cucumber mosaic virus* (*CMV*) and *Zucchini yellow mosaic virus* (*ZYMV*) in pumpkin from 26 pumpkin breeding lines.

Kone *et al.* (2017) stated that pumpkin was found to be affected by three different viruses namely, *CMV, ZYMV* and *PRSV.*

2.5. CMV in general

Agrios (1978) and Francki *et al*. (1979) stated that Cucumber mosaic virus has three functional pieces of single-stranded RNA, packaged in three icosahedral particles about 28 nm in diameter. The molecular weight of *CMV* falls in the range of 5.8 to 6.7 million, of which 18 percent is RNA and the remaining 82 percent protein. *Cucumber mosaic virus* exists in numerous strains that differ somewhat in their hosts, in the symptoms they produce, in the ways they are transmitted, and in other properties and characteristics.

Douine *et al.* (1979) reported that *CMV* infecting pumpkin has very wide host range and a worldwide distribution, it is known to effect 775 species representing 85 families.

Roossinck *et al.* (1999) reported that *Cucumber mosaic virus (CMV)* is one of the most widespread plant viruses in the world.

Kobori et al. (2000) reported that Cucumber mosaic virus (*CMV*) involve in cellular membrane changes that impede the diffusion or transport of infective virus particles from cell to cell, and or inhibit virus particle replication in the leaf tissue of resistant host plants or restriction of *CMV* movement at the interface of cell.

Roossinck (*et al.* (2002) stated that *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.

Chen (2003) stated that *CMV* has a very broad host range of wild and cultivated plants with more than 1,200 known hosts, including some monocotyledons and a great number of dicotyledons.

Zitter and Murphy (2009) stated that *Cucumber mosaic virus* (*CMV*) was first described in detail in 1916 on cucumber and other cucurbits, but is now known to occur worldwide in both temperate and tropical climates, affecting many agricultural and horticultural crops. They also added that *Cucumber mosaic virus* is responsible for reduced terminal vine growth and yellowing with misshapen and mottled cucumber fruit. Misshapen and mottled cucumber fruit due to Cucumber mosaic virus infection.

2.6. Occurrence, distribution and Identification of pumpkin viruses

Davis and Mizuki (1987) reported that identification of pumpkin virus 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.

Kader *et al.* (1997) reported that 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 for serological detection of viruses by dot-immunobinding assay. Out of the six different samples fern leaf, mosaic, vein-clearing and leaf curl were found to be positive against antisera of *PRSV*-P. Chlorotic spot and interveinal chlorosis were found positive against the antisera of *PRSV*-W and *WMV*-2 respectively.

Krstiã *et al.* (2002) showed that identify the major viruses infecting pumpkins (*Cucurbita pepo*) grown in Serbia. Virus- infected plants showed different symptoms. Due to the great variability of the symptoms, the causal viruses could not be fully and precisely determined by visual examination only.

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

Farhangi *et al*. (2004) reported cucurbits are threatened by many viruses. They determine 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. Infected plants show symptoms such as: mosaic, yellowing, deformation, shoestring of leaves and fruit deformation and yield reduction. Distribution of *CMV, ZYMV* and *WMV* were determined by DAS-ELISA. 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.

Coutts and Jones (2005) reported a survey was done to determine the incidence and distribution of virus diseases infecting cucurbit crops growing in the field at Australia Overall, 43 cucurbit-growing farms and 172 crops of susceptible cultivars were sampled. All samples were tested by enzyme-linked

immunosorbent assay (ELISA) using antibodies to *Cucumber mosaic virus* (CMV), *Papaya ringspot virus-cucurbit strain* (PRSV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (*WMV*), and *Zucchini yellow mosaic virus* (*ZYMV*).

Dukić *et al.* (2006) done a cucurbit disease survey, severe symptoms resembling those caused by viruses were observed on bottlegourd (*Lagenaria siceraria* (Molina) Standl.) in the Vojvodina region of Serbia. Symptoms included stunting, mosaic, green vein banding, blistering, yellowing, chlorotic spots, leaf deformation, and fruit distortion.

Jossey and Babadoost (2008) showed to identify the viruses infecting pumpkin and squash in Illinois. *Cucumber mosaic virus* (*CMV*), *Papaya ringspot virus* (*PRSV*), *Watermelon mosaic virus* (*WMV*), *Zucchini yellow mosaic virus* (*ZYMV*), and unknown potyviruses were detected in pumpkin, squash, and gourd fields during the surveys, 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, and was the most prevalent virus throughout the state.

Zitikaitė *et al.* (2011) stated that *Cucumber mosaic virus* (*CMV*) causing viral diseases of many important plants worldwide have been isolated from pumpkin (*Cucurbita pepo* L.) plant leaves collected in Ukraine. Diseased plants had light green mottled foliage. Leaves were smaller than normal, yellow mottled and crinkled. 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).

2.7. Incidence of virus disease

Yuki *et al.* (2000) observed that in Brazil *PRSV*-W and *ZYMV* were the most common viruses infecting pumpkin and other cucurbitaceous crops, accounting

49.1 and 24.8% of incidence, while *CMV* and *WMV*-2 were detected as 6.0 and 4.5%, respectively.

Coutts and Jones (2005) reported a survey was done to determine the incidence and distribution of virus diseases infecting cucurbit crops growing in the field at Australia Overall, 43 cucurbit-growing farms and 172 crops of susceptible cultivars were sampled. Overall, 72% of farms and 56% of crops sampled were virus-infected. The growing areas with the highest incidences of virus infection were Darwin and Carnarvon, and those with the lowest incidences were Katherine and Perth. For WA, overall 78% of farms and 56% of crops were virus-infected, and in the NT the corresponding figures were 55% of farms and 54% of crops. The most prevalent viruses were *ZYMV* and *PRSV*, each being detected in 5 and 4 of 6 cucurbit-growing areas, respectively, with infected crop incidences of <1–100%. *SqMV* was detected in 2 cucurbit-growing areas, sometimes reaching high incidences (<1–60%). *WMV* and *CMV* were found in 3 and 4 of 6 cucurbit-growing areas, respectively, but generally at low incidences in infected crops (<1–8%).

Köklü and Yilmaz (2006) carried out a survey for the detection of C*ucumber mosaic virus* (CMV), *Papaya ringspot virus*-W (*PRSV*-W), *Squash mosaic virus* (*SqMV*), *Melon necrotic spot virus* (*MNSV*), *Cucumber green mottle mosaic virus* (CGMMV), *Zucchini yellow mosaic virus* (*ZYMV*) and *Watermelon mosaic virus*-2 (*WMV*-2) in June and July 2005, covering 17 melon fields and 19 watermelon fields in the Tekirdag, Edirne and Kırklareli provinces of Turkish Thrace. The following rates of incidence of tested viruses on watermelon were found: *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 at 16.7% and 11.4%, respectively.

2.8. Symptoms of pumpkin viruses

In many cases each individual virus produces symptoms on the host unique or particular virus (Bos, 1978; Holmes, 1964). 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 (Yora *et al.* 1983). However, symptoms in all cases may not identify the causal virus but its use in preliminary diagnosis of many plant viruses have been well emphasized by many scientists (Bos, 1978; Holmes, 1964; Matthews, 1981 and Smith, 1972).

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

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* (Choi *et al*., 1990; Bilgrami and Dube, 1996)

In field-collected samples of *Cucurbita maxima* a mild mosaic symptom was recorded which was confirmed as PRSV using DIBA (Somowiyarjo, 1993). In contrast to this a strain of PRSV isolated from Taiwan was found to cause more severe symptoms than usual in cucurbits (Provvidenti, 1996).

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

Brunt *et al.* (1997) noted that the symptoms of *PRSV*-W were mosaic, systemic chlorotic mottling, green blistering or spotting, leaves and fruit malformed etc. different cucurbitaceous crops. The electron microscopy of *PRSV* revealed that

the virions are filamentous; not enveloped; usually flexuous; with a clear modal length of 760-800 nm; 12 nm wide.

Dahal *et al.* (1997) identified the most common symptoms induced by *PRSV* on papaya and cucurbits as severe mosaic, leaf distortion, oily streaks or spots on papaya fruits, whereas and shoe-string, mosaic or yellow mosaic, blisters on cucurbits.

Kader *et al.* (1997) found that the samples which showed positive reaction to PRSV-P antisera 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 .

Singh *et al.* (2003) stated that symptoms of *PRSV* differed in some of the cucurbits. In a study of host range of PRSV, 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.

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

Ozaslan *et al.* (2006) stated cucurbit growing is affected negatively due to diseases caused by cucurbit viruses. In order to prevent this damage cucurbit virus was identified by serologically. Due to this study, it is usually difficult to give definitive diagnosis based on symptoms but 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.

Bananej 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. 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%).

Jossey and Babadoost (2008) showed *CMV, PRSV, SqMV, TRSV, ToRSV, WMV, ZYMV* and other potyviruses produced 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 shoestring on leaves and color breaking and deformation of fruit.

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%).

2.9. Serology for identification of pumpkin virus

Serodiagnosis has been highly evaluated as effective and quick method. Several serological methods have been developed and applied in plant virus research. Like enzyme-linked immunosorbent assay (ELISA) developed by Clark and Adams (1977). The ELISA has been extensively used since its introduction to plant virology for rapid diagnosis of viruses from field sample (Akanda *et al.*, 1991; Clark and Bar- Joseph, 1984; Clement *et al.*, 1986). However, in the recent years DIBA has been recommended by many scientists for diagnosing viruses from field samples due to several merits like high sensitivity, rapidness, reliable, economic etc. over ELISA (Akanda *et al*., 1991; Banttari and Goodwin, 1985; Powell, 1987).

Yeh *et al.* (1984) carried out research on the serological comparison of *papaya ringspot virus* (*WMV*-1). Difference between *PRSV* and *WMV*-1 was that former infected papaya but the later did not. All the isolates of *PRSV* and *WMV*-1 tested were serologically indistinguishable as determined by Agar gel immunodiffusion test with antisera to *PRSV* and *WMV*-1 They concluded that PRSV isolates have similar biological and serological properties irrespective of geographic region.

Richter *et al.* (1989) conducted an experiment and found unsatisfactory results 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. The indirect ELISA could detect *CMV* from the samples and therefore, recommended for serial detection of *CMV* in crude leaf extracts of different cucurbits, but only in the absence of other cucumoviruses.

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

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

Krstiã *et al.* (2002) tested infected samples were by the biotest, as well as by two serological methods, ELISA and EBIA. Polyclonal antibodies raised against *Cucumber mosaic* virus (*CMV*), *Zucchini yellow mosaic* potyvirus (*ZYMV*), watermelon mosaic potyvirus 1 (*WMV*-1), Watermelon mosaic potyvirus 2 (*WMV*-2) and Squash mosaic comovirus (*SqMV*) were used. In each of the 50 collected samples one or two viruses were detected. The most prevalent viruses infecting pumpkins were *ZYMV* (62%) and *CMV* (58%). *WMV*-2 was extremely rare.

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. *Zucchini yellow mosaic virus* (*ZYMV*), *Papaya ringspot 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 detected by enzyme-linked immunosorbent assay (ELISA), and *Cucurbit yellow stunting disorder virus* (*CYSDV*), *Beet pseudo-yellows virus* (*BPYV*) and *Cucumber vein yellowing virus* (*CVYV*) by reverse transcription polymerase chain reaction (RT–PCR). *ZYMV* was 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. *CYSDV* was detected in most greenhouse cucumber samples with yellowing symptoms (88.1%), whereas *BPYV* and *CVYV* were found in only 2.4% and 9.5%, respectively, of samples. *CMV* and *SqMV* were not detected in any cucurbitaceous crop during this survey.

Dukić *et al.* (2006) collected leaf samples from 25 symptomatic plants were collected from two localities for virus identification using mechanical transmission and serological testing. Field-collected bottlegourd and inoculated plants were tested using double-antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA). Positive reactions were obtained on collected and inoculated plants with polyclonal antiserum (Loewe Biochemica,

Sauerlach, Germany) 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) 502 melon and watermelon samples were tested for the presence of seven viruses with ELISA tests using polyclonal antisera. Overall, 333 out of 502 samples tested positive for the investigated viruses: 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 six out of the seven tested viruses were present in the Thrace region of Turkey.

Bgum *et al.* (2016) conducted ELISA 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 antisera 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 antisera tested.

2.10. Vector transmission of pumpkin virus

Simons (1955) found that Southern *CMV* was transmitted by *Myzus persicae* and *Aphis rumicis* and acquisition there should period was between 5 and 10 minutes for both the aphids.

Franki *et al*. (1979) reported that *CMV* is transmitted primarily by aphids, and also by seed, cucumber beetles, parasitic plants, humans, and mechanically. He also added that *CMV* is transmitted by more than 60 species of aphid, notably *Aphis gossypii* and *Myzus persicae*, in the non-persistent manner, and is readily transmissible through plant sap. *CMV* can be acquired in 5-10 seconds and transmitted in less than 1 minute. The ability of *CMV* to be transmitted declines

after about 2 minutes and is usually lost within 2 hours. In addition, some isolates can lose their transmissibility by one aphid species but retain their transmissibility by another.

Brunt *et al.* (1996) stated that *CMV* was transmitted in a non-persistent manner by more than 60 species of insect vector including *M. persicae* and *A. coraccivora*. They also reported that *PRSV* was also transmitted in a non-persistent manner by *M. persicae* and *A. gossypii.*

Brunt *et al.* (1997) reported that the *Papaya ringspot virus*-watermelon strain (*PRSV*-W) is transmitted in nature by insect vectors belonging to the Aphidiae. The vectors were in a non-persistent manner. Virus transmitted by mechanical inoculation; not transmitted by seed.

Singh *et al.* (2003) reported *ZYMV* virus was transmitted by *Myzus persicae* and *Aphis gossypii* in a non-persistent manner.

Biswas and Varma (2012) reported host range of WMV-A was restricted to the family cucurbitaceae and it could produce symptoms on *Luffa* sp. *WMV*-A was transmitted through sap and by aphid vectors; but no seed transmission was recorded. *Aphis craccivora* was found to be a more efficient vector than *Myzus persicae* in transmitting *WMV- AWMV-A* was found to produce amorphous cytoplasmic inclusions in the infected leaves of pumpkin and the aggregation of chloroplasts was also observed. On the basis of various properties the present isolate (*WMV*-A) was identified to be an isolate of *PRSV*-W.

Fuchs (2014) described that the transmission mechanism by which aphids transmit viruses is well characterized. It takes a limited time (a few seconds to less than one minute) for the virus to be acquired and further transmitted from plant to plant. Often a few plants become infected early in the season and these initially infected plants serve as primary virus source for secondary aphid-mediated spread. A viruliferous aphid can retain the virus for a short time (less than one hour). However, it can potentially carry it over long distance.

2.11. Aphid population influenced by temperature and humidity

The optimum temperature for development of aphid population was 20-25^{0}C as reported by Broadbent *et al.* (1951) and Broadbent (1962).The migration of *M. persicae* in the plains of West Bengal normally started from the end of December and early January and their movement continued till February. The population builds up of aphids and their subsequent flight is greatly influenced by ecological conditions in different areas.

Cadman and Chambers (1960) and Beemster (1961) found that the aphid population is exterminated by high or low temperature prior to the growing period where lower temperatures and windy conditions prevail in the growing period. These conditions cause a delay in population development and restrict the opportunities for flights of winged aphids.

Khan and Bari (1981) described that aphid flying started from November and reached to the peak during the third week of February in Bangladesh. But *M. persicae* started appearing from the third week of December and its population reached to the peak in the fourth week of February. The increase of *M. persicae* had significant correlation with the total aphid flight in 1980-81 was three week earlier than that of 1979-80. The delay of the population peak in 1979-80 might be attributed to the heavy precipitation (8.2cm) occurring during the third week of November. The heavy rainfall of 1979-80 might have disrupted the aphid life cycle.

Kone *et al.* (2017) reported that mean prevalence and severity index of viral infection of cucurbits were significantly higher in the dry season than in the rainy season and also reported of higher prevalence of virus disease in dry season than in rainy season in Cote d'Ivoire. It has been reported that seasonal changes can affect hosts, pathogens and vectors in ways that alter components of the basic reproductive numbers that determine the rate at which infected hosts are produced (Altizer *et al*., 2006). These mechanisms include those that influence parasite transmission, in part by altering the behaviour of hosts, the biology of vectors or parasite infectious stages in the environment (Altizer *et al*., 2006). In addition, high temperatures that are usually experienced in dry

seasons could increase the susceptibility of host plants to virus infection and rather accelerate the fitness of viruses to cause infection, as reported by Harvell *et al*. (2002) and Mitchell *et al*. (2005).

2.12. Work done in Bangladesh

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

Rahman *et al.* (2008) conducted studies in 1500 pumpkin plants to find out the prevalence of *Papaya ringspot virus* – 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 ringspot virus*-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. Virus incidence and severity of test lines ranged from 0.00 to 79.90 % and 0.00 to 83.3 % respectively. She detected four viruses such as *PRSV-W*, *ZYMV*, *CMV* and *WMV*-2. 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.

Chapter 3

Materials and Methods

CHAPTER 3

MATERIALS AND METHODS

This chapter includes a brief description of the experiment site, experimental period, climatic condition, crop or planting materials, land preparation, experimental design and layout, crop growing procedure, treatments, intercultural operations, data collection and plant samples along with statistical analysis.

3.1. Location and research period

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 November'2016 to March'2017. The experimental plot number was 26. 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).

3.2. 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 in the Rabi season (October-March). There was very low or no rainfall during the month of December, January. The average maximum temperature during the period of investigation was 29.88° c and the average minimum temperature was 13.64° c. Details of the meteorological data in respect of temperature, rainfall and relative humidity the period of experiment were collected from Bangladesh Meteorological Department, Agargaon, Dhaka (Appendix III).

3.3. Soil type

The soil of the experimental site was medium high land belonging to the Modhupur tract under the agro ecological zone (AEZ) 28. The soil texture was silty loam, non-calcarious, dark grey soil of Tejgaon soil series with a pH 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, and Dhaka (Appendix II).

3.4. Seed collection

Seed samples were collected from four districts from farmers saved seed. These locations are namely – Narayanganj (Rupganj), Narshingdi (Belabo), Dhaka (Savar) and Gazipur (Kashimpur).

Collected seeds are named as –

S_1 = Narayanganj (Rupganj)
S_2 = Narshingdi (Belabo)
S_3 = Dhaka (Savar)
S_4 = Gazipur (Kashimpur)

3.5. Raising of seedlings

The collected seed were sown in polybag (15 x 10 cm). Each polybag received 2 kg soil which was mixed with decomposed cow dung. Three replications were in per treatment and 2 seeds were sown in each polybag. At the age of 20 days the seedlings were transplanted in the main field. Intensive care was taken to produce healthy seedlings.

Figure1: Raising of seedling in polybag.

3.5.1. Sowing time

First sowing: 25th October, 2016.

Second sowing: 5th November, 2016.

3.6. Land preparation (for transplanting)

The selected land for the experiment was first opened on 2nd November, 2016 by disc plough. After opening the land with a tractor it was plough and cross-ploughed six times with a power tiller and each ploughing was ploughed was followed by laddering to break the clods to obtain good tilth and to level the land. After final land preparation, the experimental plot was laid out.

The experimental field was upland plot located at a high elevation with drainage facility. The land were thoroughly prepared by ploughing and cross ploughing with power tiller followed by laddering to have a good tilt. All types of weeds and debris of previous crop will be removed.

3.7. Design and layout

The experiment was laid out in RCBD (Randomized Complete Block Design) with three replications. Raised beds were prepared carefully to provide better drainage. About 25 cm deep drain was dug around the field. 20 cm height, 2 m width and 2.5 m long beds were prepared.

3.8. 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). Half of the cow dung and the entire amount of TSP, Gypsum and Boron were applied at the time of final land preparation.

3.9. Pit preparation

Pits of 45 cm x 45 cm x 40 cm size was dug maintaining row to row and pit to pit spacing of 2.0 m and 2.0 m, respectively. Between beds 1 m width irrigation

and drainage channels were made. Pits were prepared in each bed one week before transplanting.

3.10. Seedling transplanting

20 days after seed sowing, raised healthy seedlings were transplanted in the experimental field.

The experiment was splited into two experiments to evaluate the effect of sowing date on pumpkin viral diseases.

Early transplanting: 15th November, 2016

Late transplanting: 25th November, 2016

3.11. Intercultural Operation

The seedlings were always kept under careful observation. Necessary intercultural operations were done through the cropping season for proper growth and development of the experimental plants.

3.11.1. Thinning and gap filling

The seedlings were thinned out and gap filled after transplanting.

3.11.2. Irrigation

The plot was irrigated as and when needed.

3.11.3. Weeding and mulching

Weeding is necessary to keep the plots free from weeds for ease aeration and mulching to conserve soil moisture. Total five weeding were done to keep the plots free from weeds. Rice straw was used for mulching.

3.11.4. Drainage

Stagnant water was effectively drained out at the time of heavy rains.

3.12. Identification of viruses

Pumpkin plants grown in the experimental field were checked at 50 days after transplanting and gradual symptoms were recorded. The recorded symptoms include fern leaf, mosaic, chlorosis and yellowing, leaf distortion. Individual plants showing visible symptoms of virus diseases were recorded. Photographs of the symptoms were taken and compared with standard literatures (Zitter, 1996).

3.13. Harvesting

Fully ripen fruits were harvested and data on fruit yield, yield contributing characters were recorded.

3.14. Parameters assessed

Data collection on the basis of growth and yield contributing characters of infected plant or plant parts. They are as follows-

- Number of infected leaf/plants
- Number of healthy leaf/plant
- Number of aphid association
- Number of flower /plants
- Number of fruits /plants (Infected and healthy)
- Fruit weight (kg)
- Length of the fruits (cm)
- Breadth of the fruits (cm)
- Thickness of fleshy part in fruit (cm)
- Thickness of placenta (cm)

3.15. Collection of data

For data collection on different morphological parameters from the selected plants different measures are taken. Data over the parameters were taken in the following ways-

3.15.2. Number of infected leaves per plant

Number of infected leaves of selected infected plants from each plot at 50, 65, 80, 95 and 110 days after sowing (DAS) was recorded. Calculating the average number of infected leaves, the average number was recorded.

3.15.3. Number of flowers per plant

Mean number of flower of selected plants from each plot as per treatment combination was recorded.

3.15.4. Number of fruits per plant

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

3.15.5. Number of aphid association

In each plot, one plant was selected. From there, mean average aphid population was recorded by selecting 10 opposite leaves and by calculating their mean.

3.15.6. Disease incidence (%) per plant

Incidences of viral disease were recorded at before and after flowering. One plant was selected randomly from each plot and the disease symptoms were observed carefully for collection of data. Data on disease incidence were recorded at an interval of 20 days commencing from first incidence and continued up to four times.

3.15.7. Disease severity (%) per plant

Severity of disease was recorded at before and after flowering. One plant was selected randomly from each plot and the disease symptoms were observed carefully for collection of data. Data on disease severity were recorded at an interval of 20 days commencing from first severity and continued up to four times.

3.15.8. Yield (kg) and yield contributing parameters

3.15.8.1. Yield (kg)

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

3.15.8.2. Fruit number

Mean number of fruit of selected plants from each plot as per treatment combination was recorded.

3.15.8.3. Fruit weight (kg)

Mean fruit weight of selected plants from each plot as per treatment combination was recorded.

3.15.8.4. Fruit diameter cm)

Mean fruit diameter (cm) of selected plants from each plot as per treatment combination was recorded.

3.15.8.5. Flesh thickness (cm)

Mean flesh thickness (cm) of selected plants from each plot as per treatment combination was recorded.

3.15.8.5. Placental thickness (cm)

Mean placental thickness (cm) of selected plants from each plot as per treatment combination was recorded.

3.16. Identification of viruses using DAS-ELISA

Virus identification was done by using standard double antibody sandwiched enzyme-linked immunosorbent assay (DAS-ELISA) as described by Clark and Adams (1977) using polyclonal antisera raised against CMV (Bioreba Ag, Switzerland). The test was carried out in Plant Pathology lab of BARI. The dried leaf samples were homogenized (dilution 1:50 g/ v) in extraction buffer (8.0 g NaCl, 0.2 g KH_2PO_4, 1.1 g Na_2HPO_4, 0.2 g KCl/L, pH 7.4) containing

0.05% v/v Tween 20, and 2% w/v polyvinylpyrolidone. Microtiter plate was coated with *CMV* antiserum. The IgG antibodies were diluted in the coating buffer (Na2CO3+NaHCO3+NaN2) at a recommended dilution of 1:1000 according to the manufacturer's instructions. Hundred microliters of the dilution was distributed in the wells and incubated at 37 °C for 3 h. The microplates were washed thrice with the phosphate buffer saline-Tween 20 (PBS-T). The homogenized leaf samples were added and incubated overnight at 4°C.

After washing the plates three times with PBS-T, they were incubated with the enzyme conjugate (alkaline phosphatase conjugate, diluted at 1/1000 in PBS-T+BSA+NaN2) at 37 °C for 2 h. After washing the plates three times with PBS-T, they were incubated for one hour at room temperature with freshly prepared phosphate substrate solution (100 µL per well). The substrate was p-nitrophenyl phosphate (pNPP) tablet (Sigma-Aldrich Co. LLC) applied at 1.0 mg Ml−1 in 9.87% diethanolamine, pH 9.8. Plate 1 shows some picture from the conducted DAS-ELISA test (A-H).

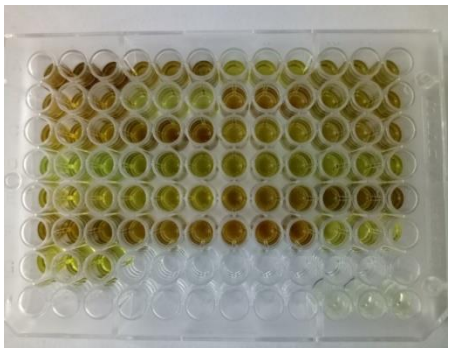
A. ELISA kit box

B. Microtiter plate coated with CMV

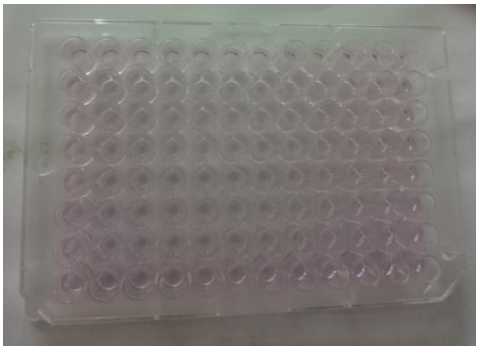
C. Incubation of microtiter plate

D. Washing of the plate with PBS-T

E. homogenization of leaf samples with extraction buffer



F. Homogenized leaf samples in each well



G. Addition of pNPP in each well

H. Final color observed visually

Plate 1. Protocol of DAS-ELISA test by using CMV antiserum (A-H)

3.17. Disease incidence and disease severity

Disease incidence was determined based on the symptoms on diseased plants. Data on disease incidence and disease severity of virus disease in experimental field were recorded through frequent visit after appearance of symptoms. Disease incidence was estimated using a standard formula (Agrios, 2005)

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

Disease severity was expressed in percent disease index (PDI). The PDI was computed using a standard formula (Piper *et al*., 1996) as shown below:

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

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 (Xu *et al*., 2004).

3.17. 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 was used for this purpose.

Chapter 4

Results

CHAPTER 4

RESULTS

Two split experiments were conducted in the field condition for evaluation the effect of sowing date on disease incidence and severity of pumpkin viruses. The results of both experiments are given below in the following heading and sub-heading-

Experiment No. 1: Effect of early sowing on viral disease incidence and severity of pumpkin collected from different sources of Bangladesh

4.1. Effect of sowing date (early) on viral disease incidence (%) of pumpkin

Disease incidence (%) was recorded at 50, 65, 80, 95 and 110 days after transplanting. Significant variations were found among the treatments at different days after transplanting (DAT). Disease incidences of different treatments are presented in Table 1.

At 50 DAT, the highest (34.85) disease incidence (%) was found in S_2 (Narshingdi) and the lowest (11.93) disease incidence (%) in S_3 (Dhaka). At 65 DAT, the highest (64.94) disease incidence (%) was found in S_2 (Narshingdi) and the lowest (18.90) disease incidence (%) in S_4 (Gazipur). At 80 DAT, the highest (76.78) disease incidence (%) was found in S_2 (Narshingdi) and the lowest (43.02) disease incidence (%) was found in S_1 (Narayanganj). Whereas at 95 DAT, the highest (88.60) disease incidence (%) was found in S_2 (Narshingdi), and the lowest (63.65) disease incidence (%) was found in S_3 (Dhaka). On the other hand, at 110 days after transplanting, the highest (92.41) disease incidence (%) were found in S_2 (Narshingdi) and the lowest (63.33) disease incidence (%) in S_1 (Narayanganj).

Table 1. Effect of early sowing date on viral disease incidence of pumpkin

Treatment	50 DAT	65 DAT	80 DAT	95 DAT	110 DAT
S_1	16.90 b*	25.49 b	43.02 b	65.65 ab	63.33 b
S_2	34.85 a	64.94 a	76.78 a	88.60 a	92.41 a
S_3	11.93 b	22.77 b	50.41 b	63.65 b	73.52ab
S_4	13.96 b	18.90 b	52.10 b	68.71ab	77.67 ab
LSD value	9.470	13.93	19.18	25.76	28.49
CV (%)	24.42 %	20.11%	17.12%	17.99%	19.02%

S_1 = Narayanganj, **S_2** = Narshingdi, **S_3** = Dhaka and **S_4** = Gazipur;
*Significant at 5% level of probability

4.2. Effect of early sowing on viral disease severity (%) of pumpkin

Disease severity of different treatments is presented in Table 2. There were significant differences found among the treatments.

At 50 DAT, the highest (31.11) disease severity (%) was found in S_2 (Narshingdi) and the lowest (6.67) disease severity (%) was found in S_1 (Narayanganj). At 65 DAT, the highest (55.08) disease severity (%) was found in S_2 (Narayanganj) and the lowest 22.22 disease severity (%) was found in S_4 (Gazipur). At 80 DAT, the highest (81.83) disease severity (%) was found in S_2 (Narshingdi) and the lowest (51.12) disease severity (%) was found in S_1 (Narayanganj). Whereas at 95 DAT, the highest (92.88) disease severity (%) was found in S_2 (Narshingdi), whereas, the lowest (68.56) disease severity (%) in S_1 (Narayanganj). On the other hand, at 110 days after transplanting, the highest disease severity was found in S_2 (97.33 %) and the lowest (81.61) disease severity (%) was found in S_1 (Narayanganj).

Table 2. Effect of early sowing date on disease severity of pumpkin

Treatment	50 DAT	65 DAT	80 DAT	95 DAT	110 DAT
S_1	6.67 b*	26.67 b	51.12 b	68.56 b	81.61 b
S_2	31.11 a	55.08 a	81.83 a	92.88 a	97.33 a
S_3	19.56 ab	33.34 b	63.61 ab	77.37 b	87.98 ab
S_4	8.89 ab	22.22 b	53.09 b	70.91 b	85.18 b
LSD value	22.78	17.65	25.15	12.04	11.09
CV (%)	58.88%	20.12%	17.12%	17.99%	19.02%

S_1 = Narayanganj, **S_2** = Narshingdi, **S_3** = Dhaka and **S_4** = Gazipur;
*Significant at 5% level of probability

4.3. Effect of temperature on aphid population at pumpkin field during experimental period at early sowing

Aphid populations with monthly temperatures at early sowing are presented in Table 3. There were significant differences found between the treatments.

In November, the highest (20.00) aphid population was found in S_2 (Narshingdi) and the lowest (9.00) in S_1 (Narayanganj). On the other hand, in December, the highest (22.00) aphid population was found in S_2 (Narshingdi) and the lowest (14.00) in S_4 (Gazipur). In January, the highest (47.00) aphid population was found in S_2 (Narshingdi) and the lowest (3.00) was found in S_1 (Narayanganj). In February, the highest (56.00) aphid population was found in S_2 and the lowest (30.00) was found in S_4 (Gazipur). In March, the highest (32.00) aphid population was found in S_2 (Narshingdi) and the lowest (27.00) in S_3 (Dhaka).

Table 3. Effect of temperature on average aphid population at pumpkin field during experimental period at early sowing

Treatment	November (26.5^0c)	December (23^0c)	January (21.5^0c)	February (23.5^0c)	March (24.5^0c)
S_1	9.00 b*	18.00 ab	30.00 b	50.00 ab	29.00 a
S_2	20.00 a	22.00 a	47.00 a	56.00 a	32.00 a
S_3	12.00 ab	19.00 ab	31.00 b	48.00 b	27.00 a
S_4	15.00 a	14.00 b	33.00 b	30.00 c	30.00 a
LSD value	8.80	6.18	5.44	7.09	7.02
CV (%)	31%	16.96%	7.78%	7.71%	11.90%

S_1 = Narayanganj, **S_2** = Narshingdi, **S_3** = Dhaka and **S_4** = Gazipur;
*Significant at 5% level of probability

4.4. Relationship between aphid population and monthly temperature (^{0}c) in the pumpkin field at early sowing

Relationship between the aphid population and monthly temperature (^{0}c) in the pumpkin field are shown in Figure 2. The aphid population increased gradually from mid-January to February and gradually decreased in March. The highest aphid population was found in S_2 (Narsingdi) and it was found in 23^0c

temperature February. The lowest aphid population was found from mid-November to mid-January and after March.

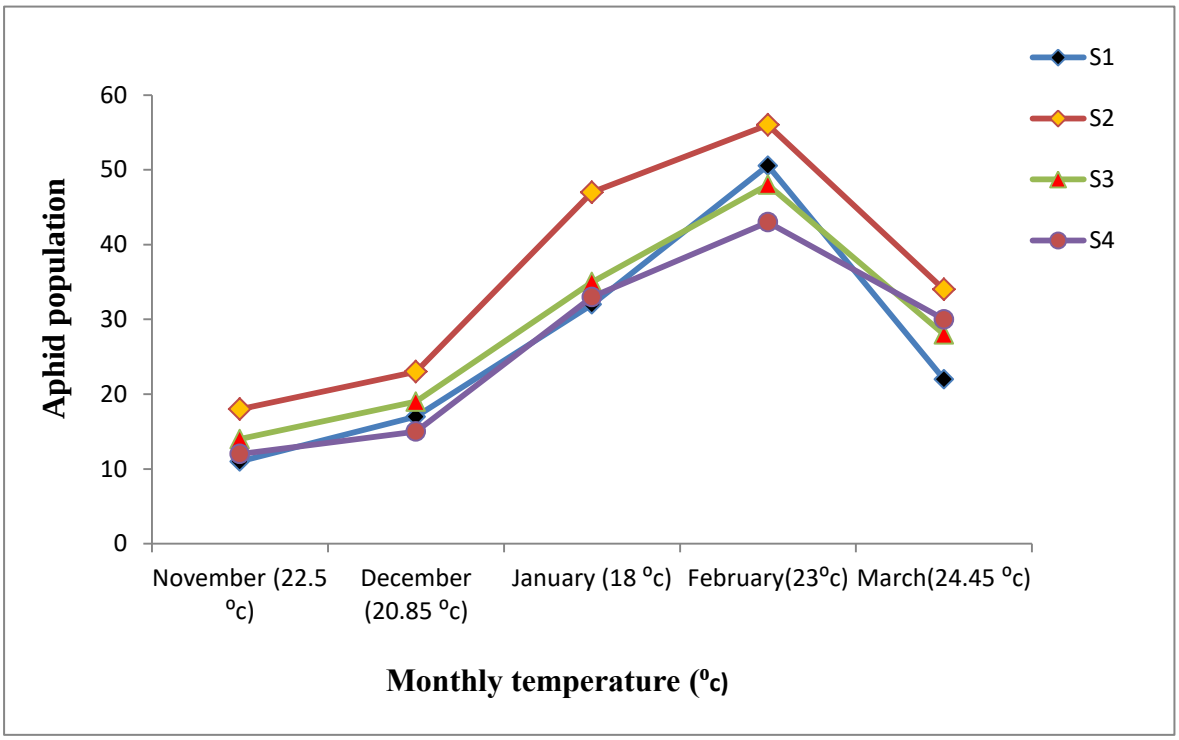


Figure 2. Relationship between aphid populations and monthly temperature (^{0}c) at early sowing of pumpkin field

4.5. Relationship between the viral disease incidence (%) `at 110 DAT and aphid population in March at early sowing

Relationship between the viral disease incidence (%) at 110 DAT and aphid population in March at early sowing the field are shown in Figure 3. A positive correlation exists between the disease incidence (%) and aphid population. It means that with the increase of aphid population, disease incidence (%) can be increased. The coefficient of multiple determination (R^2 = 0.5213) indicated that 52.13 % disease incidence in pumpkin would be affected by aphid population.

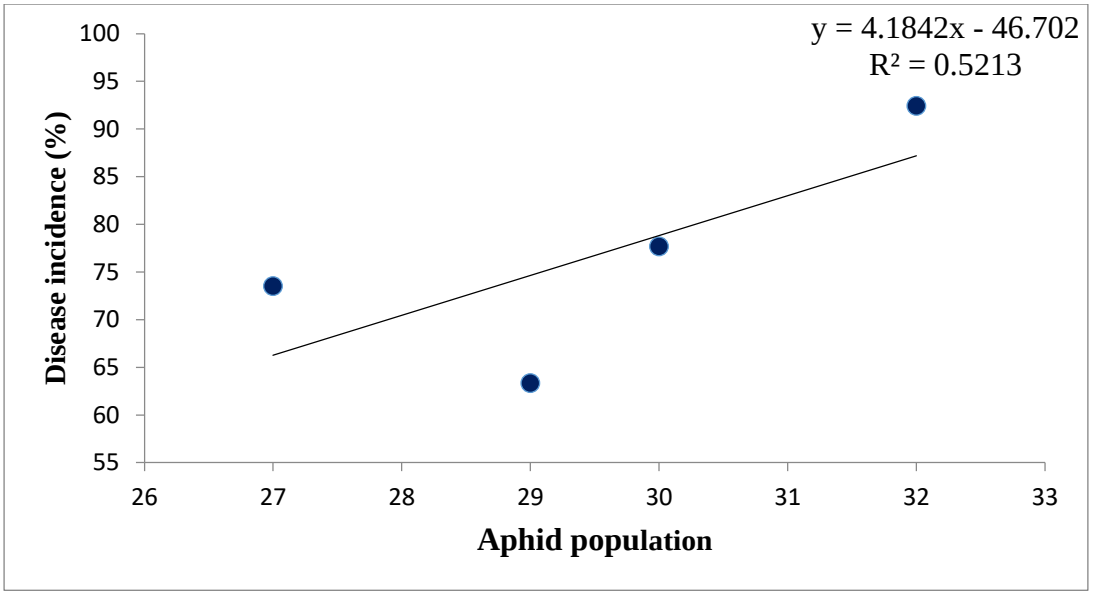


Figure 3. Relationship between the disease incidence (%) at 110 DAT and aphid population in March at early sowing

4.6. Relationship between the disease severity (%) at 110 DAT and aphid population in March at early sowing

Relationship between the disease severity (%) at 110 DAT and aphid population in March at late sowing the field are shown in Figure 4. A positive correlation exists between the disease severity (%) and aphid population. It means that with the increase of aphid population, disease severity (%) can be increased. The coefficient of multiple determination (R^2 = 0.3585) indicated that 35.85% disease severity in pumpkin would be affected by aphid population.

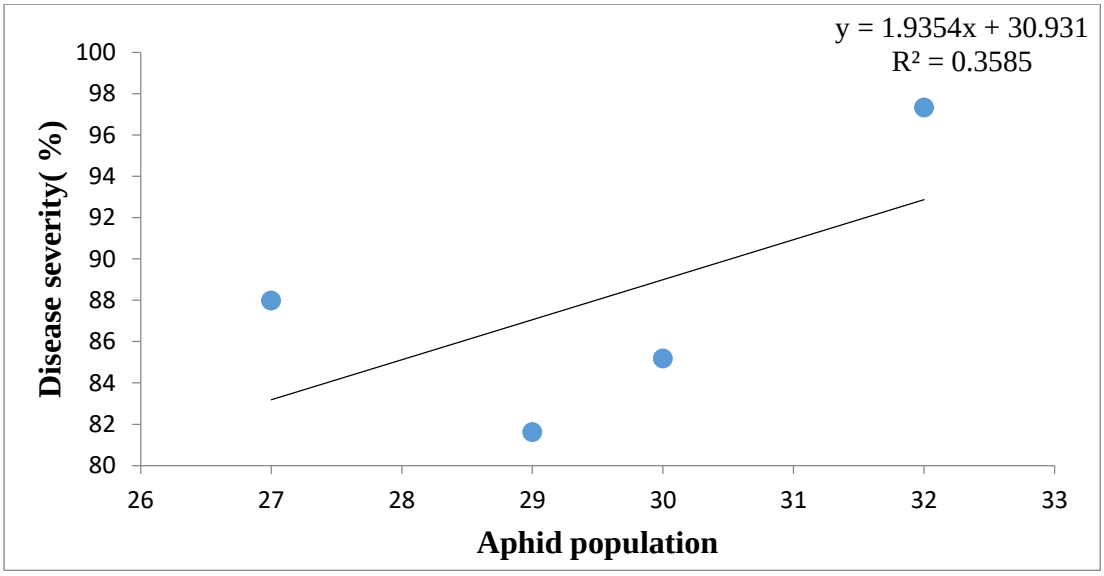


Figure 4. Relationship between the disease severity (%) at 110 DAT and aphid population in March at early sowing

4.7. Effect of pumpkin viruses on growth parameters at early sowing

Different growth parameters such as leaf area, vine length, number of branch, number of male flower and number of female flower and were recorded at 50, 65, 80, 95 and 110 days after transplanting. Significant variations were found between the treatments at different days after transplanting (DAT) among growth parameters. Variation in different growth parameters of different treatments at early sowing are presented in Table 4. The data of the parameters are follows-

4.7.1. Leaf area (cm^2)

There were significant differences found among the treatments. The highest (117.7) leaf area (cm^2) was found in S_3 (Dhaka), whereas the lowest (96.04) leaf area (cm^2) in S_4 (Gazipur).

4.7.2. Vine length (cm)

Significant differences were found among the treatments. Maximum (198.6) vine length was found in S_3 (Dhaka), whereas minimum (126.9) vine length (cm) in S_1 (Narayanganj).

4.7.3. Number of branch

There were significant differences found among the treatments. The highest (9.33) number of branch was found in S_3 (Dhaka) and the lowest (6.36) germination in S_2 (Narshingdi).

4.7.4. Number of male flower

There were no significant differences found among the treatments. The highest (49.33) number of male flower was found in S_3 (Dhaka) and the lowest (45.33) germination in S_1 (Narayanganj).

4.7.5. Number of female flower

There were no significant differences found among the sources. The highest (19.67) number of female flower was found in S_4 (Gazipur), whereas the lowest (17.67) in S_1 (Narayanganj).

Table 4. Effect of pumpkin viruses on growth parameters at early sowing

Treatment	Leaf area (cm2)	Vine length (cm)	No. of branch	No. of male flower	No. of female flower
S_1	104.40 ab*	126.9 b	7.00 ab	45.33 a	17.67 a
S_2	103.30 ab	190.40 a	6.33 b	46.00 a	18.00 a
S_3	117.70 a	198.60 a	9.33 a	49.33 a	18.67 a
S_4	96.04 b	188.60 a	7.66 ab	47.67 a	19.67 a
LSD value	18.18	53.91	2.42	19.54	4.650
CV (%)	8.64%	15.17%	16.00%	20.77%	12.58%

S_1 = Narayanganj, **S_2 =** Narshingdi, **S_3 =** Dhaka and **S_4 =** Gazipur;
*Significant at 5% level of probability

4.8. Effect of pumpkin viruses on yield and yield contributing parameters ate early sowing

Different yield and yield contributing parameters such as number of fruit, fruit weight, yield, fruit diameter, flesh thickness and placental thickness (cm) were recorded at 50, 65, 80, 95 and 110 days after transplanting. Significant differences were found among the treatments. Different yield and yield

contributing parameters different treatments at early sowing are presented in Table 5. The data of the parameters are follows-

4.8.1. Number of fruit

There were significant differences found between the treatments. Maximum (7.00) number of fruits was found in S_3 (Dhaka), whereas minimum (3.23) number of fruits in S_1 (Narayanganj).

4.8.2. Fruit weight (kg)

There were significant differences found between the treatments. The highest (1.87) fruit weight (kg) was found in S_3 (Dhaka), whereas the lowest (1.40) fruit weight (kg) in S_4 (Gazipur).

4.8.3. Yield (kg)

There were significant differences found between the treatments. The highest (13.07) yield (kg) was found in S_3 (Dhaka), whereas the lowest (4.45) yield (kg) in S_1 (Narayanganj).

4.8.4. Fruit diameter (cm)

There were significant differences found between the treatments. Maximum (14.03) fruit diameter (cm) was found in S_3 (Dhaka) and minimum (12.99) fruit diameter (cm) was found in S_1 (Narayanganj).

4.8.5. Flesh thickness (cm)

There were significant differences found between the treatments. The highest (4.00) flesh thickness (cm) was found in S_3 (Dhaka), whereas the lowest (3.26) fruit thickness (cm) was found in S_1 (Narayanganj).

4.8.6. Placental thickness (cm)

No significant difference of placental thickness was found among the treatments. The highest (10.03) placental thickness (cm) was found in S_3 (Dhaka) and the lowest (9.72) placental thickness (cm) was found in S_1 (Narayanganj).

Table 5. Effect of pumpkin viruses on yield parameters at early sowing

Treatment	No. of fruit	Fruit weight (kg)	Yield (kg)	Fruit diameter (cm)	Flesh thickness (cm)	Placental thickness (cm)
S_1	3.23 b*	1.47 ab	4.45 c	12.99 b	3.27 c	9.72 a
S_2	3.33 b	1.77 ab	5.59 c	13.72 ab	3.75 ab	9.97 a
S_3	7.00 a	1.87 a	13.07 a	14.03 a	4.00 a	10.03 a
S_4	5.33 a	1.40 b	7.74 b	13.11 b	3.35 bc	9.77 a
LSD value	1.73	0.44	1.84	0.8262	0.43	0.46
CV (%)	18.23%	13.55%	11.80%	3.07%	6.01%	2.38%

S_1 = Narayanganj, **S_2 =** Narshingdi, **S_3 =** Dhaka and **S_4 =** Gazipur;
*Significant at 5% level of probability

Experiment No. 2: Effect of late sowing on viral disease incidence and severity of pumpkin collected from different sources of Bangladesh

4.9. Effect of sowing date (late) on viral disease incidence (%) of pumpkin

Disease incidence (%) of different treatments was recorded at 50, 65, 80, 95 and 110 days after transplanting. Significant variations were found among the treatments at different days after transplanting (DAT). Disease severity of different treatments is presented in Table 6. At 50 DAT, no significant difference was found. The highest (16.42) disease incidence (%) was found in T_2 (Narshingdi) and the lowest (7.57) disease incidence (%) was in S_4 (Gazipur). Variations were found in 65, 80, 95 and110 DAT. At 65 DAT, the highest (31.79) disease incidence (%) was and in S_2 (Narshingdi) and the lowest (14.73) disease incidence (%) was in S_1 (Narayanganj). At 80 DAT, the highest disease incidence was found in S_1 (34.79 %) and the lowest (19.25) disease incidence (%) was in S_2 (Narshingdi). Similarly, at 95 DAT, the highest (52.73) disease incidence (%) was found in S_1 (Narayanganj), whereas, the lowest (36.58) disease incidence (%) was found in S_2 (Narshingdi). At 110 DAT, the highest (74.14) disease incidence (%) was found in S_1 (Narayanganj) and the lowest (57.49) disease incidence (%) was found in S_2 (Narshingdi).

Table 6. Effect of late sowing on disease incidence of pumpkin at 50, 65, 80, 95 and 110 DAT

Treatments	50 DAT	65 DAT	80 DAT	95 DAT	110 DAT
S_1	9.87 a*	14.73 b	19.25 b	36.58 b	57.49 b
S_2	16.42 a	31.79 a	34.79 a	52.73 a	74.14 a
S_3	9.13 a	22.27 ab	28.79 ab	44.34 ab	65.37 ab
S_4	7.57 a	20.45 ab	27.24 ab	49.82 ab	69.75 ab
LSD value	16.87	11.55	10.96	13.30	12.95
CV (%)	28.57 %	20.11 %	19.93%	14.51 %	9.72%

S_1 = Narayanganj, **S_2** = Narshingdi, **S_3** = Dhaka and **S_4** = Gazipur;
*Significant at 5% level of probability

4.10. Effect of different treatments at sowing date (late) on viral disease severity (%) of pumpkin

In case of disease severity of different treatments are presented in Table 7. There were significant differences were found among the treatments.

At 50 DAT, the highest (35.63) disease severity (%) was found in S_2 (Narshingdi) and the lowest (9.67) disease severity (%) was found in S_1 (Narshingdi). Similarly, at 65 DAT, the highest (52.75) disease severity (%) was found in S_2 (Narshingdi) and the lowest (21.67) disease severity (%) was found in S_1 (Narayanganj). But at 80 DAT, the highest (72.66) disease severity (%) was found in S_2 (Narshingdi). and the lowest disease severity was found in S_4 (50.79 %). At 95 DAT, the highest (86.71) disease severity (%) was found in S_2 (Narshingdi), whereas, the lowest (71.15) disease severity (%) was found in S_1 (Narayanganj). Similarly, at 110 DAT, the highest (97.33) disease severity (%) was found in S_2 (Narshingdi) and the lowest (82.26) disease severity (%) was found in S_1 (Narayanganj).

Table 7. Effect of late sowing on disease severity of pumpkin

Treatment	50 DAT	65 DAT	80 DAT	95 DAT	110 DAT
S_1	9.67 b*	21.67 b	48.71 b	71.15 ab	82.26 b
S_2	35.63 a	52.75 a	72.66 a	86.71 a	97.32 a
S_3	12.22 b	22.22 b	43.45 b	61.70 b	84.05 b
S_4	13.33 b	26.44 ab	50.79 b	77.38 ab	92.94 ab
LSD value	18.65	15.29	18.79	23.31	10.74
CV (%)	43.02	20.28 %	17.45%	15.72 %	6.03%

S_1 = Narayanganj, **S_2** = Narshingdi, **S_3** = Dhaka and **S_4** = Gazipur;
*Significant at 5% level of probability

4.11. Effect of temperature on aphid population at late sowing in pumpkin field

Aphid populations with monthly temperatures are presented in Table 8. In November, no significant differences were found among the treatments. The highest (18.00) aphid population was found in S_2 (Narshingdi), whereas the lowest (11.00) aphid population was in S_1 (Narayanganj). There were significant differences found among the treatments in December, January, February and March. In December, the highest (23.00) aphid population was found in S_2 (Narshingdi) and the lowest (15.00) aphid population was found in S_1 (Narayanganj). In January, the highest (44.00) aphid population was found in S_2 (Narshingdi), whereas the lowest (32.00) aphid population was in S_1 (Narayanganj). On the other hand, in February, the highest (58.00) aphid population was found in S_2 (Narshingdi), whereas the lowest (43.00) aphid population in S_4 (Gazipur). In March, the highest (34.00) aphid population was found in S_2 (Narshingdi) and the lowest (22.00) aphid population was found in S_1 (Narayanganj).

Table 8. Effect of temperature on aphid population at pumpkin field during experimental period at late sowing

Treatment	November (26.5^0c)	December (23^0c)	January (21.5^0c)	February (23.5^0c)	March (24.5^0c)
S_1	11.00 a*	17.00 ab	32.00 b	50.67 ab	22.00 b
S_2	18.00 a	23.00 a	44.00 a	58.00 a	34.00 a
S_3	14.00 a	19.00 ab	35.00 b	48.00 ab	28.00 ab
S_4	12.00 b	15.00 b	33.00 b	43.00b	30.00 ab
LSD value	7.02	7.56	7.59	9.03	10.74
CV (%)	23.81 %	20.46 %	10.33%	9.10 %	18.97%

S_1 = Narayanganj, **S_2** = Narshingdi, **S_3** = Dhaka and **S_4** = Gazipur;
*Significant at 5% level of probability

4.12. Relationship between the aphid population and monthly temperature (^{0}c) in pumpkin at late sowing

Relationship between aphid populations and monthly temperature (^{0}c) in the pumpkin field are shown in Figure 5.

The aphid population increased gradually from mid-January to February and gradually decreased in March. The highest aphid population was found in S_2 (Narsingdi) and it was found month of February (23°c). The lowest aphid population was found from mid-November to mid-January and after March.

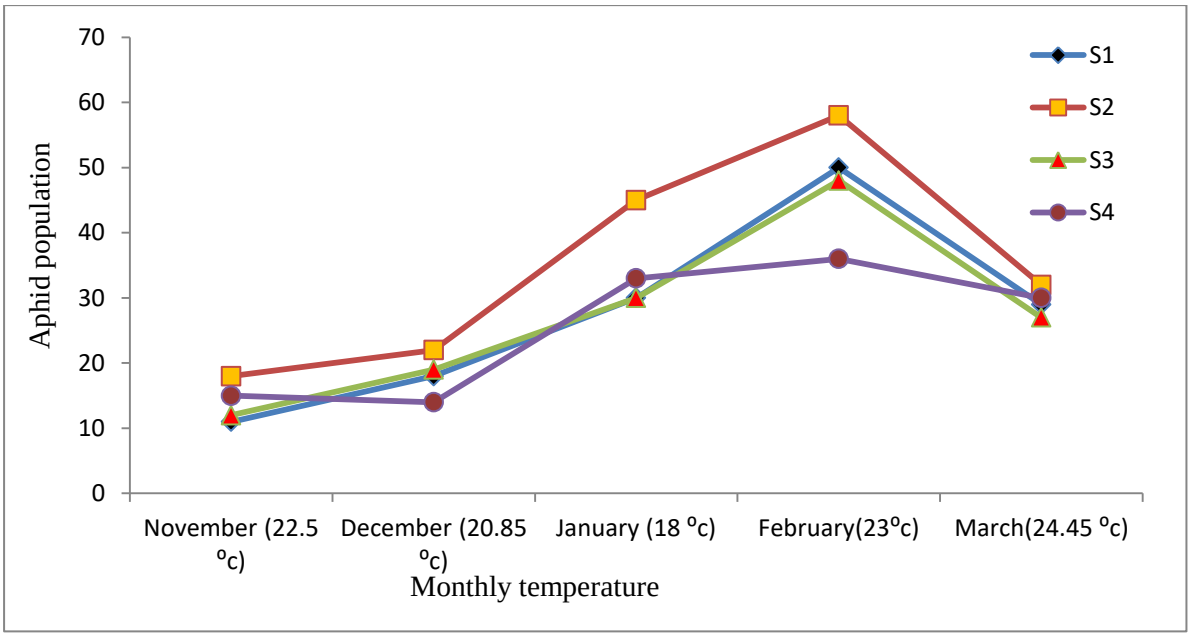


Figure 5. Relationship between aphid population and monthly temperature (^{0}c) in pumpkin at early sowing

4.13. Relationship between viral disease incidence (%) at 110 DAT and aphid population in March at late sowing

Relationship between viral disease incidence (%) at 110 DAT and aphid population in March at early sowing the field are shown in Figure 6. A strong positive correlation exists between the disease incidence (%) and aphid population. It means that with the increase of aphid population, disease incidence (%) can be increased. The coefficient of multiple determination (R^2 =

0.991) indicated that 99.10% disease incidence in pumpkin would be affected by aphid population.

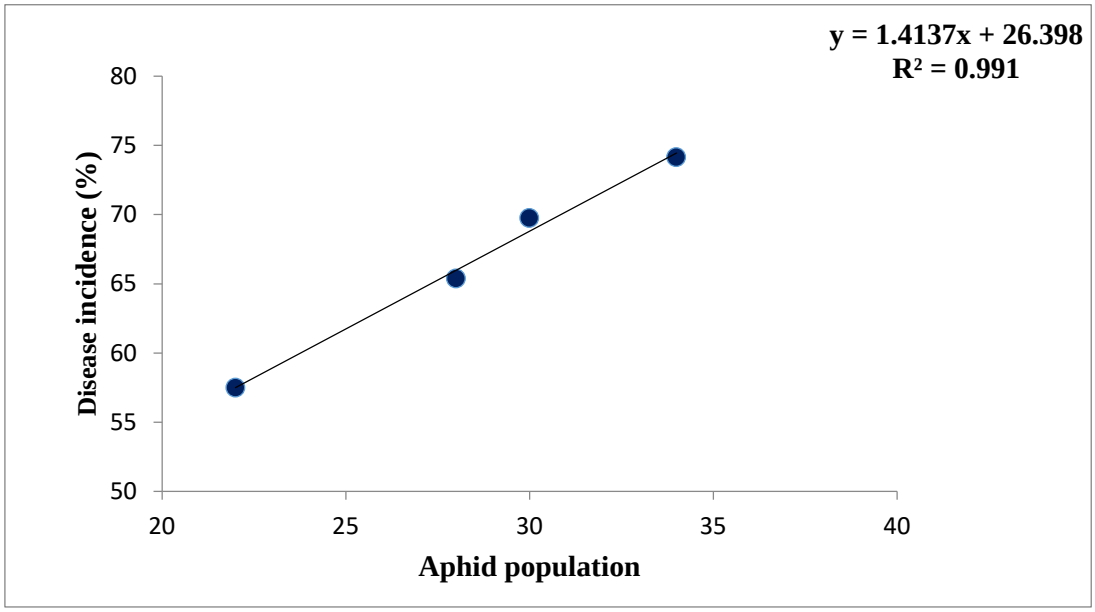


Figure 6. Relationship between viral disease incidence (%) at 110 DAT and aphid population in March at late sowing

4.14. Relationship between viral disease severity (%) at 110 DAT and aphid population in March at late sowing

Relationship between viral disease severity (%) at 110 DAT and aphid population in March at early sowing the field are shown in Figure 7. A strong positive correlation exists between the disease severity (%) and aphid population. It means that with the increase of aphid population, disease severity (%) can be increased. The coefficient of multiple determination (R^2 = 0.8276) indicated that 82.76% disease severity in pumpkin would be affected by aphid population.

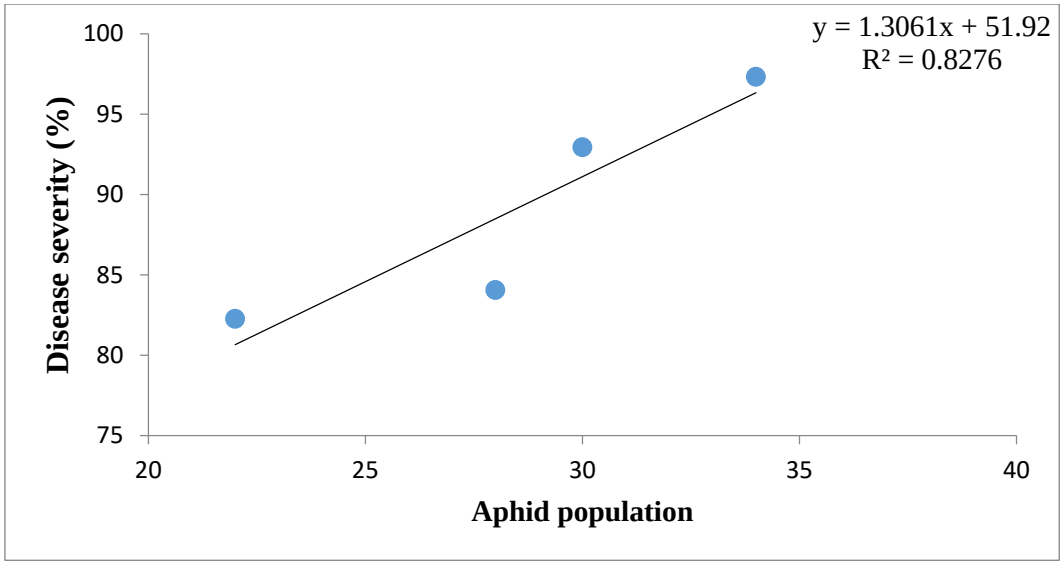


Figure 7. Relationship between viral disease severity (%) at 110 DAT and aphid population in March at late sowing

4.15. Effect of viral diseases on growth parameters at late sowing of pumpkin

Different growth parameters such as leaf area, vine length, number of branch, number of male flower and number of female flower were recorded at 50, 65, 80, 95 and 110 days after transplanting. Different growth parameters of different treatments are presented in Table 9. The data of the parameters are follows-

4.15.1. Leaf area (cm^2)

There were significant differences found between the treatments. The highest (92.80) leaf area (cm^2) was found in S_2 (Narshingdi) and the lowest (67.03) was found in S_4 (Gazipur).

4.15.2. Vine length (cm)

There were significant differences found between the treatments. Maximum (199.6) vine length (cm) was found in S_3 (Dhaka) and minimum (164.3) was found in S_1 (Narayanganj).

4.15.3. Number of branch

Significant variations were found between the treatments. The highest (8) number of branch was found in S_3 (Dhaka) and the lowest (6.67) was found in S_2 (Narshingdi).

4.15.4. Number of male flower

Significant differences were found between the treatments. Maximum (46.33) male flower was found in S_1 (Narayanganj) and minimum (32.67) was found in S_3 (Dhaka).

4.15.5. Number of female flower

There were significant differences found between the treatments. The highest (32) female flower was found in S_1 (Narayanganj) and the lowest (16.33) was in S_4 (Gazipur).

Table 9. Effect of late sowing time on pumpkin at different growth parameters

Treatment	Leaf area (cm2)	Vine length (cm)	No. of branch	No. of male flower	No. of female flower
S_1	80.92 b*	199.6 a	7.33 a	46.33 a	32.00 a
S_2	81.89 b	184.8 b	6.67 b	34.33 a	26.33 b
S_3	92.80 a	164.3 c	8.00 a	32.67 a	16.67 c
S_4	67.03 c	188.9 b	7.00 a	39.67 a	16.33 c
LSD value	7.14	7.68	2.42	13.91	3.14
CV (%)	4.43%	2.09%	16.74%	18.20%	6.89%

S_1 = Narayanganj, **S_2** = Narshingdi, **S_3** = Dhaka and **S_4** = Gazipur;
*Significant at 5% level of probability

4.16. Effect of sowing date (late) on pumpkin at different yield and yield contributing parameters

Different yield and yield contributing parameters such as number of fruit/plant, fruit weight (kg), yield (Kg), fruit diameter (cm), flesh thickness (cm) and placenta thickness (cm) were recorded at 50, 65, 80, 95 and 110 days after transplanting. Yield parameters of different treatments at late sowing are presented in Table 10.The data of the parameters are follows-

4.16.1. Number of fruit/plant

There were significant differences found between the treatments. The highest (6) number of fruits was found in S_3 (Dhaka) and the lowest (3.67) in S_2 (Narshingdi).

4.16.2. Fruit weight (kg)

Significant differences were found between the treatments in case of fruit weight (Kg). Maximum (1.79) fruit weight (kg) was found in S_3 (Dhaka) and minimum (1.37) was found in S_1 (Narayanganj).

4.16.3. Yield (kg)

There were significant differences found between the treatments. The highest (10.67) yield was found in S_3 (Dhaka) and the lowest (5.26) was in S_4 (Gazipur).

4.16.4. Fruit diameter (cm)

There were significant differences found between the treatments. Maximum fruit diameter (cm) was found in S_3 (13.80) and minimum was in S_1 (12.80).

4.16.5. Flesh thickness (cm)

There were significant differences found between the treatments. The highest (3.73) flesh thickness (cm) was found in S_3 (Dhaka) and the lowest (3.03) was in S_4 (Gazipur).

4.16.6. Placental thickness (cm)

No significant differences were found between the treatments. Maximum (10.07) fruit diameter (cm) was found in S_3 (Dhaka) and minimum (9.67) in S_1 (Narayanganj).

Table 10. Effect of late sowing date on pumpkin at different yield and yield parameters

Treatment	No. of fruit	Fruit weight (kg)	Yield (kg)	Fruit diameter (cm)	Flesh thickness (cm)	Placental thickness (cm)
S_1	4.33 b*	1.37 ab	6.08 bc	12.80 b	3.13 b	9.67 a
S_2	3.67 b	1.69 a	6.12 b	13.53 ab	3.50 ab	10.03 a
S_3	6.00 a	1.79 ab	10.67 a	13.80 ab	3.73 a	10.07 a
S_4	4.67 ab	1.32 b	5.26 c	12.87 b	3.03 b	9.83 a
LSD value	1.37	0.44	0.85	0.78	0.50	0.54
CV (%)	14.73%	14.19%	6.07%	2.95%	7.56%	2.74%

S_1 = Narayanganj, **S_2 =** Narshingdi, **S_3 =** Dhaka and **S_4 =** Gazipur;
*Significant at 5% level of probability

4.17. Detection of pumpkin infecting viruses by visual observation

Various types of symptoms developed on pumpkin due to infection with different viruses are shown in Table 11. Virus symptoms showed only on young leaves of the plants. The observed symptoms were classified into 4 symptom categories. They were fern leaf, mosaic, chlorosis and yellowing, leaf distortion. 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, ZYMV,* and *PRSV-W*. Photographs of virus infected leaves and healthy leaves showing typical symptoms were taken and are presented in Plate 2 (A-E).

Table 11. Categories of symptoms identified from infected pumpkin in field condition

Symptoms category	Description of the symptoms
1	Fern leaf
2	Mosaic
3	Chlorosis and yellowing
4	Leaf distortion

4.17.1. Fern leaf symptoms

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 (Plate-2 A). The symptoms so far noted on pumpkin and named as fern leaf were identical with the symptoms produced by *Papaya ringspot virus* both watermelon strain or papaya strain (PRSV-W/P) in papaya and cucurbits as reported by Purcifull *et al.* (1984). Considering the symptoms of PRSV as per literature and the symptomatological observation noted on pumpkin genotypes in the experiment suggested that the causal virus might be PRSV.

4.17.2. Mosaic symptoms

In initial stage, mosaic symptoms were observed in growing leaves. Further development of the symptoms was characterized by chlorosis or yellowing started from leaf tip and edge of the leaves resulting yellowing/chlorosis of the most of the area of the infected leaves. From the observed symptoms and literature (Lovisolo, 1980; Purcifull, 1984), it may be *Cucumber mosaic virus* (CMV) (Plate 2 B). Serological test confirmed the identification of the virus.

4.17.3. Chlorosis and yellowing symptoms

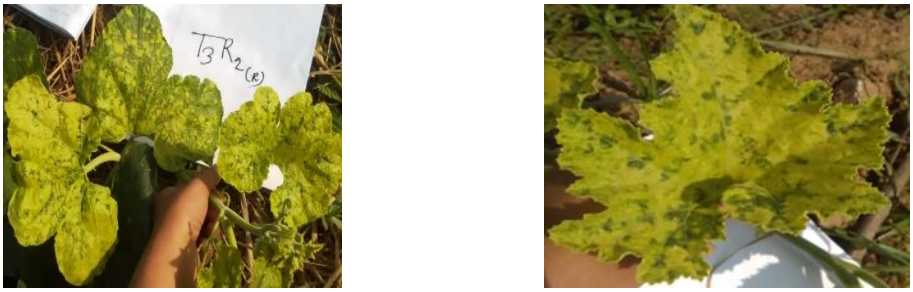
The first symptoms were yellow green spots with mottling. There were alternative yellow green patches on leaves, which enlarged rapidly and covered the entire leaf. With the aged of the plant, the infected leaves developed chlorosis, yellow patches and distortion (Plate-2 C). The plants were stunted; mosaic and stunting (Begum *et al*., 2016; Brunt, 1996) may be for *Cucumber mosaic virus* (CMV). Serological test confirmed the identification of the virus.

4.17.4. Leaf distortion symptoms

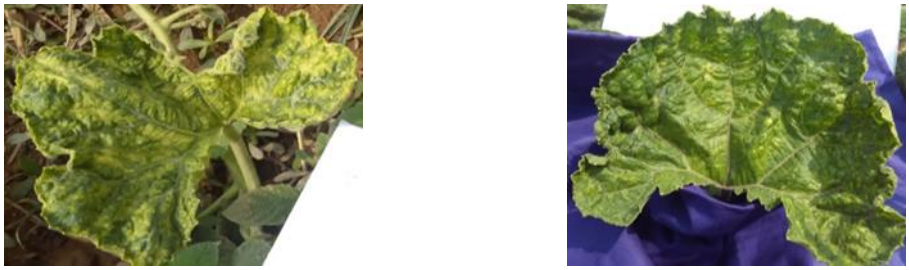
Pumpkin leaf showed mosaic symptom at early stage of infection. But at later stage of infection leaves showed yellow mosaic and leaf distortion. Especially fern leaf and shoestring type leaf distortion was appeared at later stage of infection when pumpkin plant was infected by *Zucchini yellow mosaic virus* (ZYMV) (Begum *et al*., 2016; Bilgrami and Dub, 1996; Choi *et al*., 1990) (Plate-2 D).

A. Fern leaf symptoms

B. Mosaic symptoms



C. Chlorosis and yellowing



D. Leaf distortion

E. Healthy leaves

Plate 2. Various types of symptoms appeared on pumpkin plants (A-E)

4.18. Identification of pumpkin virus through Serological test

Serological test of healthy and diseased leaves of 4 sources with four different categories of symptoms were performed using only CMV antiserum. Among all treatments at both sowing dates, two categories of symptoms (mosaic, chlorosis and yellowing) showed positive to serological test with CMV antiserum. Others symptoms like fern leaf and leaf distortion which showed symptoms in field condition but negative reaction against CMV antiserum. Based on results of DAS-ELISA in the present study indicate that pumpkin plants at both sowing timees were infected with CMV.

Table 12. Response of pumpkin sources against CMV by DAS-ELISA

Symptoms	*CMV*
Fern leaf	-
Mosaic	+
Chlorosis and yellowing	+
Leaf distortion	-

Chapter 5

Discussion

CHAPTER 5

DISCUSSION

Pumpkin (*Cucurbita moschata*) is a common vegetable crop in Bangladesh. It is grown round the year in Bangladesh and has the longest storability among all the vegetables. The well matured fruits (ripe fruits) can be stored 2 to 4 months under ambient conditions (Yawalkar, 1991).The fleshy large fruits of pumpkin can be consumed at mature and immature stages as vegetable. The delicate shoots and leaves are used as delicious and nutritious vegetable also. Seeds are very nutritious (contains 40-50% oil and 30% protein) and eaten in many countries of the world (Tindall, 1987). About 14% vegetables come from pumpkin in respect of total vegetable requirement. It may certainly contribute to improve nutritional status of the people, particularly the vulnerable groups in respect of vitamin A requirement. Low yield and crop loss in pumpkin is caused by several pathogens such as fungi, bacteria, viruses, nematodes and mycoplasmas etc. 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 (Provvidenti, 1996). It could also be due to the use of uncertified seeds by the farmers, the misuse of pesticides which causes the resistance of the aphid vector to the chemical and the lack of knowledge on viral diseases by the farmers (Afouda *et al*., 2013; Ayo- John *et al*., 2014). Among various pumpkin diseases, virus diseases (*CMV, PRSV, ZYMV, WMV2* and other potyviruses) are significant ones (Lisa and Lecoq, 1984). Among these viruses *Cucumber mosaic virus* (*CMV*) is an important and widespread virus (Chen, 2003*).*

5.1. Percent Disease incidence and severity

There were significant variations found among the sources at two sowing time. At early sowing, disease incidence ranges from 63.33-92.41%. The highest incidence (92.41%) was found in S_2 (Narshinghdi) and the lowest incidence (63.33%) was found in S_1 (Narayangonj) at 110 DAT. At late sowing, disease incidence ranges from 57.49-74.14 %. The highest incidence (74.14%) was

found in S_2 (Narshinghdi) and the lowest incidence (57.49%) was found in S_1 (Narayangonj) at 110 DAT.

In case of disease severity, significant variations were found among the treatments at two sowing times. At early sowing, disease severity ranged from 63.33-92.41%. Maximum severity (97.32%) was found in S_2 (Narshinghdi) and minimum severity (81.61%) was found in S_1 (Narayangonj) at 110 DAT. At late sowing, disease severity ranges from 82.26-97.32 %. The highest severity was found in S_2 (97.32%) and the lowest severity was found in S_1 (82.26%) at 110 DAT. Periodic increment of viral disease incidence and severity was found at two sowing times**.** Similar findings were also reported by Begum *et al*. (2016), Mitchell *et al.* (2005), Harvell *et al*. (2002). They worked on different pumpkin virus incidence and severity at field condition.

5.2. Aphid population

Significant variations were found among average number of aphid population and monthly temperature during experimental period. The aphid population increased gradually from mid-January to February and gradually decreased in March. In both experiment, the highest aphid populations (58 and 56 at early and late sowing, respectively) was found in S_2 (Narsingdi) and it was found month of February (23°c). The lowest aphid populations (43 and 30 at early and late sowing, respectively) were found from mid-November to mid-January and after March. Similar research was done by Mitchell *et al.* (2005), Harvell *et al*. (2002), Khan and Bari (1981). Kone *et al.* (2017) conducted similar study on aphid-borne viruses in cucurbits and found that sowing time has an important influence on aphid population growth and pumpkin diseases development.

5.3. Growth parameters

Different growth parameters such as leaf area, vine length, number of branch, number of male flower and number of female flower were recorded. There were significant variations found among all the treatments at two sowing time.

At early sowing, among all growth parameters significant variations are found in leaf area, vine length and number of branch. The highest leaf area (117.70 cm^2) in S_3 (Dhaka) and the lowest leaf area (96.04 cm^2) was found in S_4 (Gazipur). Maximum vine length (198.60 cm) was found in S_3 (Dhaka) and minimum (126.90 cm) was found in S_1 (Narayanganj). The highest number of branch (9.33) was found in S_3 (Dhaka) and lowest (7.00) was found in S_1 (Narayanganj). Similar work was also done by Begum *et al*. (2016 and 2015). They reported that virus infection decreased different growth parameters which were similar to this finding.

5.4. Yield parameters

Different yield and yield contributing parameters such as number of fruit, fruit weight (Kg), yield (Kg), fruit diameter (cm), flesh thickness (cm) and placental thickness (cm) were recorded. There were significant variations found among all the treatments at two sowing times.

At early sowing, among all yield parameters significant variations are found in number of fruit, fruit weight (Kg), yield (Kg), fruit diameter (cm), flesh thickness (cm). The highest fruit weight (1.87 kg) in S_3 (Dhaka) and the lowest fruit weight (1.40 Kg) was found in S_4 (Gazipur). Maximum yield (13.07 kg) was found in S_3 (Dhaka) and minimum (4.45 kg) was found in S_1 (Narayanganj). The highest fruit diameter (14.03 cm) was found in S_3 (Dhaka) and lowest (12.99) was found in S_1 (Narayanganj). Maximum flesh thickness (4.00 cm) was found in S_3 (Dhaka) and minimum (3.27 cm) was found in S_1 (Narayanganj).

At early sowing, among all yield parameters significant variations are found in number of fruit, fruit weight (Kg), yield (Kg), fruit diameter (cm), flesh thickness (cm). The highest fruit weight (1.79 kg) in S_3 (Dhaka) and the lowest fruit weight (1.32 Kg) was found in S_4 (Gazipur). Maximum yield (10.67 kg) was found in S_3 (Dhaka) and minimum (5.26 kg) was found in S_1 (Narayanganj). The highest fruit diameter (13.80 cm) was found in S_3 (Dhaka)

and lowest (12.80) was found in S_1 (Narayanganj). Maximum flesh thickness (3.73 cm) was found in S_3 (Dhaka) and minimum (3.13 cm) was found in S_1 (Narayanganj).

Similar work 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. Both in experiment the highest growth parameters were recorded in S_3 (Dhaka).

5.5. Relationship between viral disease incidence (%) and severity (%) with aphid population

Significant relationship was found in (%) disease incidence and severity with aphid population at two sowing dates. At early sowing, a strong positive correlation exits between disease incidence and severity with aphid population. The contribution of the regression (R^2 = 0.5213 and 0.3585) indicated that 52.13 % and 35.85 % disease incidence and disease severity respectively in pumpkin would be affected by aphid population.

At late sowing, a strong positive correlation exits between disease incidence and severity with aphid population. The coefficient of multiple determination (R^2 = 0.991 and 0.8276) indicated that 99.10 % and 82.76 % disease incidence and disease severity respectively in pumpkin would be affected by aphid population. Similar research was done by Fuchs, 2014; Mitchell *et al.* (2005), Singh *et al*., 2003; Harvell *et al*. (2002), Burnt *et al*., 1996 and 1997; Khan and Bari (1981).

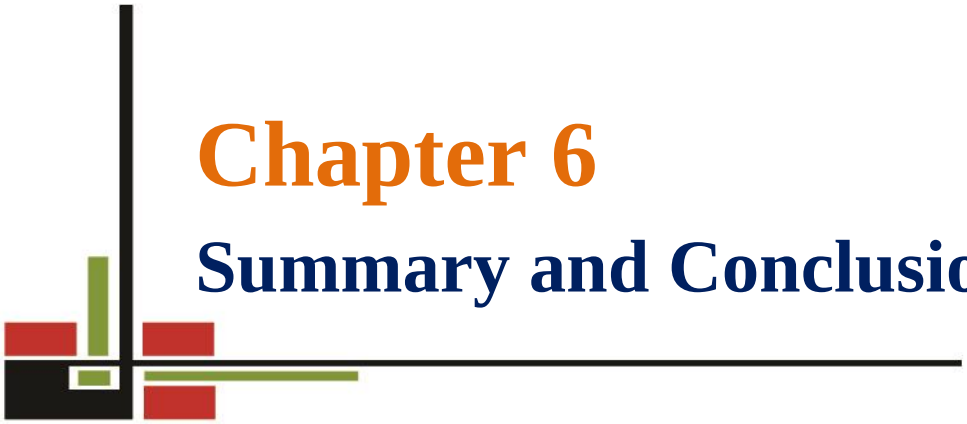
5.5. Virus identification by visual observation

Four different categories of symptoms were found in the experimental field such as fern leaf, mosaic, chlorosis and yellowing, leaf distortion. The symptoms described under the present investigation were confirmed with the symptoms of *CMV ZYMV,* and *PRSV-W* as mentioned by different researchers on various cucurbits. Considering the symptoms of all categories as per literature and the symptomological observation noted on pumpkin genotypes in

the experiment suggest that the causal virus might be *CMV, PRSV* and *ZYMV*. Similar study was also done by Begun *et al.* (2016), Zitter (2009), Jossey and Babadost, 2008; Lecoq (2001); Akanda (1991), Percifull *et al*. (1984) and Lovisolo (1980) which were similar to these symptoms.

5.6. Serological test

In Serological test, only one type antiserum *CMV* was used to identify pumpkin virus. By observing the color of DAS-ELISA kit, it was concluded that mosaic, chlorosis and yellowing symptoms produced by *CMV*. Similar result was found by Begum *et al*. (2016), Yilmaz and Sherwood (2000).



Chapter 6

Summary and Conclusions

CHAPTER 6

SUMMARY AND CONCLUSIONS

Pumpkin is a common virus in Bangladesh. It is highly nutritious vegetable crop. Viral diseases are a major constraint to cucurbit production worldwide. Control of virus may be difficult due to unavailability of virus resistant or tolerant 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. Therefore present study was carried out to evaluate the effect of sowing times on pumpkin infecting viral disease incidence (%) and severity (%). 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 November'2016 to March'2017. The experiment was laid out in Randomized Complete Block design with three replications at two sowing dates. The experiment consists of 4 treatments which are collected as 4 pumpkin sources and split into two experiment. These genotypes were sown in two sowing date that were 25th October'2016 and 5th November' 2016, respectively. There were twelve plots for four treatments and three replications for each sowing date. Data were analyzed using MSTATC and the mean differences among the treatments were compared by Least Significant Difference (LSD) at 5% level of significance.

Significant variations were recorded for all (disease incidence (%), disease severity (%), aphid population, growth parameters, yield and yield contributing parameters).

Disease incidence and disease severity were recorded at 50, 65, 80, 95 and 120 days after transplanting. Significant variations were found between the treatments at different days after transplanting (DAT).

In early sowing, at 110 days after transplanting, the highest disease incidence (%) and severity was found in treatment S_2 (Narshingdi), which were 92.41and

97.33 respectively. On the other hand, the lowest disease incidence (%) and severity was found in treatment S_1 (Narayanganj), which were 63.33 and 81.61 %), respectively.

In late sowing, at 110 days after transplanting, the highest disease incidence (%) and severity was found in S_2 (Narshingdi), which were 74.14 and 97.32%, respectively. On the other hand, the lowest disease incidence (%) and severity was found in S_1 (Narayanganj), which were 57.49 and 82.26%, respectively.

Aphid population at early sowing, the highest aphid population (56.00) was found in S_2 (Narshingdi) in February and the lowest aphid population (30.00) was in S_4 (Gazipur) in same month. On the other hand, at late sowing, the highest aphid population (58.00) was found in S_2 (Narshingdi) and the lowest aphid population (43.00) was found in S_4 (Gazipur) in same month.

Growth parameters (vine length, leaf area and number of branch) varied significantly due to virus infection at both sowing time. The highest growth parameter was found in S_3 (Dhaka) in leaf area, vine length and number of branches which were 117.7cm^2, 198.6 cm and 9.33, respectively at early sowing time. The highest growth parameter was found in S_3 (Dhaka) in leaf area and number of branches which were 92.80 cm^2 and 8.00, respectively whereas 199.6 cm vine length in S_1 (Narayanganj) at late sowing time. There were no significant variations found in male and female flower at both sowing times.

Yield and yield contributing characters (fruit weight, yield, fruit diameter, flesh thickness) varied significantly due to virus infection. S_3 (Dhaka) gave the highest yield parameters viz. fruit weight (kg), yield (kg), fruit diameter (cm) and flesh thickness which were 1.87, 13.07, 14.03 and 4.00, respectively at early sowing time. Whereas, S_3 (Dhaka) gave the highest yield parameters viz. fruit weight (kg), yield (kg), fruit diameter (cm) and flesh thickness which were 1.79, 10.67, 13.80 and 3.73, respectively at late sowing time There were no significant differences found in fruit number and placental thickness (cm) at both sowing times.

Significant relation was found in disease incidence and severity with aphid population at two sowing times. A strong positive correlation exits between disease incidence and severity with aphid population at both sowing times.

Four different categories of symptoms were found in the experimental field such as fern leaf, mosaic, chlorosis and yellowing, leaf distortion. The symptoms described under the present investigation were confirmed with the symptoms of *CMV ZYMV,* and *PRSV-W* as mentioned by different researchers on various cucurbits. Considering the symptoms of all categories as per literature and the symptomological observation noted on pumpkin genotypes in the experiment suggest that the causal virus might be *CMV, PRSV* and *ZYMV*.

In Serological test, only one type antiserum *CMV* was used to identify pumpkin viruses. By observing color of DAS-ELISA kit, it was concluded that mosaic, chlorosis and yellowing symptoms produced by *CMV*.

CONCLUSIONS

Based on the results of the present study the following conclusions may be concluded

1. At early sowing, the highest disease incidence and severity were found (92.41% and 97.33%, respectively) in S_2 (Narshingdi) and the lowest disease incidence and severity were found (63.33% and 81.61%, respectively) in S_1 (Narayanganj).

Similarly in late sowing, the highest disease incidence and severity were found (94.14% and 97.32%, respectively) in S_2 (Narshingdi) and the lowest disease incidence and severity were found (57.49% and 82.26%, respectively) in S_1 (Narayanganj).

2. In both sowing time, the highest aphid populations were found in month of February and the lowest in March.

3. There was a positive correlation exists between (%) disease incidence and severity with aphid populations both in early and late sowing. It means disease incidence and severity increased with the increase of aphid population.

4. Four different categories of symptoms were found in the experimental field such as fern leaf, mosaic, chlorosis and yellowing, and leaf distortion by visual

observation. These viruses were identified as *CMV*, *ZYMV*, and *PRSV-W* by symptomology.

5. In Serological test, among four types of symptoms mosaic, chlorosis and yellowing confirmed as *CMV* by using *CMV* antiserum through DAS-ELISA.

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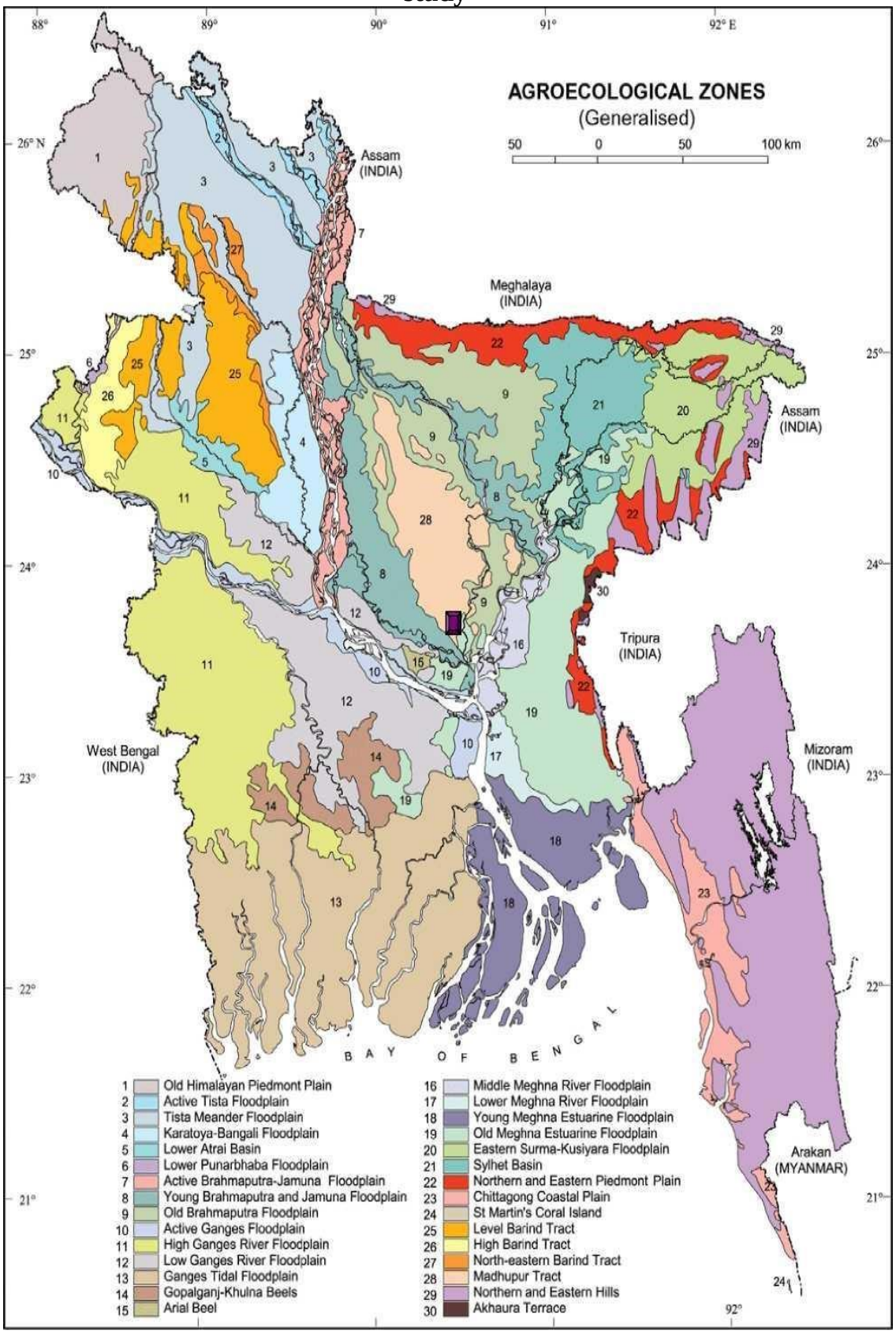
Appendices

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 (September, 2016 to May, 2017)

Name of months	Temperature (^{0}C)			Relative humidity (%)
	Maximum	Minimum	Mean	
October, 2016	36	24	30	75
November, 2016	34	19	26.5	71
December, 2016	30	16	23	68
January, 2017	29	14	21.5	71
February, 2017	32	15	23.5	76
March, 2017	32	17	24.5	83

Source: Timeanddate.com/weather/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	0.5g
Calcium	20g
Iron	0.8g
Beta-carotene	210µg
Thiamine	0.05mg
Riboflavin	0.05mg
Niacin	15mg
Water	90ml

Source: Tindall, 1987

Appendix V: Different steps in pumpkin production in field

A. Seedling raising in polybag

B. Plot view of field

C. Growth stage of plant

D. Male flower

E. Female flower

F. Aphid population

G. Sex pheromone in field for controlling fruit fly

H. Mature fruit