EFFECTS OF PLANT EXTRACTS AND CULTURAL PRACTICES ON *CUCUMBER MOSAIC VIRUS* (CMV) DISEASE MANAGEMENT IN SQUASH (*Cucurbita pepo* L.)

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This is to certify that the thesis entitled ***"EFFECTS OF PLANT EXTRACTS AND CULTURAL PRACTICES ON CUCUMBER MOSAIC VIRUS (CMV) DISEASE MANAGEMENT IN SQUASH (Cucurbita pepo L.)''*** *submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE in PLANT PATHOLOGY****, embodies the result of a piece of bona-fide research work carried out by* ***Tithi Sarker, Registration No.: 15-06685*** *under my supervision and guidance. No part of the 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.

Professor Dr. Fatema Begum
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Dated:

Dhaka, Bangladesh

Dedicated to My Beloved

Parents

and Supervisor

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ABSTRACT

An experiment was set up at the field of Sher-e-Bangla Agricultural University from December, 2020 to March 2021. The present study was aimed to evaluate effectiveness of several cultural practices viz. inter cropping by coriander, border cropping by wheat, black polythene mulch, reflective aluminum mulch and plant extracts viz. neem leaf, papaya leaf, mahogany bark for the management of viral disease of BARI squash 1. The virus in squash was detected through biological method and DAS-ELISA test. Most of the samples reacted positively against the antiserum of *Cucumber mosaic virus*. In response to different selected treatments against the viral disease, growth and growth contributing characters, yield and yield attributes of squash were recorded. Significant variations were found among different treatments against virus disease management in squash. Based on the last observation, it was found that viral infection varied from 22.22 % to 83.33% and 11.10% to 83.30% in terms of disease incidence and disease severity, respectively. Maximum prevalence (disease incidence 83.33% and disease severity 83.30%) was found in T_0 (Control) and minimum prevalence (disease incidence 22.22% and disease severity 11.10%) was observed in T_4 (Reflecting mulch) followed by T_5 (Neem leaf extract) (disease incidence 44.44% and disease severity 24.96%). The effect of different treatments on yield and yield contributing characters also showed significant variations among them, the highest yield (ton/ha) was found in T_4 (Reflecting mulch) followed by T_5 (Neem leaf extract) and the lowest yield (ton/ha) was found in T_0 (control). It was also found that the percent of disease incidence and severity were positively correlated with the number of insect vector. On the contrary, the yield (ton/ha) was negatively correlated with the value of percent disease incidence and severity and insect population in the field of squash. From the findings of the present study, it may be concluded that reflecting mulch (aluminum foil) and neem leaf extracts can be used as ecofriendly approaches for the management of viral disease of squash. However, further investigations are needed to justify the present findings to get more reliable result.

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ABBREVIATIONS AND ACRONYMS

AERI = Agribusiness Education & Research International

AEZ = Agro ecological zone

BARI = Bangladesh Agricultural Research Institute

CABY virus = *Cucurbit Aphid-Borne Yellow virus*

cm = Centimeter

CV % = Percent Coefficient of Variation

^{0}C = Degree Celsius

et. al.= And others

FAOSTAT = Food and Agriculture Organization Statistics

g = Gram

ha = Hectare

hrs. = Hours

i.e. = id est (L), that is

Kg = Kilogram

mg = Gram

min = Minute

ml = Milliliter

nm = Nano meter

No. = Number

% = Percentage

SAU = Sher-e-Bangla Agricultural University

SLCV = *Squash Leaf Curl Virus*

µl = Microliter

ABBREVIATIONS AND ACRONYMS (Cont'd)

µg = Microgram

viz. = Videre licet (L), it is permitted to see

w/v = Weight/Volume

B = Boron

Ca = Calcium

Fe = Iron

K = Potassium

K_2O = Potassium oxide

P = Phosphorus

P_2O_5 = Phosphorus pentoxide

Mg = Magnesium

N = Nitrogen

Na = Sodium

S = Sulphur

Zn = Zinc

CHAPTER I

INTRODUCTION

Squash (*Cucurbita pepo* L.) is a kind of vegetable in gourd family which are widely cultivated as vegetables and for livestock feed. Squash belongs to the family of Cucurbitaceae and genus of *Cucurbita* (Schaffer, 2003). Gourds are in the same family as squashes. There are two main types of squash: winter squash and summer squash. Squashes are native to the New World, where they were cultivated by indigenous peoples before European settlement. Its origins are central Mexico, Peru, and the United States. It is found from the southern temperate zone of North America to the northern subtropical zone of South America. The squash has been an important crop in the Andes mountains since the pre Columbian Era (Kathiravan, 2006).

Squashes, such as zucchini, are quick-growing, small-fruited, no trailing or bush varieties of *Cucurbita pepo*. Plants are upright and spreading, produce a great diversity of fruit forms, from flatten through oblong to elongate and crooked, colored from white through cream to yellow and green. The fruits develop very rapidly and must be harvested a few days after they form (before the seeds and rinds harden). Although squash is a fruit according to its botanical classification, it is generally considered as a vegetable in food preparation (Berrin *et al*., 1997). Squash are good sources of vitamin A, C, and B vitamins; they're high in antioxidants; and they're rich in minerals such as potassium, magnesium, and manganese. And, of course, as whole plant foods, they're also rich in fiber and water, making them both hydrating and good for the gut. Summer squash is rich in the carotenoid's beta carotene and lutein (Tamer *et al*., 2010). Carotenoids are integral to eye health, and not only improve night vision, but also decrease the risk of macular degeneration and cataracts (Mohammad *et al*., 2011). In addition to a wide variety of other nutrients, summer squash contents fiber, which improves colon health and decreases the risk of certain cancers.

Worldwide, summer squash ranks high economically among other vegetables produced. The squash cultivation method is popular nowadays. According to FAO Statistical Corporate Database (FAOSTAT, 2014), the world's largest production of squash reached 27.9 million tones with harvested area two million hectares based on the continent region.

Asia region still became the biggest producer of squash and gourds in 2020 with production about 16.7 million tones. Europe in the second place with production around 4.8 million tones. America region in the third place with production around 3.3 million tones and Africa in the fourth place with production around 2.7 million tones. Squash is an exotic vegetable, in nature. Squash cultivation is now practiced commercially (AERI, 2020). It is also suitable in Bangladesh. Local market demand for this squash is strong. This vegetable has a wide range of nutritional value and good prices and so many people are inclined to grow this vegetable now. The total vegetable production of Bangladesh is 3365 metric tons (Bangladesh Bureau of Statictics, 2014), but new introduction of squash production is low compared with other vegetables due to many causes. Among them viral attack is the most destructive one which causes serious crop failure. According to the different estimates, 3-5% of overall vegetable production is lost due to virus infections, but losses can be occasionally very high, where pest control is insufficient, especially in developing countries (Caciagli, 2010). Virus disease is a worldwide problem for cucurbit production and cause serious economic losses.

Indeed, more than 35 different viruses have been isolated from cucurbits (Provvidenti, 1996). These viruses constitute complex and dynamically changing problems as described by (Namath *et al*., 1986). These include *Cucumber mosaic virus* (CMV) (Kurcman, 1977), *Watermelon mosaic Potyvirus-2* (WMV-2) (Nogay & Yorgancı, 1984), *Zucchini yellow mosaic Potyvirus* (ZYMV) (Davis. & Yılmaz, 1984), *Papaya ringspot Potyvirus-watermelon strain* (PRSV-W) (Erdiller. & Ertunç. 1988), *Cucumber vein yellowing Ipomovirus* (CVYV) (Yılmaz *et al*., 1991), *Cucurbit aphidborne yellows Polerovirus* (Yılmaz *et al.,* 1992), *Melon mosaic virus* (MMV) (Yılmaz *et al*., 1995). These viruses are transmitted by insect vectors including whitefly, aphid etc. (Lecoq *et* *al*., 1992). Plants virus show symptoms such as mosaic, mottling, vein clearing, blistering distortion, shoestring, stunting or yellowing and fruit discoloration and deformation when attacked by virus (Panno *et al*., 2016).

Cucumber mosaic virus (CMV) is efficiently can transmit in a nonpersistent manner by more than 75 species of aphids (Palukaitis *et al.*, 1992). Aphids, including the generalist herbivore *Myzus persicae*, transmit *cucumber mosaic virus* (CMV). The infection affects *M. persicae* feeding behavior and performance on cucurbits in varying ways. CMV decreases host quality and inhibits prolonged feeding by aphids in cucurbits, which may enhance virus transmission rates. In contrast, aphids on CMV-infected plants exhibit increased survival and reproduction. Plant viruses are dependent on vectors for their horizontal transmission, and aphids are the most common and important group of plant virus vectors causing huge yield loss. Aphids transmit at least 275 plant viruses, and approximately 75% of these viruses are transmitted in a nonpersistent manner, or stylet borne in cucurbits (Powell, 2005). A successful aphid transmission event is dependent upon the uptake of virus, stable retention of the acquired virions, the release of retained virions from regions within the mouth parts of the vector, and their delivery to a site of infection in the plant (Perry, 2004). The CMV is efficiently transmitted by both *Aphis gossypii* and *Myzus persicae* (Perry, 1998; Chen and Franki, 1990) in cucurbits.

Now it's a big challenge for the farmers to grow squash without disease infestation. The frequent development of the disease and high yield loss lead to a total crop failure and have drawn attention to the scientists to develop effective management program against the disease for profitable production of squash. Various strategies have been suggested to minimize the disease infestation to have satisfied yield. But it is very difficult to control viral diseases. There are very limited options to manage the viral disease. Insecticides applying against the aphid vectors are not so effective in reducing viral disease because aphid transmits virus before insecticides application act to kill them.

Pest management is being confronted with immense economic and environmental issues worldwide because of depending on massive utilization and over-reliance on pesticides. The antiviral activity of different plant extracts like mahogany (*Swietenia macrophylla*), neem (*Azadirachta indica*), papaya (*Carica papaya*) against aphid-borne mosaic virus is reported (Rajseskaran, 2013). Many plant-based pesticides have been discovered now. Neem leaf extract is very effective for controlling mosaic virus (Sadasivam *et al*., 1991). Neem-leaf powder treated plants are less vulnerable to diseases occasioned by viruses. It was observed that application of plant leaf extract conferred the highest tolerance to virus diseases (Aliyu *et al*., 2010; 2011.). Papaya leaf extracts are the most potent in suppressing the abundance of aphids and the damages caused to the leaves in squash. This might be due to the different insecticidal constituents contained in these plant materials (Murovhi *et al*., 2020.). Aphid populations migrating into summer squash plantings were reduced by the use of aluminum and plastic mulches (Wyman, 1979.), reflective mulches (Boyhan *et al.*, 2000; Brown *et al*., 1993). Synthetic reflecting mulch has been reported effective to control virus in various crops (Csizinszky *et al*., 1997). Using of border and intercrop with the target crop act as a trap for the insects and thus reduce the viral infection. Intercropping in close proximity with the main crop either in alternative row or ground cover between the rows of main crop has impact on nonpersistent viruses (Sammons, 2009).

Considering the environmental issue and residual activity of chemicals scientists are emphasizing on alternate way to manage the viral disease of plants like the above cultural practices using different mulching, border cropping, intercropping and plant extracts etc. The effective and safe practices are very important in this purpose to gain profitable production of squash in our country and to minimize the losses of the producer. As virus control is very difficult taking the preventive measures is a great step to control the vector and the disease. Considering the importance of the above background the present research program was conducted to know the effect of different cultural practices, plant extracts on prevalence of vectors and viral diseases of squash.

The proposed research work was conducted to achieve the following specific objectives:

- To detect the squash-infecting *Cucumber Mosaic Virus* (CMV) by biological and serological methods
- To evaluate the CMV disease intensity and their effects on growth and yield of squash; and
- To find out an effective Cultural practice and Plant extract to control the vector for the management of CMV disease in squash.

CHAPTER II

REVIEW OF LITERATURE

Squash (*Cucurbita pepo* L.) belongs to the family Cucurbitaceae and is grown in both temperate and tropical climatic zones throughout the world. In Bangladesh, this crop is relatively new but is increasingly gaining high levels of economic importance both in case of income and provision of nutritional value. Squash is cultivated in Bangladesh during the winter season. In this time rainfall is scanty and its growth and development requires optimum temperature within 18-25 ^{0}C (Albert, 2018). But it suffers much by different viral diseases like *Cucumber mosaic virus* (CMV). *Watermelon mosaic Potyvirus-2* (WMV-2), *Zucchini yellow mosaic Potyvirus* (ZYMV), *Papaya ringspot Potyvirus watermelon strain* (PRSV-W) etc. which are widely distributed throughout the world wherever squash is grown. Due to this, its yield becomes lower. The studies with respect to effect of different cultural practices and plant extracts on viral disease and yield are taken into consideration while reviewing the literature. According to the literature pertaining to the above aspects is presented here.

2.1. NUTRITIONAL VALUE OF SQUASH

Consumption of squash plays an important role in the reduction of obesity, heart disease, stroke, cancer, and other chronic diseases (Boffeta *et al.,* 2010).

It is rich in nutrients and bioactive compounds contents such as phenolics, flavonoids, vitamins (including β-carotene, vitamin A, vitamin B2, α-tocopherol, vitamin C, and vitamin E), amino acids, carbohydrates and minerals (especially potassium), and it is low in amount of energy content (about 17 Kcal/100 g of fresh squash) and has huge amount of fiber (Tamer *et al*., 2010).

Squash has various health benefits to human as well as medicinal potentials in it (Mohammad *et al*., 2011).
Squash provides a variety of nutrients essential for human development, maintenance and repair such as vitamins, minerals, dietary fiber, protein and fat (Barthel, 2011).

Dari (2016) stated that butternut squash is an emerging economic crop in Ghana with ready market and high nutritional value. The main objective of the study was to determine the proximate composition and storability of butternut squash to provide nutritional information. Recommended methods of the association of official analytical chemist were used for the assessment of fat, fiber, protein, ash, carbohydrate, moisture and vitamin C. From the results of the proximate composition, butternut squash contained moisture content (82.15g), ash content (9.9g), carbohydrate (5.51g), crude fiber (1.45g), crude protein (0.86g), crude fat (0.13g) and 15.33 mg of vitamin C which can provide the nutrient needs for normal body function, maintenance and reproduction especially for persons cutting down or reducing the consumption of fats and proteins.

Maria (2020) had worked on the potential contribution to the required daily intake by compiling the most recent works found in the literature. The beneficial health effects of summer squash are attributed to their different bioactive compounds contributing positively to the daily nutritional intake and it also protects against some diseases, including diabetes, cardiovascular diseases, accelerated aging, and some types of cancer. This chapter provides about knowledge of nutritional composition, antioxidant properties, as well as the health benefits of the eight-summer squash morphotypes (pumpkin, vegetable marrow, cocozelle, zucchini, acorn, scallop, crookneck, and straight neck).

2.2. VIRAL DISEASES IN SQUASH

2.2.1. HISTORICAL BACKGROUND; DISTRIBUTION AND ECONOMIC IMPORTANCE OF VIRAL DISEASES IN SQUASH

Freitag (1956) stated that the mosaic disease of squash is very common in the coastal regions and also occurs in the interior of California, and investigations on the properties, host range and transmission of the causal virus are here recorded.

Virus disease is a major problem for crop cultivation. Almost all crops suffer from more than one virus disease caused serious yield and quality loss of the crops (Harrison, 1970).

Tobias *et al*. (2008) found that pumpkin infecting *Cucumber mosaic virus* is one of the most common viruses occurring in vegetables. The infection in pepper and melon may reach 80-100% depending on environmental condition.

Lovisolo (1980) reviewed the virus disease of Cucurbitaceous crops and found that these crops were highly susceptible to virus disease and more than 25 viruses, including at least seven potyviruses, infects cucurbits family. The most important viruses are *Papaya ringspot* v*irus (*PRSV), *Watermelon mosaic Poty*v*irus* (WMV), *Zucchini yellow yeck Poty*v*irus* (ZYFV), *Cucumber mosaic* v*irus* (CMV), *Squash mosaic* v*irus* (SqMV).

Several virus diseases inducing mosaic symptoms were previously inscribed including *Watermelon mosaic Poty*v*irus* (WMV), *Zucchini yellow mosaic Poty*v*irus* (ZYMV), *Papaya ringspot Poty*v*irus* of watermelon strain (PRSV-W), *Zucchini yellow yeck Poty*v*irus* (ZYFV), and *Cucumber mosaic* v*irus* (CMV) (Makkouk and Lesemann,1980; Lesemann *et a*l., 1983; Katul, 1986).

Papaya ringspot virus-Type W (PRSV-W), *Watermelon mosaic virus* 2 (WMV2), *Watermelon mosaic virus* – Morocco (WMV-M), *Zucchini yellow mosaic virus* (ZYMV) and *Cucumber mosaic virus* (CMV) can severely reduce the production of squash (Lovisolo, 1980).

CMV was found widely distributed throughout Greece, infecting a large number of cultivated and wild plants stated by Kyriakopoulos (1982). Damage due to the disease was especially severe on Cucurbitaceae and mainly on squash.

During a 3-yr study by Davis (1987) found that different viruses were associated with severe disease symptoms, depending on the year. In 1983, *Cucumber mosaic virus* caused the most severe disease in squash (*Cucurbita pepo*) although *Watermelon mosaic virus 2* was the most prevalent in that region. In 1984, the watermelon mosaic which is a strain of Papaya *ringspot virus* caused a destructive disease of squash.

Fraser (1987) stated that virus infection in plants affects the cellular components like chlorophyll, beta-carotene, organic carbon, potassium and phosphorus and alters the physiology of the host, thus reduces the quality of the vegetables.

Biochemical alteration of the cellular components is directly related to the morphological deviation of the virus-infected plant and the extent of crop loss (Sreenivasulu *et al.*, 1989).

Greber *et al*. (1988); Coutts and Jones (2005); Coutts *et al*. (2011) found that Cucurbit plants infected with these viruses have deformed leaves with mosaic pattern and fruit are small, lumpy and distorted. When plants are infected early in the cultivation, there are substantial yield and quality losses as the fruit produced is unmarketable.

Delgadillo *et al*. (1989) stated that *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Tobacco ringspot virus* (TRSV), *Squash mosaic virus* (SqMV), *Papaya ringspot virus* - Type W (PRSV-W) and *Zucchini yellow mosaic virus* (ZYMV) were detected in cucurbit in Mexico

Verma *et al*. (1989) found that yield decreases with the insect population in cucurbits when they studied the viral disease.

Akanda *et al*. (1991) reported that in Bangladesh pumpkin was found to be affected by two viruses: PRSV and WMV-2 and may cause 70-100% yield reduction in cucurbits depending upon the stage of infection (Rahman *et al*., 2010).

Lima and Viera (1992) stated that PRSV-W is predominant and SqMV is the least frequent virus occurring in cucurbitaceous crops in Brazil.

Three yellow crookneck squash (*Cucurbita pepo* var. *melopepo*) and five cantaloupe (*Cucumis melo*, Reticulatus group) lines used for developing the resistancy to *Zucchini yellow mosaic potyvirus* and *Watermelon mosaic virus* [*Watermelon mosaic 2 potyvirus*], were field tested by Clough and Hamm (1995) in 1993 and 1994, respectively. During both years, plants were inoculated with virus before transplanting so that it can provide a high virus threat to the plants. Yield was assessed in 1993 and disease progression, yield in the end-of-season. In both years, plant disease development was rated per week.

More than 35 viruses have been isolated from cucurbits by Provvidenti (1996). These viruses constitute complex and dynamically changing problems in cucurbits as described by Nameth *et al*. (1986).

Luis *et al*. (1998) reported that in Spain CMV and WMV-2 were the most frequently found viruses in cucurbits. But PRSV-W and ZYMV were found fewer in sites.

Silva and Delgadillo (1990) found the highest degree of occurrence and widest distribution of CMV, WMV and PRSV-W in Mexico.

A survey was conducted by Abou-Jawdah *et al.* (1999) in 1996-1997 of the major cucurbit cultivation areas along the Lebanon. Viral diseases are the common cause of economic losses in commercial cucurbit production in Lebanon. This survey informed that *Zucchini yellow mosaic Poty*virus (ZYMV) and *Cucurbit aphid-borne yellows* v*irus* (CABYV) are the most common viruses of field-grown squash in Lebanon. Severe infections of field-grown squash are the major reason for which most of the farmers on the Lebanese coastal region no longer grow summer and fall squash.

Boyhan (2000) found that yield decreases with the viral disease infection in melons. Zucchini and yellow summer squash are very common grown vegetables in the Mid-western U.S. However, summer squash growers in Illinois often suffer very much for the economic losses due to mosaic virus diseases (Walters *et al*. 2003).

The most problematic virus in southern Illinois is *Watermelon mosaic virus.* However, other viruses including *Cucumber mosaic virus* (CMV), *Papaya ringspot virus* (PRSV), *Squash mosaic virus* (SqMV*), and Zucchini yellow mosaic virus* (ZYMV) can also be prob-lematic (Walters *et al*., 2003).

Desbiez (2003) carried out an experiment that *Zucchini yellow mosaic potyvirus* (ZYMV), first isolated in Italy in 1973, described in 1981, and then identified in all continents within a decade. Now, it is one of the most economically important viruses of cucurbit crops. A research by Desbiez (2003) stated that *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) are two major potyviruses infecting cucurbits. WMV is the causal organism of one of the first described cucurbit mosaic virus diseases, while ZYMV emerged as a major cucurbit pathogen causing severe yield losses only in the late 1970s.

Diseases of cucurbit crops throughout horticultural cropping regions of tropical and subtropical Australia, greatly reducing both yield and quality of produce, and seriously damaging industry profitability (Greber, 1969; Coutts and Jones, 2005). The virus that currently possess the most serious threats are two non-persistently aphid-borne potyviruses, *Zucchini yellow mosaic virus* (ZYMV) and *Papaya ringspot virus* (PRSV).

Delmiglio and Pearson (2006) studied the effect and incidence of *Cucumber mosaic virus, Watermelon mosaic virus* and *Zuccini yellow mosaic virus* in cucurbit. They found that the symptoms included leaf distortion, mosaic, vein chlorosis, reduced the leaf size reduced yield up to 72% in New Zealand.

Pathogenicity of the detected viruses was detected for CMV, PRSV, SqMV, WMV, and ZYMV on summer squash, pumpkin and processing pumpkin. *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic potyvirus* (ZYMV), and *Watermelon mosaic virus 2 potyvirus* (WMV 2) from squash were analyzed in the field by Marc and David (2007) for their reaction to mixed infections by these three viruses and for the production of fruit. Test plants were opened to natural inoculations via aphids in trials simulating the introduction of viruses by secondary spread from mechanically infected susceptible border row plants. This study concludes that virus-resistant lines are economically viable even if they are affected by viruses other than those to which they are resistant.

A research done by Al-Musa et al (2008) in Israel found that the highest disease incidence (95%) was recorded in Dir Alla area, whereas disease incidence was exceeding 69% in squash samples collected from North Ghor.

Al-Musa *et al.* (2008) described that *Squash leaf curl virus* (SLCV) was detected for the first time in Jordan using degenerated oligonucleotide primers. Two isolates of the virus in squash, SLCV-E and SLCV-R, were detected using specific oligonucleotide primers in symptomatic *Cucurbita pepo*. SLCV was also found to occur naturally in *Malva parviflora*, which cause severe leaf curling, yellowing and stunting of the whole plants.

Gyoutoku *et al*. (2009) found the Cucurbit chlorotic yellow virus (CCYV) primarily in melon plants in Japan.

Dennis and Savarni (2010) stated that the term papaya ringspot (PRS) was first coined by Jensen in 1949 in Hawaii. Previously described diseases such as papaya mosaic (caused by *Papaya mosaic virus*) and watermelon mosaic (caused by *Watermelon mosaic virus1*) were observed recently to be caused by Papaya ringspot virus (PRSV). The virus (PRSV) causes a major disease in cucurbits and is found in all areas of the world where cucurbits are cultivated.

Viral diseases cause important economic losses throughout the world stated by Palukaitis (1992). The author stated that *Cucumber mosaic virus* (CMV), the type member of the *cucumovirus* group, was first reported in 1916 as the causal agent of a disease of cucumber and muskmelon in Michigan and cucumber in New York. Since then, CMV has been found in most countries of the world, predominantly in the temperate zones, but increasingly more often in the tropical countries.

Tali and Moshe (2011) stated that *Squash leaf curl virus* (SLCV) and *Watermelon chlorotic stunt virus* (WmCSV) are cucurbit-infecting bipartite begomoviruses. Both viruses can be seen in the eastern Mediterranean basin. In the summer, the yield is decreased by 22%, on average. Dual-inoculated plants gave a synergistic interaction between the two viruses. They developed disease symptoms that were more predominant than WmCSV alone, with plants being shorter than control plants by 20 to 25% regardless of season. Moreover, the yield of dual-inoculated plants was decreased on average by 21% in the spring and 54% in the summer, and fruit appearance was adversely affected.

Lecoq and Desbiez (2012) stated that Cucurbit crops may be affected by at least 28 different viruses in the Mediterranean basin. Some of these viruses are widely distributed and cause severe yield reduction.

Virus diseases cause serious yield and quality losses in the field of cucurbit crops worldwide. In Australia, the main viruses that attack cucurbits are *Papaya ringspot virus* (PRSV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV). Plants infected early have severely deformed fruit. High infection incidences, of ZYMV and PRSV in crops makes losses of marketable fruit of up to 100% and infected crops are often abandoned (Coutts *et al*., 2011).

According to Selangga *et al.* (2022) the yield loss due to curling disease on individual plants was 56.3%, and the disease caused a reduction in the quality of harvest fruits. Yield loss assessment caused by the disease in fields with different levels of disease intensity (DI) ranged from 10.02 to 25.83%. It is found that the correlation between curling disease severity and yield loss was high.

2.2.2. SYMPTOMS OF VIRAL DISEASES IN SQUASH

Currently severe yellowing symptoms of older leaves of cucumber, melon and squash were found in open fields and in greenhouses (Lasermann, 1983).

Chakraborty and Sinha (1994) found a mosaic disease of pumpkin ib Uttar Pradesh, India and characterised by the presence of raised dark green blisters on the leaves, chlorotic lamina.

Zitter *et al*. (1996) found virus symptoms like mosaic, chlorotic mottle, vein clearing, leaf distortion and malformation. These viruses may cause a reduction of plant growth, flower abortion and fruit can exhibit mosaic, depressed areas and distorted development rendering them unmarketable.

A study was carried out by Gracia (2007) in August 1996, a severe viral disease occurred in squash produced in the subtropical Province of Salta, Argentina. Plants of *Cucurbita pepo* L. (zucchini) and *Cucurbita maxima* L. anywhere affected. Approximately 50% of the plants showed severe yellow mosaic, necrosis, and foliar distortion symptoms. Most of the fruits on infected plants were small, with scattered glossy yellow knobs over a green background, and some looked additional fruit malformation.

Al-Musa *et al.* (2008) reported that SLCV was also found to occur naturally in *Malva parviflora*, which produced severe leaf curling, yellowing and stunting of the whole plants.

Abraha *et al*. (2013) found that Symptoms on cucurbits include brittleness, thickening, and interveinal yellowing of leaves that usually appear on basal leaves of the plant, but slowly expand to the new foliage and they are indistinguishable from those caused by nutrition deficiencies.

Panno *et al*. (2016) found that Zucchini squash (*C. pepo*) is one of the most important horticultural crops grown in Italy. It has been a representative threat for zucchini squash cultivation. Normally, virus infected zucchini squash reveals typical symptoms including severe curling, yellow mosaic and vein swelling of young leaves, shortening of internodes, rough skin, and reduced size of fruit.

Medina-Hernández *et al.* (2019) zucchini squash plants infected with SLCuV exhibit typical symptoms which includes a severe chlorotic mosaic or mottle on foliar, curling, malformation and thickened vein-banding of the leaf, stunting, flower drops and fruit set failure.

2.2.3. IDENTIFICATION OF CUMBER MOSAIC VIRUS(CMV) BY ELISA TEST

A virus was isolated from squash *(Cucurbita pepo* L.) plants with severe malformations and discolorations by Kyriakopoulos (1982) and identified as a strain of *Cucumber mosaic virus* (CMV). Identification was carried out based on the host range and reaction, aphid transmissibility, particle size and morphology, physical properties, and serology. From highly purified preparations, a pure antiserum of high titre (1:1024) was prepared.

Mosaic virus was detected for the first time in New Jersey in 1985 by Davis (1987) and he found that it caused severe production losses in squash and other cucurbit crops. In field samples which were infected by the various mixtures of CMV, ZYMV, WMV-2 and PRSV-W, ZYMV usually predominated after inoculation of susceptible test plants and detection by ELISA. The assay of field samples was more reliable as the detection of cucurbit viruses in New Jersey.

Silva and Delgadillo (1990) reported that CMV, WMV, PRSV-W, TRSV and ZYMV viruses were identified on pumpkin in Mexico.

Before and after transplanting, serological testing (ELISA) was done by Clough and Hamm in 1995 to obtain baseline information on transformed plants and to confirm field virus infection.

Juan (2007) to identified five viruses which infects cucurbitaceous crops, *Zuccini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), *Cucumber mosaic virus* (CMV), *Papaya ringspot virus-watermelon strain* (PRSV-W) and *Squash mosaic virus* (SqMV).

Krstia *et al*. (2002) used a serological method, ELIZA to identify the virus in infected samples of pumpkins. Polyclonal antibodies raised against *Cucumber mosaic* *virus* (CMV), *Zuccini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* 1 (WMV-1), *Watermelon mosaic virus* 2 (WMV-2) and *Squash mosaic virus* (SqMV) were used.

Surveys were conducted by Jossey and Babadoost (2007) during 2004 to 2006 to detect the viruses infecting pumpkin and squash in Illinois. In 2004, 16 jack-o-lantern pumpkin (*Cucurbita pepo*) samples and one squash (*C. pepo*) sample were gathered from 11 counties. In 2005, 85 jack-o-lantern pumpkin, 12 processing pumpkin (*Cucurbita moschata*), 37 squash, and six gourd (*C. pepo*) samples were collected from 54 countries. In 2006, 85 jack-o-lantern pumpkin, 16 processing pumpkin, 51 squash, and 18 gourd samples were collected from 47 countries by them. *Cucumber mosaic virus* (CMV), *Papaya ringspot virus* (PRSV), *Squash mosaic virus* (SqMV), *Tobacco ringspot virus* (TRSV), *Tomato ringspot virus* (ToRSV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), and unknown potyviruses were detected in pumpkin, squash, and gourd fields during the surveys they conducted, using enzyme-linked immunosorbent assay (ELISA). SqMV was detected in more countries than any other virus because it was identified in 65 and 88% of the counties during survey of 2005 and 2006, respectively.

Jossy and Babadoost (2007) identified viruses in pumpkin and squash in Illinois. They found *Zucchini yellow mosaic Potyvirus* (ZYMV), *Watermelon mosaic virus* (WMV), *Cucumber mosaic virus* (CMV), *Papaya ringspot Potyvirus-watermelon strain* (PRSV-W) and *Squash mosaic virus* (SqMV) by ELIZA (Enzyme linked immunosorbent assay).

A study was performed by Yesil (2014) in Turkey like in some Mediterranean countries and Germany, Hungary, Austria and China. He observed that viral diseases are very destructive especially on squash (*Cucurbita pepo* L.). He determined the virus infections in major squash growing areas in Konya province (Turkey). 334 plant samples were collected with common virus symptoms like mosaic, curling, blistering, mottling, distortion, shoestring, stunting and vine decline from squash plants during

2013. The viruses were identified by DAS-ELISA test. It showed that 80.53 % of plant samples were infected with *Zucchini yellow mosaic Potyvirus* (ZYMV), *Watermelon mosaic Potyvirus-2* (WMV-2), *Cucumber mosaic virus* (CMV), *Papaya ringspot Potyvirus-watermelon strain* (PRSV-W) and *Squash mosaic Comovirus* (SqMV). ZYMV was predominant in the research area.WMV-2 was detected in the ratio 52.99% in squash and it was second important virus disease in that area.

In Bangladesh, *Squash leaf curl virus* was identified from whiteflies collected in a cucurbit field (Zobayer.2020). During the survey for gemini virus in August 2019, author observed mild leaf curl and mosaic symptoms on pumpkin in a commercial field in Chittagong (22°N, 91°E) in southeastern Bangladesh and collected one representative leaf sample. The presence of SLCV in pumpkin has become a new potential threat to cucurbit production and should be monitored in Bangladesh. During a survey for gemini virus in August 2019, author observed the mild leaf curl and mosaic symptoms on pumpkin in a commercial field in Chittagong (22°N, 91°E) in southeastern Bangladesh and collected one representative leaf sample.

Cucumber mosaic virus (CMV) associated with severe cucumber (*Cucumis sativus* L.) mosaic disease was identified in leaf samples collected from 13 districts of Bangladesh by (Tridib *et al*., 2022) during the period 2017–2020. Leaves and fruits possessing the typical mosaic disease symptoms were observed under field conditions. Infected leaves were first screened by DAS-ELISA test. Molecular characterization of *Cucumber mosaic virus* subgroup II isolate associated with cucumber in Bangladesh had been done by the researchers.

2.2.4. TRANSMISSION OF VIRUS

Freitag (1956) conducted tests on insect transmission, nine species of Aphids, including four that occur on squash in the field, five Cicadellids, *Anasa tristis* (Deg.) and *Lygus* sp. all failed to transmit the virus from infected to healthy squash, but it was transmitted by adults of *Acalymma trivittata* (Mannh.) and, less readily, *Diabrotica undecimpunctata* Mannh. *A. trivittata* acquired the virus in feeding periods of five minutes after starving for three days, and acquired and transmitted it in less than ten hours. When transmitted daily to healthy squash plants, infective adults of *A. trivittata* retained the virus for 17 days, and those of *D. undecimpunctata* for 20. One example of *A. trivittata* infected 9 of 28 plants in daily transfers. Fluid regurgitated by the beetles after feeding on infected plants was reported to be highly infectious, which suggests that it is the most likely source of virus in transmission.

Aphids are an important factor in the transmission of viruses both within a field and over long distances. In some cases, it has been noted that infected plants seem to be more favorable to rapid vector development than healthy plants (Swenson, 1968).

WMV2, WMV-M, ZYMV and CMV have been reported previously in South Africa (Vander Meer, 1985; Vander Meer & Garnett, 1987). These viruses are quickly transmitted in the stylet-borne manner by aphids (Shukla *et al.*, 1994). Non-colonizing aphids (i.e., aphids which probe, then reject a plant as a host) can be important in the ecology of two viruses that attacks squash: PRSV-W and WMV2 (Zitter *et al.*, 1977).

Desbiez and Lecoq (2011) stated that aphids transmit ZYMV in the field and once the virus is introduced to a cucurbit planting its spread within the field is generally very rapid.

ZYMV is seed-borne at low levels in zucchini and squash (*Cucurbita pepo*), and in Delicia-type butternut squash suggested by many authors (Davis and Mizuki, 1986; Greber *et al*., 1988; Fletcher *et al*., 2000; Riedle-Bauer *et al.*, 2002; Tobias and Palkovics, 2003; Tobias *et al*., 2008; Coutts *et al*., 2010).

Viruses that cause similar yellowing symptoms on cucurbits have been stated in several countries, and they are transmitted either by aphids (Lecoq *et al*., 1992) or by whiteflies (Wisler *et al.*, 1998).

A number of aphid species have been reported as feeding on members of the Cucurbitaceae. The following records, covering sub-Saharan Africa, was compiled by Millar (1994): *Aphis gossypii* Glover, *A. craccivora* Koch, *A. fabae* Scopoli, *Myzus persicae* Sulzer and *Macrosiphum euphorbiae* Thomas.

According to Zitter *et al*. (1996) aphids are responsible for transmitting most of the important viruses that infect squash.

ZYMV, PRSV, WMV and SqMV transmit readily from infected to healthy cucurbit plants by direct leaf contact. ZYMV survives and remains virulent on diverse surfaces for up to 6 hours but can be inactivated by some disinfectants. ZYMV transmit to pumpkins by aphids was greater downwind than upwind of a virus source (Coutts *et al*., 2011).

Gupta (2000) studied a negative correlation between the whitefly population and yield of tomato. He found that the yield decreased with the increasing of the number of whiteflies.

According to Desbiez (2003) ZYMV is aphid-transmitted in a nonpersistent manner and it is also seed-borne in zucchini squash, which could have led to its rapid spread worldwide.

Fabre *et al.* (2014) described that the viruses can be transmitted via virus-infected seeds, causing their spread beyond the limits of time and place. This is because of the seed transmission is the way of allowing viruses to spread through generations, to overcome geographical limitations of horizontal transmission and to survive from longer periods of time than within vectors. zucchini squash seeds can be transmitted to other healthy plants by sap of germinated seedlings.

According to Gallet *et al.* (2018), plant viruses can be transmitted by insect vectors and sap of infected plants in fields, which are restricted to horizontal transmission.

2.2.5. VIRAL DISEASE INCIDENCE AND DISEASE SEVERITY

The incidence of leaf mosaic and deformation symptoms on squash and cucumber and the loss of yield are so high that summer and fall crops have been largely abandoned in the region of coastal area of Lebanon (Katul, 1986).

Field visits by Aboul-Jawdah *et al*., (2000) revealed a high disease incidence of mosaic and leaf deformation symptoms with sometimes prominent yellowing of aged leaves in open fields. They clearly showed that ZYMV and CABYV are the widest spread cucurbit viruses in open-field crop production. Their incidence reached about 64 and 65% in squash.

Aboul-Ata (2000) found negative correlation between the whitefly population and disease severity; whitefly population and yield; disease severity and yield during the study of leaf curl disease in okra. He found that disease severity reduced the fruiting in plant.

Papayiannis *et al*. (2005) found that ZYMV was the most prevalent virus in cucurbits in Cyprus with the overall incidence of 45%. PRSV-W, CABY and WMV were detected in 20.8%, 20.8% and 7.8% of the samples, respectively.

Coutts and Jones (2005) stated that a survey was done to determine the incidence and distribution of virus disease infecting cucurbits in Australia. They reported that the most prevalent viruses were ZYMV and PRSV with infected crop incidence of 1-100%. SqMV was found reaching the incidence 1-60% in two cucurbit growing areas in Australia.

Damicone *et al*. (2007) found the reductions in disease incidence ranged from 43 to 96% ($P \leq 0.05$) in pumpkin field using the border crop.

Al-Musa *et al.* (2008) detected the *Squash leaf curl virus* (SLCV) for the first time in Jordan using degenerated oligonucleotide primers. The highest disease incidence (95%)

was found in Dir Alla area, whereas disease incidence did not exceed 69% in squash samples collected from North Ghor.

Teresia *et al*. (2008) observed Nov 2006, zucchini leaves were observed showing mottling, curling, and crumpling symptoms. These symptoms, typical of viral infection, were significantly more damaging on squash growing on white synthetic mulch but the cumulative disease incidence is lower where reflective mulch was used.

Bananej and Vahdat (2008) screened for 11 cucurbit viruses and found that *Cucurbit aphid-borne yellow virus* (CABY) was the most common overall occurring in 49, 47, 40 and 33% in incidence of cucumber, squash, melon and watermelon 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*, *Zuccini yellow mosaic virus* (ZYMV) (38%).

According to Al-Ani *et al*. (2009) at least, 61 plant viruses, belonging to 39 different viral groups, were reported to infect cucurbits, including zucchini squash and they caused a huge amount of disease incidence and severity.

Al-Kuwaiti (2017) conducted a research on Squash leaf curl disease (SqLCD), caused by SLCuV and found this disease had become a threat to zucchini squash in recent growing seasons. SqLCD symptoms were seen in zucchini plants in growing areas in Baghdad and Babylon provinces with a high disease incidence (ca. 100%).
Rahman *et al*. (2020) found positive correlation between aphid population and disease incidence in cucumber during their study of management of Cucumber mosaic disease.

2.2.6. EFFECTS OF DIFFERENT CULTURAL PRACTICES ON VIRAL DISEASES OF SQUASH

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

Aphids are mostly responsible for transmitting most of the important viruses that infect squash (Zitter *et al*., 1996). Control of aphids using contact insecticides has little influence on the incidence of virus diseases since they do not kill aphids before virus transmission occurs (Broadbent, 1957; Zitter *et al*., 1996).

Use of pesticides to control the vector of virus disease cause environmental pollution and risk of pesticides resistance. For this reason, the present emphasized on different cultural practices and plant extracts to control the virus disease in squash.

2.2.6.1. MULCHING

Smith *et al*. (1964) carried out an experiment with reflective mulch to control virus. They found that reflective aluminium foil or paper-backed aluminium foil reduced the disease incidence of aphid borne virus.

Living mulches provide both food resources (honey and pollen) and shelter for natural enemies that could contribute to the reduction of pest populations (Root. 1973). It has been reported as the reduction in the number of adult insects and thus reduce the virus disease in crops.

Chalfant *et al*. (1977) resulted that the newer reflective, metallic mulches and reflective white mulches have been shown to elicit various levels of detergency of aphids and thus reduce the incidence of mosaic on yellow summer squash.

Aphid populations migrating into summer squash plantings between February and May were reduced by 96 and 68% by aluminum and plastic mulches, respectively. Aluminum and white plastic mulches increased 43%. Of total fruit yield. Yield responses were particularly evident during early cultivation with 85 and 69% increases resulting from the aluminum and white plastic mulches, respectively (Wyman, 1979).

According to Greenough (1990) more aphids were found in the no mulch treatments than in the black or white mulch treatments. Fewer aphids were found on black mulch compared to white mulch, but no distinguishing were observed between the two squash cultivars with respect to aphid number. A negative correlation was shown between the aphid number and early total yield. The black and white mulches produced greater marketable and total squash yields compared to the no mulch treatments. More fruit with WMV symptoms were observed on no mulch compared to either the black or white mulch regardless of cultivar. Greater marketable and total squash production were found when the white mulch was used compared to black mulch.

Synthetic UV-reflective mulch has been reported to successfully controls various vegetable crops against insect pests and to reduce the incidence of viral diseases (Csizinszky *et al*. 1997). These mulches protect the crop during the early growing season from insect herbivores and delay the onset of insect-vectored viruses.

Several management practices for the control of virus diseases of cucurbits have been reported including the plastic mulch by Brown *et al*., in 1993 and Vander Meer *et al*., in 1987 and found the desirable result of controlling the disease

Living mulches have also been shown to reduce the possibility of insects from locating their hosts and subsequently reduce spreading of viruses (Hooks *et al.* 1998).

Five methods were tested by Cradock *et al*. (2001) for their effectiveness in reducing both aphid numbers and stylet-borne virus diseases in *Cucurbita pepo* L. fields. The reflective mulch and straw mulch were consistently better than the other treatments in controlling aphid numbers. The reflective mulch performed best in reducing disease.

Several control methods have been suggested for the control of viral diseases in cucurbits including living mulches (Hooks *et al.* 1998; Frank & Liburd. 2005), floating row covers and reflective mulches (Alderz & Everett. 1968; Greenough *et al.* 1990).

Damicone *et al. (*2007) stated that reflective mulches of white or silver color is very effective in providing disease control delaying the epidemics of viral diseases.

An integrated approach involving the use of a living or reflective mulch with a reduced-risk insecticide may provide a more sustainable and effective approach for whitefly and disease management (Liburd & Nyoike. 2008).

2.2.6.2. BORDER CROPPING AND INTERCROPPING

Several authors have suggested to use barrier crop and inter crop to reduce the viral disease in cucurbits that act as sink of non-persistent aphid viruses.

Simmons *et al.* (1957) found that border crop forms a screen around the target crop and provides protection against vector borne viral diseases. Using of sunflower as border crop in pepper reduce the potyviruses significantly.

Toba *et al*. (1997) stated that aphids which land on the barrier crop and inter crop lose their virus charging on while they probe.

Damicone *et al*. (2007) suggested different border crops to control mosaic virus in plant such as sunflower, sorghum corn, wheat, swiss chard etc. He showed that wheat as border crop around the muskmelon reduced the incidence of potyviruses by 90%. He also suggested intercropping for the control of virus in cucurbits.

Carvalho *et al*. (2017) conducted experiment against viral disease transmitted by whitefly in cucurbits using intercrop which have aeromatic behavior such as coriander and found the reduction of whitefly infestation and so the viral infection in field.
A study was done by Namikoyi *et al*. (2018) reflected that sugarcane mosaic virus (SCMV) and maize chlorotic mottle virus (MCMV) was less in maize while intercropped with coriander.

Namikoyo *et al*. (2018) conducted an experiment to find out the effectiveness of different intercrops against viral diseases of pumpkin. The crop was intercropped by elephant grass (*Pennisetum purpureum* Schumach.), coriander (*Coriandrum sativum* L.), pearl millet (*Pennisetum glaucum* (L.) R. Br.) and Gadam sorghum (*Sorghum bicolour* (L.). While it was grown with coriander tested negative for SCMV ($P < 0.001$) and reduce the disease intensity and severity.

2.2.7. EFFECTS OF DIFFERENT PLANT EXTRACTS ON VIRAL DISEASE IN SQUASH

Sadasivam *et al.* (1991) worked on the antiviral activity of extracts from *Boerhavia diffusa*, *Bougainvillea spectabilis*, *Clerodendrum aculeatum* and sorghum against tomato spotted wilt virus on gourds and *Capsicum*, cowpea aphid-borne mosaic virus [blackeye cowpea mosaic potyvirus] on cowpea and tobacco mosaic virus on tobacco is reported. All the plant products were very effective for up to 20 d for managing TSWV and CpMV and for 45 d for TMV.

Azadirachtin induced harmful metamorphic effects on aphid, such as a slow rate of pupation time as well as reduced growth from larva-to-pupa (Wisler, 1998).

A study was carried out using potted plants arranged in a randomized complete block experimental design, to evaluate the pathogenic responses of cucurbit that was inoculated with cucumber mosaic virus to soil amendment with neem leaf powder. The amendments were given at varying rates of 0.125Kg/10kg soil, 0.25Kg/10Kg soil and 0.5Kg/10Kg soil. Plants that served as control were also inoculated with the virus but were sown in soil not amended with neem leaf powder and results from the experiment showed that amendment of the soil with neem-leaf powder produced plants that were less vulnerable to diseases occasioned by viruses. The rate and time of the application of the neem leaf powder also found to be an important factor in this regard. It was concluded that application of relatively lower rate of 0.125kg neem leaf powder per 10kg of soil at two weeks prior planting conferred the highest tolerance to virus diseases, as these treatment plants had the highest growth indices and yields (Aliyu, 2010).

Aliyu *et al*. (2011) conducted an experiment with neem leaf powder against the cucumber mosaic virus in cowpea. They concluded that use of neem leaf powder in proper amount can reduce the potentiality of virus in cowpea.

Arnason *et al*. (2012) exemplified different plants that are sources of commercially available botanical etxracts include pyrethrum (*Tanacetum cinerariifolium*), neem (*Azadirachta indica*), mahogany (*Sweitenia macrophylla*), sabadilla (*Schoenocaulon officinale*), tobacco (*Nicotiana tabacum*) and ryania (*Ryania speciosa*) to control viruses.

The *C. papaya* leaf extract contains groups of cysteine protease enzymes such as papain, alkaloids, terpenoids, flavonoids, and non-protein amino acids that are notorious to plant-sucking insect pests, including aphids, spotted bollworms, and whiteflies (Abou-Jawdah, 2000); (Zobayer, 2013).

Asare *et al*. (2014) conducted an evaluation to find out the effectiveness of different aqueous plant extracts on the management of mosaic disease on okra. They used 10% (w/v) crude extract each of neem leaf, garlic, mahogany bark, chili pepper fruit, pawpaw leaf, bougainvillea leaf and the control (water) for this purpose and found a reduction of disease incidence in mosaic disease by using different plant extracts.

Extraction from neem leaf exhibited a potency of reducing virus was highly significant because of its higher azadirachtin and nimbin contents. Furthermore, neem leaf extract resulted in a significant decrease in the number of eggs laid and survival rates of adults of grains/seeds storage pests at a concentration of 1.5 mg/100 g (Ahmad, 2015).

Azadirachtin exhibits multiple modes of action, which include antifeedancy, detrimental effects on morphology, alteration in biological fitness, fecundity suppression, decreased growth, oviposition repulsive, and even sterilization of the insects (Zhang, 2018); (Asaduzzaman, 2016).

Sharma *et al*. (2017) found that different plant extracts reduce the viral diseases in watermelon. A gradual increase in number of fruits plant^{-1}, fruit diameter, fruit weight and fruit yield were found as the number of sprays of botanical biopesticides increased. They also studied the avoidable yield loss against different treatments and found that botanical biopesticides including neem extracts reduce the avoidable yield loss in watermelon.

Carica papaya L. and *T. minuta* extracts were the most potent in suppressing the abundance of aphids and destruction caused to the leaves. This might be due to the different insecticidal constituents contained in these plant materials (Murovhi, 2020).

CHAPTER III

MATERIALS AND METHODS

Description of different materials and methods required like experimental site, soil type, planting materials, layout, seed sowing etc are given as follows:

3.1. Experimental site

The experiment was conducted at the central research field of Sher-e-Bangla Agricultural University, Sher-e Bangla Nagar, Dhaka 1207. The location of the experimental site was $23^{0}74'$ N latitude and $90^{0}35'$ E longitude with an elevation of 8.2 meter from sea level (Appendix I).

3.2. Soil type

The soil of the experimental site is in agro-ecological regions of “Madhupur Tract” and the AEZ No. is 28. The texture of the top soil is clay loam and olive grey with common fine to medium distinct dark yellowish-brown mottles. The range of pH 4.47 to 5.63 and organic carbon content is 0.8 (Appendix II).

3.3. Climate

The monthly mean of maximum and minimum temperature and relative humidity at the experimental site during the experimental period of the study had been collected. (Appendix III).

3.4. Experimental period

The present study was conducted during from the month of November 2020 to March 2021.

3.5. Treatments of the experiment

Total seven treatments and control with three replications were used to achieve the stated objectives. Treatments are given as follows:

T_0-Control

T_1- Intercrop (Coriander)

T_2- Border crop (Wheat)

T_3- Black polythene mulch

T_4- Reflecting mulch (Aluminum foil)

T_5- Neem leaf extract

T_6- Papaya leaf extract

T_7- Mahogany bark extract

3.5.1. Seed Collection

BARI squash-1 released by Bangladesh Agricultural Research Institute (BARI) was used. The seeds of squash variety were collected from vegetable division, Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur and the seeds of coriander and wheat were collected from Siddik Bazar seed market, Gulistan, Dhaka.

3.5.2. Collection and application of mulches

Mulches were collected from the Kawran bazar. After final land preparation and making of unit plot, all the mulches were put in the field.

A

B

Plate 1: Photographs showing mulching in the plot

(A) Black polythene mulching

(B) Reflecting mulching (aluminum foil)

3.5.3. Collection and preparation of plant extracts

The required plant parts were collected from the Sher-e-Bangla Agricultural University campus. Then extraction was made by blending by an electrical blender adding equal amount of sterile water for 1:1 solution. Then the juice was filtered through a sterile cheese cloth. Finally, the supernatant was diluted in equal amount of sterile water for 1:2 solution.

Table 1. Collection and preparation of plant extracts with their pictures:

No.	Selected botanical	Botanical name	Picture of leaves or bark	Picture of extracts
1	Neem leaf extract	*Azadirachta indica*		
2	Papaya leaf extract	*Carica papaya*		
3	Mahogany bark extract	*Swietenia macrophylla*		

3.6. Experimental design and layout

Field layout was done after final land preparation. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Layout of the whole plot was divided into three blocks each containing eight (8) plots of 2.5 m x 2.5 m size, giving 24-unit plots. The drain was kept 1.0 m between the blocks and 0.5 m between the plots were kept. The distance between row to row and plant to plant was 65 cm and 60 cm, respectively.

3.7. Land preparation

The experimental field was thoroughly ploughed and cross ploughed and cleaned. Finally, the land was properly levelled and plots were prepared as per the design. 6 pits were made in each plot for seed sowing. The depth, diameter and width of each pit was 50 cm,50 cm and 45 cm respectively.

3.8. Application of manure and fertilizers

The sources of N, P_2O_5, K_2O as Urea, TSP and MP were applied as recommended dose of BARI. The whole amounts of TSP and MP and cow dung were applied during the final land preparation and Urea was applied in three splits at 25, 35 and 45 days after seed sowing (DAS). Pits were covered with equal amount of soil and cow dung according to BARI.

Table 2: Fertilizer dose for squash

Fertilizer	Dose	
	Dose Per hectare	Dose for recommended area (137 m^2)
Cow dung	10 ton	140 kg
TSP	175 kg	2.38 kg
Urea	175 kg	2.38 kg
MOP	150 kg	2.05 kg
Gypsum	100 kg	1.37 kg

3.9. Sowing of seeds of squash

The soil was well prepared and converted into loose friable condition to gain good tilt. All weeds, stubbles and dead roots were removed from seed bed. The seeds were soaked in water overnight. Two squash seeds were sowed in each pit. The seeds of squash were sown in the pit on 1st December 2020. Light watering and weeding were done as and when necessary to provide good condition for growth.

3.10. Sowing of seeds of coriander and wheat

The seeds of coriander and wheat were soaked in water overnight. 80 g and 1000 g of coriander and wheat seeds were sown in three plots that are selected randomly as intercrop and border crop respectively on 10th December 2020. After sowing the seeds, the plots were covered with finished light soil. Light watering and weeding were done as and when necessary to provide good condition for growth.

A **B**

Plate 2. Intercropping and border cropping in squash field

A) **Intercropping by coriander**
B) **Border cropping by wheat**

3.11. Intercultural operation

After sowing the seeds different intercultural operations were performed.

3.11.1. Irrigation and drainage

Irrigation was provided with a watering cane to the plots immediately after sowing seeds in every alternate day in the evening up to seedling establishment. Further irrigation was provided when needed. Excess water was drained out.

3.11.2. Gap filling and uprooting of squash seedling

Gap filling and uprooting was done as and when needed.

3.11.3.Weeding

Weeding was done to keep the plots clean and easy aeration of soil which provides better growth and development. The newly emerged weeds were uprooted carefully.

3.12. 4. Application of plant extracts

Plant extracts were applied in the plot after 20 days of sowing the seed. Plant extracts were applied in both upper and lower surfaces of plant leaves.

Figure 1. Plant extracts application in squash leaf

3.12. Harvesting

Fruits were harvested at 7 days intervals during maturity to ripening stage. Harvesting was started from 14 March, 2021 and completed by 21 March,2021.

Figure 2. Harvested squash fruit

3.13. Identification of the symptom

The viral diseases were identified on the basis of typical field symptoms as described by Panno *et al*., (2016) and by ELISA test described by Yesil (2014). The plants were inspected at 30, 40, 50 and 60 days after sowing to observe the appearance and development of the symptoms of virus and samples were collected and stored for ELISA test.

A

B

Plate 3: Photographs showing the healthy plant and diseased plant in the plot; Healthy plant (A) and; Diseased plant (B)

3.13.1. CMV identification by DAS-ELISA

Sample leaves were taken and analyzed using ELIZA kit to determine whether virus was present in squash plants. The ELIZA test was performed in Plant Pathology laboratory of Bangladesh Agricultural Research Institute. For that purpose, only CMV antiserum of Bioreba was used.

3.13.1.1. Steps of Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELIZA) test

According to Yesil (2014):

Step 1: Dilution of specific antibody in coating buffer i.e. 20 µl in 20 ml buffer at a recommended dilution of 1:1000 or 40 µl in 20 ml buffer at a recommended dilution of 1:500 and then addition of 200 µl to each well of the microtiter plate should be done.

Step 2: The plates should be covered and incubated at 37^0C for 2-4 hrs.

Step 3: The plate should be washed with PBS-Tween and soaked for few minutes. The washing should be repeated two times by tapping upside down on tissue paper.

Step 4: Extraction of samples 1:20 (w/v) should be done in extraction buffer. Then 200 µl aliquots of the test sample should be added to duplicate wells.

Step 5: The plates should be covered and incubated overnight.

Step 6: Then the washing of the plate should be done three times as in step 3.

Step 7: After adding 200 µl enzyme conjugate, recommended dilution should be given in the delivery note, in conjugate buffer.

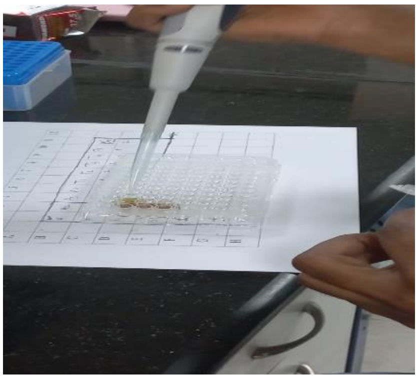
Step 8: After covering the plate, it should be incubated in 37^0C for 2-4 hrs.

Step 9: The washing of the plate should be done three times as in step 3.

Step 10: 200 µl aliquots of freshly prepared substrate (1 mg/ml para-nitrophenyl-phosphate in substrate buffer) should be added to each well.

Step 11: The plates should be covered and incubated at 37^0C for 30-60 min.

Step 12: The results should be assessed by spectrophotometric measurement of absorbance at 405 nm.



A B

C D

Plate 4: Steps of ELISA test for the confirmation of CMV

A) Extraction of sample adding extraction buffer

B) Loading of samples in ELIZA plate

C) Plates covered by aluminum foil

D) Spectrophotometric measurement

3.14. Collection of data

Three plants were selected randomly from each unit plot for data collection in such a way that the border effect could be avoided at the highest precision. Data were collected at 30 DAS (Days after sowing), 45 DAS (Days after sowing) and 60 DAS (Days after sowing). Data of the following parameters were recorded from the sample plants during the course of experiment.

3.14.1 Number of leaves plant^{-1}

Number of leaves was measured from the sample plants and recorded in 30, 45 and 60 days after sowing to observe the growth rate of the plants.

3.14.2. Area of leaf (cm^2)

The area of the leaf both health and diseased was measured by the following formula:

Area of leaf (cm^2) = Length of the leaf (cm) × Breadth of the leaf (cm)

3.14.3. Leaf area reduction (%)

Percentage of leaf area reduction was calculated by following formula used by Rahman (2010).

$$\text{Leaf area reduction (\%)} = \frac{\text{Healthy leaf area (cm2)} - \text{Infected leaf area (cm2)}}{\text{Healthy leaf area (cm2)}} \times 100$$

3.14.4. Percent of infected leaf tissue

It was recorded according to the disease rating scale of cucurbits followed by Yuosaf *et al.*, (2018).

3.14.5. Number of infected plants plot^{-1}

Number of infected plants plot^{-1} was measured from all the plants and recorded in 30, 45 and 60 days after sowing to observe the growth rate of the plants.

3.14.6. Number of fruits plant^{-1}

The number of fruits plant^{-1} was counted from the whole plot.

3.14.7. Fruit weight plant^{-1} (kg)

Fresh fruit weight (kg)/plant of plant was weighted by an electric balance after harvesting and recorded.

3.14.8. Diameter of fruit (cm)

The diameter of fruit was measured by a measuring tape from three randomly selected fruit.

3.14.9. Length of fruit (cm)

The length of fruit was measured by a scale.

3.14.10. Fruit weight plot^{-1} (kg)

An electric balance was used to take the fruit weight per plot. It was measured by the sum of fruit yield from each unit plot during the period from first to final harvest and was recorded in kilogram.

3.14.11. Fruit yield (kg/ha)

It was measured by the following formula:

$$\text{Fruit yield per hectare (kg)} = \frac{\text{Fruit yield per plot (kg)} \times 10000 \text{ m2}}{\text{Area of plot in square meter (m2)}}$$

3.14.12. Fruit yield (ton/ha)

It was measured by the following formula:

$$\text{Fruit Yield per hectare (ton)} = \frac{\text{Fruit yield per plot (kg)} \times 10000 \text{ m2}}{\text{Area of the plot in square meter (m2)} \times 1000 \text{ kg}}$$

3.14.13. Avoidable yield loss (%)

The percentage of avoidable yield loss by each treatment was calculate by following formula according to Sharma *et al*. (2017).

$$\text{Avoidable yield loss (\%)} = \frac{\text{Yield of protected plant} - \text{Yield of unprotected plant}}{\text{Yield of protected plant}} \times 100$$

3.14.14. Number of aphid plant^{-1}: Number of aphid per plant was counted from the sample plants and recorded from 30, 45 and 60 days after sowing to observe the occurrence of insect.

Figure 3. Photographs showing the aphid vectors in the dorsal site of squash leaf

3.14.16. Disease incidence (%)

Percent disease incidence was calculated using the following formula which was used by Ashrafuzzaman (2016):

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

3.14.17. Disease severity (%)

Percent disease severity was calculated using the following formula which was used by McKinney (1923):

$$\text{Disease Severity (\%)} = \frac{\text{Sum of all numerical rating}}{\text{Maximum disease grade} \times \text{Total number of plants observed}} \times 100$$

Disease scale: Percent disease severity was calculated using 0-4 scale according to Yuosaf *et al*., (2018).

Rating	Infected tissue (%)	Result
0	0	Immune
1	1-25	Moderately resistant
2	26-50	Moderately susceptible
3	51-75	Susceptible
4	76-81	Highly susceptible

3.15. Analysis of data

The data recorded for different characters were statistically analyzed using STATISTIX-10 software. Least Significant Difference (LSD) test were performed at 5% level of significance and CV (%) was performed. Tables, graphs and charts were used for interpretation of different parameters.

CHAPTER IV

RESULTS AND DISCUSSION

The present study was conducted to see the effects of different cultural practices (mulching by black polythene and reflecting aluminum foil, border cropping by wheat, inter cropping by coriander) and plant extracts of neem leaf, papaya leaf and mahogany bark against viral diseases and their impacts on yield of squash. This chapter will contain the explanation and description of the results obtained from the experiment. The results have been presented and the interpretations have been given under the following headings:

4.1. Identification of CMV disease in squash plant

4.1.1. Symptomology

Squash plants exhibiting mosaic, yellowing, curling, crinkling and stunting symptoms were observed in the field during the study. The symptom of mosaic was found clearing of vein development of mosaic patterns with dark green area alternating with light green or yellow area (Plate 5A). In leaf curl symptoms, the new growth bends and mid-vein portions of leaves were severely mottled (Plate 5B). Small patches in the field with new leaves showing yellow spots was also found which called crinkling symptom (Plate 5C).

A **B** **C**

Plate 5: Visual symptoms of virus disease in squash plant;

Mosaic on leaves (A); curling on leaves (B) and; crinkling on Leaves (C)

4.1.2. Serological identification of CMV through DAS-ELIZA test

Eight samples were collected from the field and set for ELIZA test. Only CMV antiserum was used for virus detection in collected samples. Five samples were showed positive against the antiserum of CMV. The positively reacted samples produce bright yellow color in ELISA kit. The results indicated that the rest of the samples showing virus-disease like symptoms in the field, gave negative result may had been infected with other viruses of squash which antiserum was not used.

Table 3. Optical density (A 405 nm) of CMV infected squash plant leaves extracts in ELIZA test

Samples	**OD value** [a]	Reaction
Positive control	2.9362	
Negative control	0.1909	
Sample-1	1.5246	+
Sample-2	2.1321	+
Sample-3	2.3361	+
Sample-4	2.1146	+
Sample-5	2.2064	+
Sample-6	0.1515	-
Sample-7	0.1698	-
Sample-8	0.1669	-

*The optical density (OD) of at least double that of the negative control were considered positive. [a] mean value of three wells

4.2. Effect of different treatments on CMV disease incidence (%) and severity (%) of squash

Significant differences were found in disease incidence and severity among the treatments on squash plants against virus during experimental period. The percentages of disease incidence and severity are shown in table 4 & 5.

4.2.1. Disease incidence (%)

The effect of different treatments on disease incidence (%) of virus disease in squash was observed based on visual symptoms. Disease incidence (%) was recorded three times at 30, 45, 60 days after sowing (DAS) (table 4).

At 30 DAS, the highest disease incidence (60.10%) was recorded in T_0 (control) which was identical with T_1 (Intercropping by coriander, 50.00%), T_6 (Papaya leaf extracts, 44.44%), T_7 (Mahogany bark extracts, 50.00%). The lowest disease incidence (22.22%) was recorded in T_4 (Reflecting mulch) which is identical with T_2 (Border cropping by wheat, 38.88%), T_3 (Black polythene mulch, 38.88 %), T_5 (Neem leaf extracts, 38.88%).

At 45 DAS, the highest disease incidence (61.11%) was recorded in T_0 (control) which was identical with T_7 (Mahogany bark extracts, 50.00%). The lowest disease incidence (11.11%) was recorded in T_4 (Reflecting mulch) followed by T_2 (Border cropping by wheat, 27.77%) T_5 (Neem leaf extracts, 27.77%) which were statistically similar.

At 60 DAS, the highest disease incidence (83.33%) was recorded in T_0 (control) which was identical with T_1 (Intercropping by coriander, 66.67%) and T_7 (Mahogany bark extracts, 72.22%). The lowest disease incidence (22.22%) was recorded in T_4 (Reflecting mulch). In other treatments, the disease incidence was found moderate that was in range of 55.56%- 44.44%.

Table 4: Effect of different treatments on CMV disease incidence (%) in squash

Treatments	Disease incidence (%)		
	At 30 DAS	**At 45 DAS**	**At 60 DAS**
T_0	60.10 a	61.11 a	83.33 a
T_1	50.00 ab	44.44 bc	66.67 a-c
T_2	38.88 bc	27.77 d	50.00 cd
T_3	38.88 bc	33.33 cd	55.55 b-d
T_4	22.22 c	11.11 e	22.22 e
T_5	38.88 bc	27.77 d	44.44 d
T_6	44.44 ab	44.44 bc	55.56 b-d
T_7	50.00 ab	50.00 ab	72.22 ab
CV (%)	19.63	19.49	19.81

*Means followed by same letters are not significantly differ from each other at 5% level of significant.

T_0= control; T_1 = Intercropping by coriander; T_2= Border cropping by wheat; T_3= Black polythene mulch; T_4= Reflecting mulch of aluminum foil; T_5= Neem leaf extract; T_6= Papaya leaf extract and T_7= Mahogany bark extract

4.2.2 Disease severity (%)

There were significant variations of disease severity (%) among the different treatments in squash. The effect of different treatments on disease severity (%) of virus disease in squash was observed based on visual symptoms. Disease severity (%) was recorded three times at 30, 45, 60 days after sowing (DAS) (table 5).

At 30 DAS, the highest disease severity (45.83 %) was recorded in T_0 (control). The lowest disease severity (5.56%) was recorded in T_4 (Reflecting mulch) followed by T_5 (Neem leaf extracts, 16.22%), T_2 (Border cropping by wheat, 16.64%) which were statistically similar. In remaining treatments, the disease severity was found moderate that was in range of 25.00%- 33.33%.

At 45 DAS, the highest disease severity (40.26%) was recorded in T_0 (control). The lowest disease severity (12.64%) was recorded in T_4 (Reflecting mulch) followed by T_5 (Neem leaf extracts, 13.83%), T_2 (Border cropping by wheat, 16.65%) which were statistically similar. In remaining treatments, the disease severity was found moderate that was in range of 25.00%- 41.66%.

At 60 DAS, the highest disease severity (83.00 %) was recorded in T_0 (control). The lowest disease severity (5.56%) was recorded in T_4 (Reflecting mulch) followed by T_5 (Neem leaf extracts, 16.22%), T_2 (Border cropping by wheat, 16.64%) which were statistically similar. In remaining treatments, the disease was severity found moderate that was in range of 25.00%- 33.33%.

Table 5: Effect of different treatments on CMV disease severity (%) in squash

Treatments	Disease severity (%)		
	At 30 DAS	At 45 DAS	At 60 DAS
T_0	40.26 a	45.83 a	83.00 a
T_1	29.16 c	30.53 c	58.33 b
T_2	16.64 f	16.65 f	33.33 c
T_3	25.00 e	25.00 e	37.50 c
T_4	12.64 g	5.56 h	11.10 d
T_5	16.22 f	13.83 f	24.96 cd
T_6	26.16 d	29.16 d	38.89 c
T_7	33.33 b	41.66 b	61.11 b
CV (%)	2.20	1.71	19.95

*Means followed by same letters are not significantly differ from each other at 5% level of significant.

T_0= control; T_1 = Intercropping by coriander; T_2= Border cropping by wheat; T_3= Black polythene mulch; T_4= Reflecting mulch of aluminum foil; T_5= Neem leaf extract; T_6= Papaya leaf extract and T_7= Mahogany bark extract

4.3. Effect of different treatments on growth and growth contributing characters of squash against CMV

Growth and growth contributing characters of squash were affected by virus diseases in squash under different treatments. Growth contributing characters such as number of leaves per plant, area of healthy leaf (cm^2), area of diseased leaf (cm^2), reduction of leaf area (%) showed significant differences under different treatments used. The effects of different treatments on growth and growth contributing characters of squash against viral disease are presented in table 6.

4.3.1. Number of leaves plant^{-1}

Average number of leaves of squash showed significant difference due to viral infection. The range of leaves number per plant varied from 7.33 to 13.00. Maximum no. of leaves per plant (13.00) was found in T_4 (reflecting mulch) which was identical with T_5 (neem leaf extract) with value 11.00. On the other hand, minimum no. of leaves per plant (7.33) was recorded in T_0 (control plot).

4.3.2. Area of healthy leaf (cm^2)

Area of healthy leaf (cm^2) of squash showed significant difference due to viral infection. The range of area of healthy leaf varied from 604.29 cm^2 to 920.57 cm^2. Maximum area of healthy leaf (cm^2) of squash (920.57cm^2) was found in T_4 (reflecting mulch) which was identical with T_5 (neem leaf extract) with value 816.93 cm^2. On the other hand, minimum area of healthy leaf (604.29 cm^2) was recorded in T_0 (control plot).

4.3.3. Area of diseased leaf (cm^2)

Area of diseased leaf (cm^2) of squash showed significant difference due to viral infection. The range of area of healthy leaf varied from 239.33 cm^2 to 415 cm^2. Maximum area of diseased leaf (cm^2) of squash (415 cm^2) was found in T_4 (reflecting mulch) which was identical with T_5 (neem leaf extract) with value 368 cm^2. On the other hand, minimum area of diseased leaf (239.33 cm^2) was recorded in T_0 (control plot).

4.3.4. Reduction of leaf area (%)

Percentage of average number of leaf area reduction of squash showed significant difference due to the viral infection of squash. The range of reduction of leaf area reduction (%) varied from 25.23% to 74.35%. The maximum percentage of reduction of leaf area (74.35%) was found in T_0 (control) and the minimum percentage of reduction of leaf area (25.23%) was found in T_4 (reflecting mulch) followed by T_5 (neem leaf extract) which was 50.43%.

Table 6. Effect of different treatments on growth and growth contributing characters of squash against CMV

Treatments	Average leaf no. plant^{-1}	Area of healthy leaf (cm^2)	Area of diseased leaf (cm^2)	Reduction of leaf area (%)
T_0	7.33 c	604.29 c	239.33 c	74.35 a
T_1	9.00 bc	647.97 c	246.85 c	60.90 ab
T_2	10.67 ab	799.01 b	355.20 ab	53.80 b
T_3	10.65 ab	786.07 b	335.82 b	53.83 b
T_4	13.00 a	920.57 a	415.00 a	25.23 c
T_5	11.00 ab	816.93 ab	368.00 ab	50.43 b
T_6	9.55 bc	711.63 bc	331.50 b	60.23 ab
T_7	8.00 bc	634.93 c	246.70 c	64.40 ab
CV (%)	18.01	5.52	7.60	19.13

* Means followed by same letters are not significantly differ from each other at 5% level of significant.

T_0= control; T_1 = Intercropping by coriander; T_2= Border cropping by wheat; T_3= Black polythene mulch; T_4= Reflecting mulch of aluminum foil; T_5= Neem leaf extract; T_6= Papaya leaf extract and T_7= Mahogany bark extract

4.4. Effect of different treatments on yield and yield contributing character of squash against CMV

The yield and yield contributing characters of squash were affected by virus diseases in squash under different treatments. Yield contributing characters such as average no. of fruits per plant, Length and diameter of fruit (cm), average fruit weight per plant (Kg), average fruit weight per plot (Kg), average fruit yield (ton/ha) showed significant differences under different treatments used. The effects of different treatments on yield and yield contributing characters of squash against viral disease are given in table 7.

4.4.1. Average no. of fruits plant^{-1}

The number of fruit plant^{-1} showed significant difference among the treatments. The highest number of fruits plant^{-1} (6.00) was found in T_4 (reflecting mulch) and the lowest of fruits plant^{-1} (2.83) was found in T_0 (control) which was statistically similar with T_1 (intercropping with coriander), T_6 (papaya leaf extract) and T_7 (mahogany bark extract) which were 3.45, 3.50 and 3.33, respectively.

4.4.2. Length of fruit (cm)

The length of fruit showed significant difference among the treatments. The highest (34.10cm) and the lowest (21.48cm) length of fruit were found in T_4 (reflecting mulch) and T_0 (control), respectively.

4.4.3. Diameter of fruit (cm)

The diameter of fruit showed significant difference among the treatments. The highest (24.28cm) and the lowest (13.69cm) value of diameter of fruit were found in T_4 (reflecting mulch) and T_8 (control) respectively.

4.4.4. Average fruit weight plant^{-1} (Kg)

Fruit weight is an important yield contributing character of squash. Th fruit weight per plant of squash showed significant results among different treatments. The range of fruit weight per plant varied from 1.27 kg to 6.98 kg. The highest average fruit weight per plant (6.98 kg) was found in T_4 (reflecting mulch). On the other hand, the lowest average fruit weight per plant (1.27 kg) was recorded in T_0 (control) which was statistically similar with T_7 (mahogany bark extract) with value 1.55 kg.

4.4.5. Average fruit weight plot^{-1} (Kg)

Average fruit weight per plot of squash showed significant results among different treatments. The range of average fruit weight per plot varied from 7.60 kg to 41.90 kg. The highest average fruit weight per plot (41.90 kg) was found in T_4 (reflecting mulch). On the other hand, the lowest average fruit weight per plot (7.60 kg) was recorded in T_0 (control).

4.4.6. Average fruit yield (ton/ha)

Fruit weight per plot of squash showed appreciable differences due to virus infection among different treatments. The range of average fruit yield (ton/ha) varied from 15.20 ton/ha to 83.80 ton/ha. The highest average fruit yield (83.80 ton/ha) was found in T_4 (reflecting mulch) followed by T_5 (neem leaf extract) which was 32.63 ton/ha. On the other hand, the lowest average fruit yield (15.20 ton/ha) was recorded in T_0 (control) which was statistically similar with T_7 (mahogany bark extract) which was 18.56 ton/ha. T_2 (border cropping with wheat) and T_3 (black polythene mulch) were statistically similar with each other which were 31.06 ton/ha and 30.93 ton/ha respectively. T_1 (intercropping with coriander) and T_6 (papaya leaf extract) were identical with each other which were 24.33 ton/ha and 27.90 ton/ha respectively (table 7).

Table 7. Effect of different treatments on yield and yield contributing characters of squash against CMV

Treatments	Average no. of fruits plant^{-1}	Average length of fruit (cm)	Average diameter of fruit (cm)	Average fruit weight plant^{-1} (Kg)	Average fruit weight plot^{-1} (Kg)	Average fruit yield (ton/ha)
T_0	2.83 b	21.48 d	13.69 d	1.27 e	7.60 e	15.20 e
T_1	3.45 b	24.00 cd	17.55 cd	2.03 d	12.16 d	24.33 d
T_2	4.50 ab	27.41 bc	21.06 a-c	2.59 bc	15.54 bc	31.06 bc
T_3	4.33 ab	26.36 b-d	19.50 bc	2.58 bc	15.46 bc	30.93bc
T_4	6.00 a	34.10 a	24.28 a	6.98 a	41.90 a	83.80 a
T_5	4.67 ab	30.67 ab	22.73 ab	2.72 b	16.32 b	32.63 b
T_6	3.50 b	25.10 cd	17.85 cd	2.32 cd	13.94 cd	27.90 cd
T_7	3.33b	23.58 cd	14.61 d	1.55 e	9.28 e	18.56 e
CV (%)	18.91	11.15	13.78	6.83	6.83	6.87

*Means followed by same letters are not significantly differ from each other at 5% level of significant.

T_0= control; T_1 = Intercropping by coriander; T_2= Border cropping by wheat; T_3= Black polythene mulch; T_4= Reflecting mulch of aluminum foil; T_5= Neem leaf extract; T_6= Papaya leaf extract and T_7= Mahogany bark extract

4.5. Effect of different treatments on avoidable yield loss (%) over control

Avoidable yield loss (%) over T_0 (control) showed appreciable differences among different treatments. The results of avoidable yield loss (%) over control among different treatments are shown in table 8. The highest value of avoidable yield loss (81.86%) over T_0 (control) was found in T_4 (reflecting mulch) followed by T_5 (neem leaf extract) and the lowest value was found in T_1 (intercrop).

Table 8. Effect of different treatments on avoidable yield loss (%) over control against CMV in squash

Treatments	Avoidable yield loss (%) over control
T_0	0.00
T_1	37.49 d
T_2	50.05 bc
T_3	50.81 bc
T_4	**81.86 a**
T_5	53.13 b
T_6	45.46 c
T_7	18.10 e
CV (%)	9.37

*Means followed by same letters are not significantly differ from each other at 5% level of significant.

T_0= control; T_1 = Intercropping by coriander; T_2= Border cropping by wheat; T_3= Black polythene mulch; T_4= Reflecting mulch of aluminum foil; T_5= Neem leaf extract; T_6= Papaya leaf extract and T_7= Mahogany bark extract

4.6. Effect of different treatments on Aphid infestation in squash field

The effect of different treatments on aphid number/plant in squash was observed based on their presence in field. Insects number/plant was recorded three times at 30, 45, 60 days after sowing (DAS).

At 30 DAS, the highest (14.00) insect number plant^{-1} was recorded in T_0 (control). The lowest insect number plant^{-1} (7.66) was recorded in T_4 (Reflecting mulch) followed by T_5 (Neem leaf extracts, 8.00), T_2 (Border cropping by wheat, 8.33%) which were statistically similar. In remaining treatments, insect number plant^{-1} was identical with T_4 (Reflecting mulch) ranging the value from 9.33 to 11.33.

At 45 DAS, the highest (14.67) insect number plant^{-1} was recorded in T_0 (control) which was statistically identical with T_1 (Intercropping by coriander, 12.67), T_3 (Black polythene mulch, 11.90), T_6 (Papaya leaf extracts, 12.00) and T_7 (Mahogany bark extracts, 13.33). The lowest insect number plant^{-1} (8.67) was recorded in T_4 (Reflecting mulch) followed by T_5 (Neem leaf extracts, 9.00), T_2 (Border cropping by wheat, 10.67).

At 60 DAS, the highest (21.00) insect number plant^{-1} was recorded in T_0 (control). The lowest insect number plant^{-1} (7.33) was recorded in T_4 (Reflecting mulch) followed by T_5 (Neem leaf extracts, 9.66), T_2 (Border cropping by wheat, 11.00) which were statistically identical. In remaining treatments ranged the value from 12.66 to 17.33. All the results are presented in table 9.

Table 9. Effect of different treatments on aphid infestation in squash field which transmit CMV

Treatments	Number of Aphid plant^{-1}		
	30 DAS	**45 DAS**	**60 DAS**
T_0	14.00 a	14.67 a	21.00 a
T_1	9.33 bc	12.67 a-c	15.00 bc
T_2	8.33 c	10.67 b-d	11.00 c-d
T_3	9.00 bc	11.90 a-d	12.66 bc
T_4	7.66 c	8.67 d	7.33 e
T_5	8.00 c	9.00 cd	9.66 cd
T_6	9.33 bc	12.00 a-d	14.66 de
T_7	11.33 bc	13.33 ab	17.33 ab
CV (%)	17.41	19.45	19.20

*Means followed by same letters are not significantly differ from each other at 5% level of significant.

T_0= control; T_1 = Intercropping by coriander; T_2= Border cropping by wheat; T_3= Black polythene mulch; T_4= Reflecting mulch of aluminum foil; T_5= Neem leaf extract; T_6= Papaya leaf extract and T_7= Mahogany bark extract

4.7. Relationship between the aphid population and CMV disease incidence (%) of squash

Relation between aphid population and % disease incidence of squash in the field condition are shown in figure 4. This figure showed a strong positive correlation between disease incidence (%) of CMV infection and aphid populations. With the increase of insect populations, viral infection also increased. A regression line was fitted between aphid populations and (%) disease incidence of squash. In this case, the correlation coefficient (r) was 0.9530 and the contribution of the regression (R^2 = 0.9083) indicated that 90.8 % CMV infection increased due to the aphid infestation.

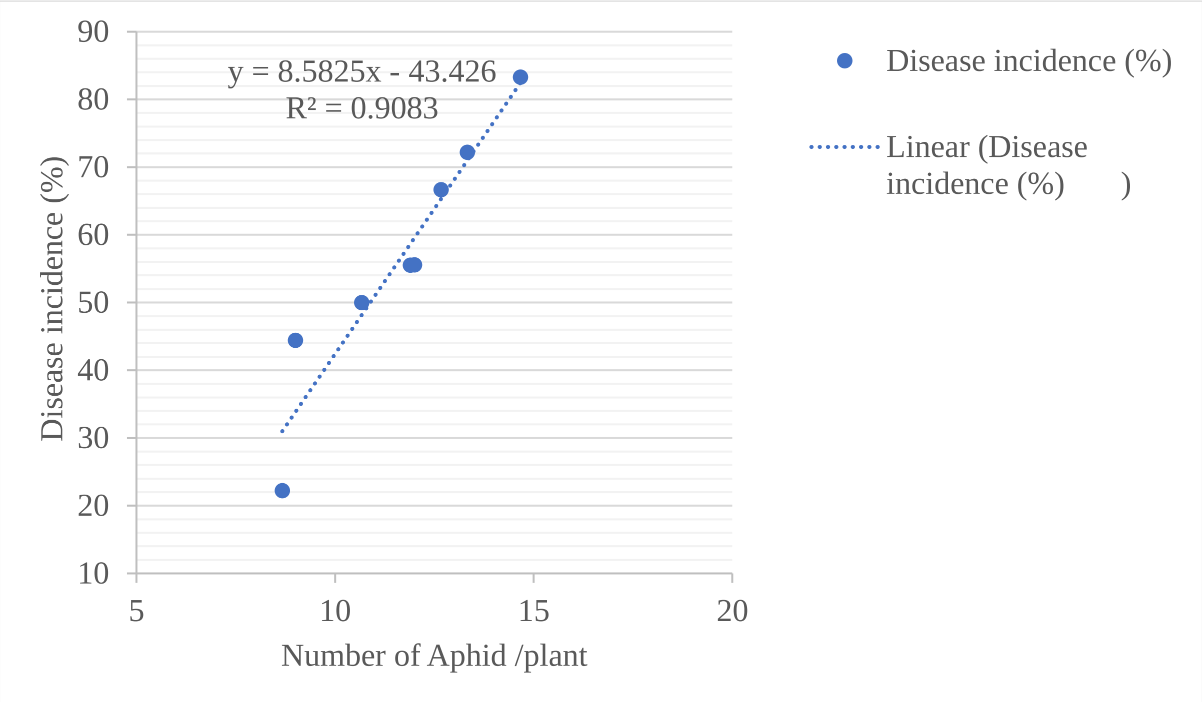


Figure 4: Relationship between the aphid population and CMV disease incidence (%) of squash

4.8. Relationship between the aphid population and CMV disease severity (%) of squash

Relation between aphid population and % disease severity of squash in the field condition are shown in figure 5. This figure showed a strong positive correlation between disease severity (%) of CMV infection and aphid populations. With the increase of aphid populations, the amount of disease due to CMV infection also increased. A regression line was fitted between aphid populations and disease severity (%) of squash. In this case, the correlation coefficient (r) was 0.9733 and the contribution of the regression (R^2 = 0.9453) indicated that 94.5 % amount of disease severity increased due to the aphid infestation.

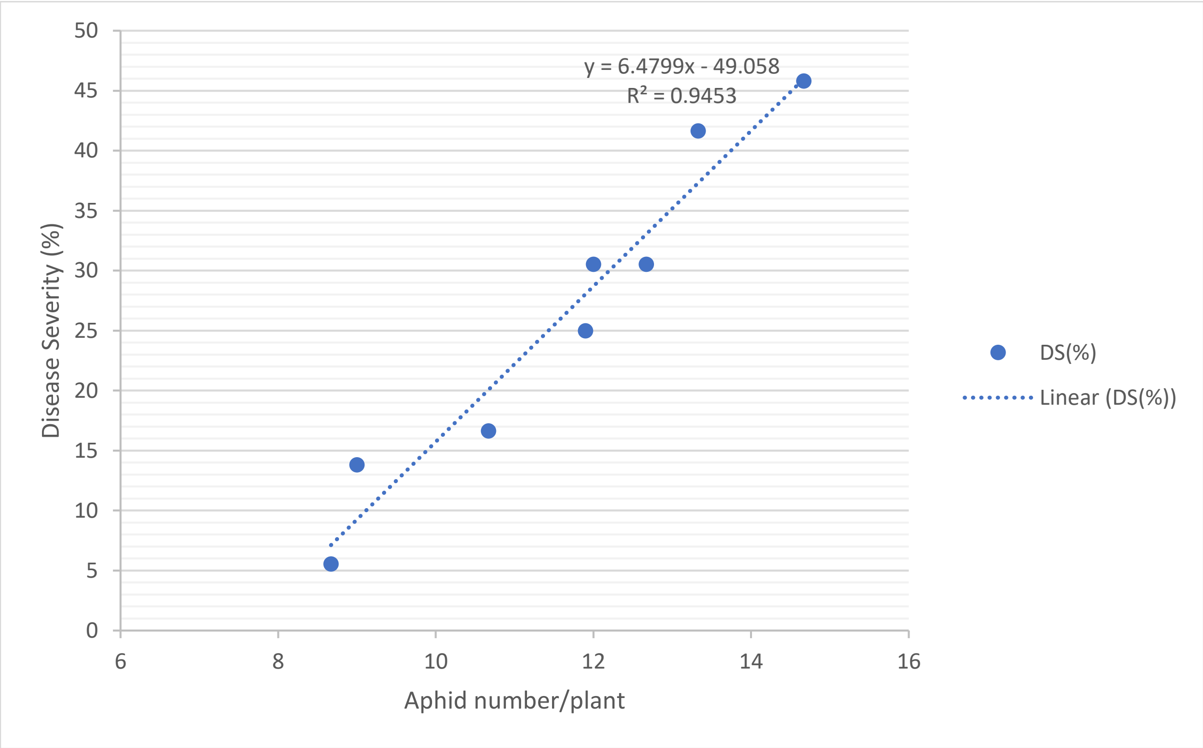


Figure 5. Relationship between the aphid population and CMV disease severity (%) of squash

4.9. Relationship between the aphid population and average yield (ton/ha) of squash

Relation between aphid population and average yield (ton/ha) of squash in the field condition are shown in figure 6. This figure showed a strong negative correlation between average yield (ton/ha) of squash rage yield (ton/ha) of squash and insect populations. With the increase of aphid populations, the average yield (ton/ha) of squash decreased and with the decrease of aphid populations, the average yield (ton/ha) of squash increased. A regression line was fitted between aphid populations & average yield (ton/ha) of squash. In this case, the correlation coefficient (r) was 0.7649 and the contribution of the regression (R^2 = 0.5851) indicated that 58.5% yield in squash would be affected due to the aphid infestation.

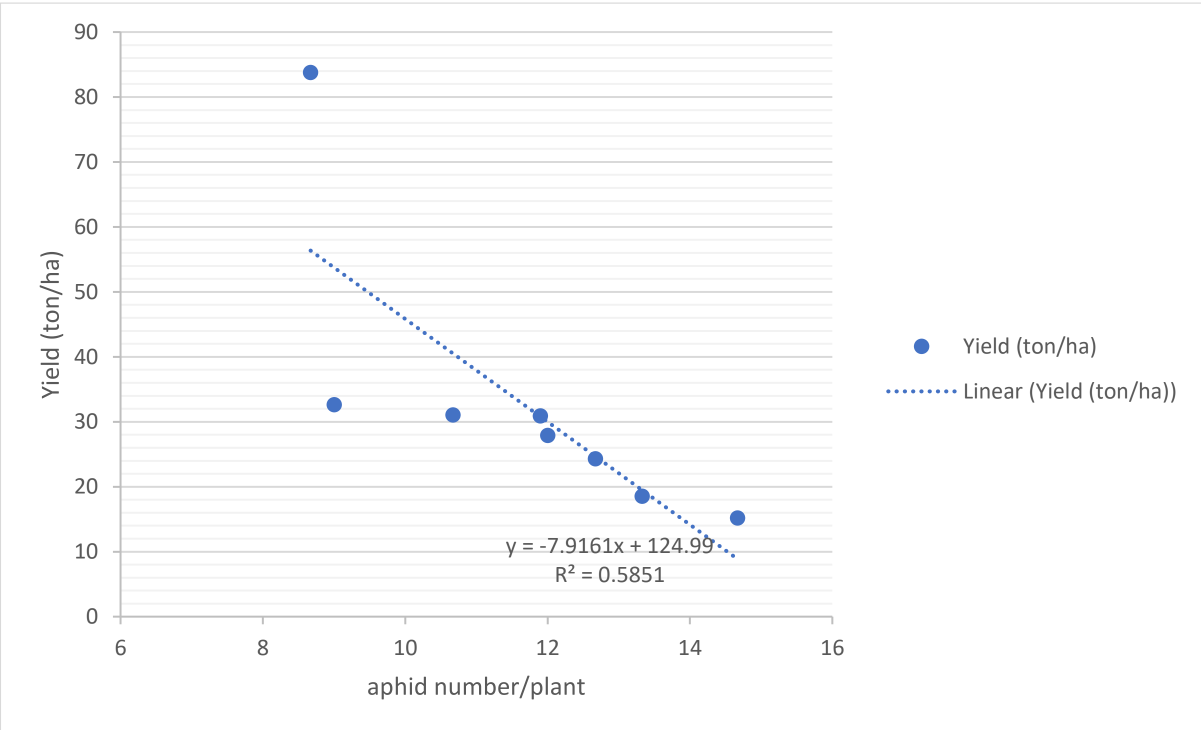


Figure 6. Relationship between aphid population and average yield (ton/ha) of squash

4.10. Relationship between the CMV disease incidence (%) and average yield (ton/ha) of squash

Relation between (%) disease incidence and yield (ton/ha) of squash in the field condition are shown in figure 7. This figure showed a strong negative correlation between disease incidence (%) of viral infection and yield (ton/ha) of squash. With the increase of disease incidence (%), the yield (ton/ha) decreased. A regression line was fitted between % disease incidence and yield (ton/ha) of squash. In this case, the correlation coefficient (r) was -0.8972 and the contribution of the regression (R^2 = 0.805) indicated that 80.5 % yield in squash would be affected by viral infection.

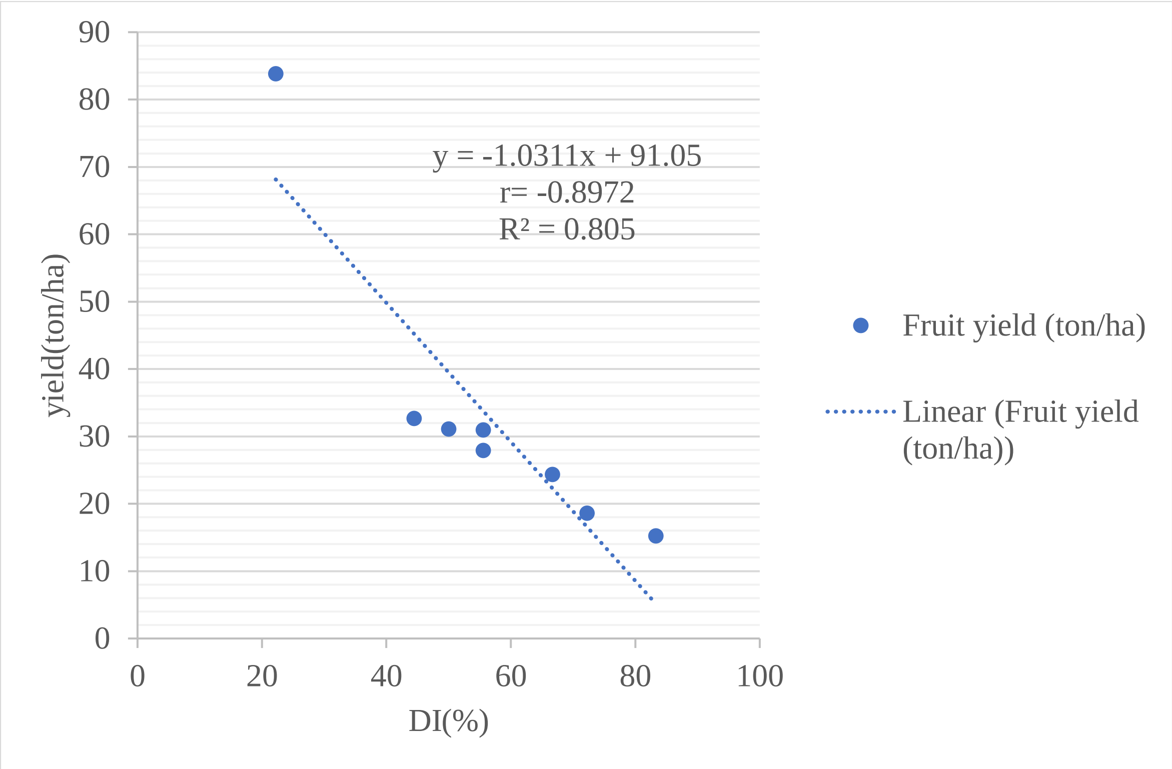


Figure 7. Relationship between the CMV disease incidence (%) and yield (ton/ha) of squash

4.11. Relationship between CMV disease severity (%) and average yield (ton/ha) of squash

Relation between disease severity (%) and yield (ton/ha) of squash in the field condition are shown in figure 8. This figure showed a strong negative correlation between disease severity (%) of viral infection and yield (ton/ha) of squash. With the increase of disease severity (%), the yield (ton/ha) decreased. A regression line was fitted between % disease severity and yield (ton/ha) of squash. In this case, the correlation coefficient (r) was -0.7999 and the contribution of the regression (R^2 = 0.639) indicated that 63.9% yield in squash would be affected by the increase of severity of viral infection.

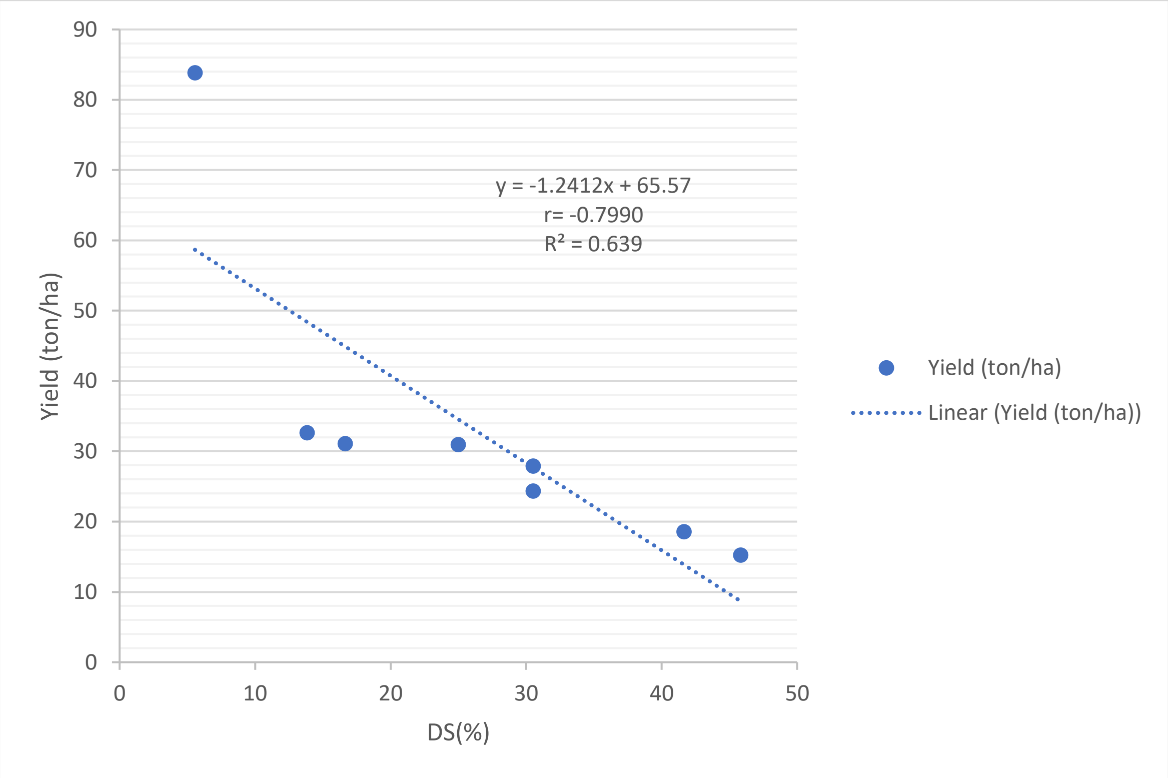


Figure 8. Relationship between the CMV disease severity (%) and average yield (ton/ha) of squash

4.12. Relationship among the CMV disease severity (%); reduction of leaf area (%) and average yield (ton/ha) of squash

From the relationship study among disease severity (%); reduction of leaf area (%) and average yield (ton/ha) revealed that in case of every treatment, the amount of average yield (ton/ha) decreased with the increase reduction of leaf area (%) and disease severity. On the contrary, the amount of average yield (ton/ha) increased with the decrease of reduction of leaf area (%) and disease severity (%) (Figure 9).

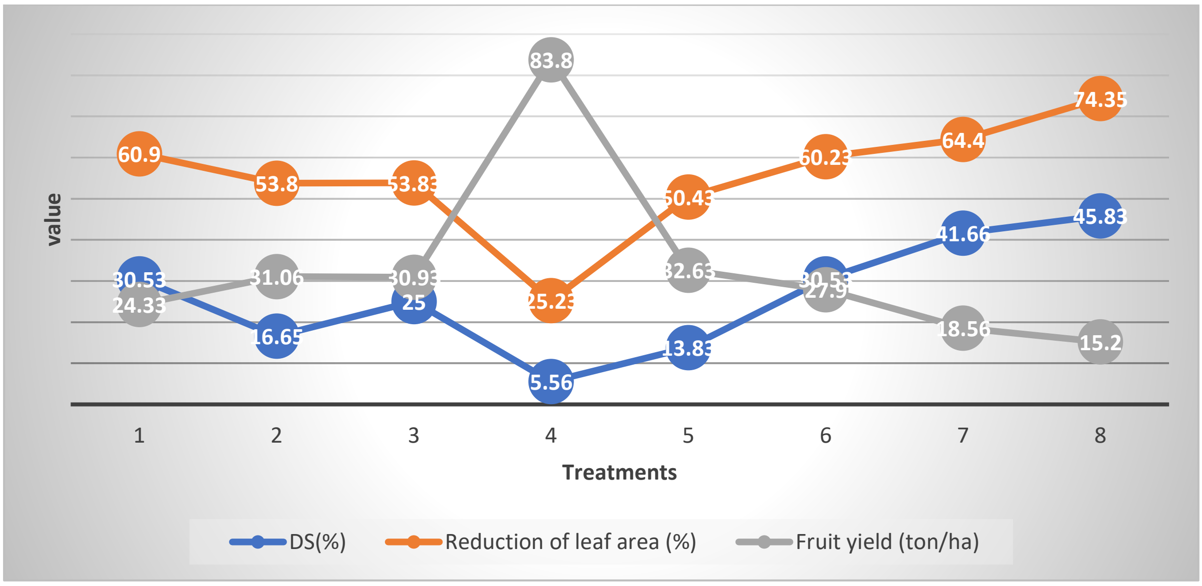


Figure 9. Relationship among the CMV disease severity (%); reduction of leaf area (%) and average yield (ton/ha) of squash

Here, 1= Intercropping by coriander (T_1); 2= Border cropping by wheat (T_2); 3= Black polythene mulch (T_3); 4= Reflecting mulch of aluminum foil (T_4); 5= Neem leaf extract (T_5); 6= Papaya leaf extract (T_6); 7= Mahogany bark extract (T_7) and 8= control (T_0)

4.13. Relationship between the CMV disease severity (%) and the average no. of fruits plant^{-1} in squash

From the relationship study between average disease severity (%) and average number of fruits plant^{-1} revealed that in case of every treatment, the average number of fruits plant^{-1} increased with the decrease of average disease severity (%) and vice-versa (Figure 10).

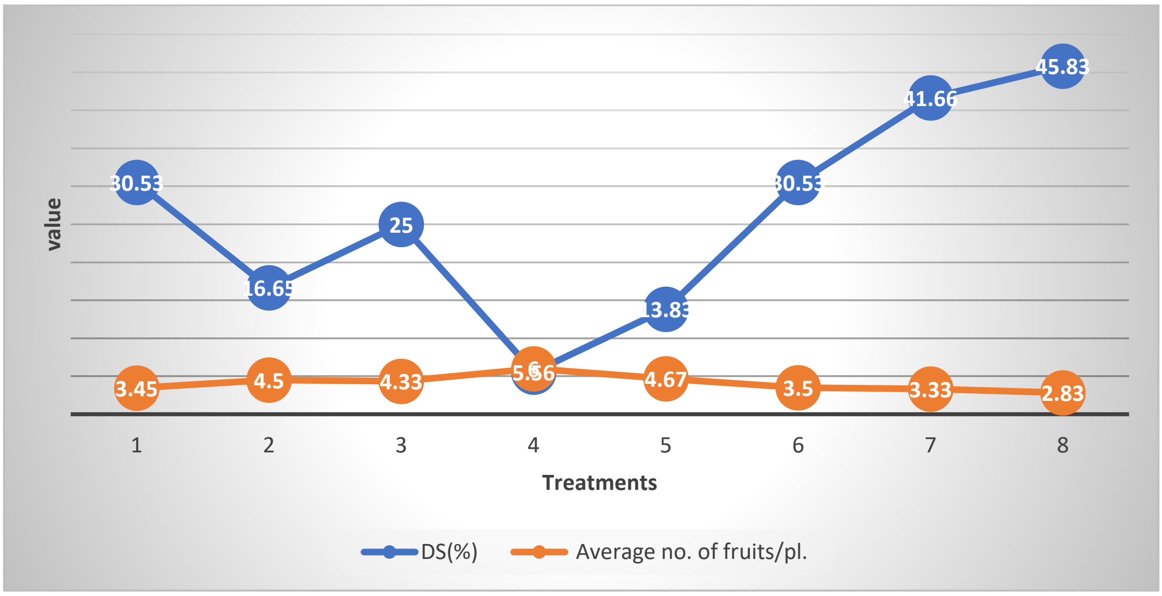


Figure 10. Relationship between the CMV disease severity (%) and average no. of fruits plant^{-1} of squash

Here, 1= Intercropping by coriander (T_1); 2= Border cropping by wheat (T_2); 3= Black polythene mulch (T_3); 4= Reflecting mulch of aluminum foil (T_4); 5= Neem leaf extract (T_5); 6= Papaya leaf extract (T_6); 7= Mahogany bark extract (T_7) and 8= control (T_0)

DISCUSSION

Squash is now popular vegetable in Bangladesh. It has a high nutritional value with carbohydrates, vitamins and minerals. The production of squash is increasing in our country day by day and it has high market value in our country. But the crop suffers important losses from infection by different virus which considered as a major problem affecting squash production in many of the old world, tropical and subtropical countries Virus infection is a major problem during the production of squash and it causes a great yield loss (Provvidenti, 1996).

The aim of this study was to evaluate the effect of different eco-friendly approaches on viral diseases of squash and its impact on yield of squash. The study was conducted at the research farm of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207, during the period of December 2020 to March 2021. Different cultural practices (inter cropping by coriander, border cropping by wheat, reflecting mulch, black polythene mulch) and plant extracts (neem leaf, papaya leaf, mahogany bark) were used in this experiment.

For the identification of virus disease in the squash field, both the biological method and serological test were done (Yesil, 2014; Bos, 1969). Different virus borne disease symptoms like mosaic, leaf crinkling, leaf curling, growth stunting etc. were found in the field and observed. Then the samples were collected and set for the serological test in BARI (Bangladesh Agricultural Research Institute). Among the eight samples, five samples were found positive against the antiserum of CMV. The samples which reacted negatively might be the other virus disease for which the antiserum was not used. The result was more or less similar with and Begum *et al.* (2016) and; Rahman *et al*. (2020).

The result of this study revealed that treatment T_4 (Reflecting mulch) showed better performance among the other treatments in case of disease incidence (%), disease severity (%), growth and yield contributing characters.

The disease incidence (%) at 60 DAS ranged from 22.22% to 83.33%. The highest disease incidence (%) (83.33%) was found in T_0 (Control) which was identical with T_1 (Intercropping by coriander, 66.67%) and T_7 (Mahogany bark extracts, 72.22%) and the lowest disease incidence (%) (22.22%) was found in T_4 (Reflective mulch) followed by T_5 (Neem leaf extracts, 44.44%). The remaining treatments were moderate in disease incidence (%). The disease severity (%) based on last observation at 60 DAS ranged from 11.10% to 83.30% among different treatments. The highest disease severity (83.30%) was found in T_0 (Control). The lowest disease severity (%) was found in T_4 (Reflective mulch) followed by T_5 (Neem leaf extracts, 24.96%) which were statistically identical with each other. The remaining treatments were moderate in disease severity (%) ranged from 33.33% to 61.11%. Almost such type of investigation on viral disease incidence (%) and severity (%) in field was observed by Csizinszky *et al*. (1997) using reflecting mulch in vegetable crops; Chalfant *et al*. (1077) using the reflective mulch in summer squash and Brown *et al*. (1993) using mulch in cucurbits. Sharma *et al.* (2017) also founded that viral disease incidence and severity (%) reduced in watermelon while treating with different plant extracts including neem. Almost such type of result was founded by Damicone *et al*. (2007) using wheat as border crop in muskmelon against the mosaic disease.

In case of growth contributing characters, average leaves number per plant varied from 7.33 to 13.00. Maximum no. of leaves per plant was found in T_4 (reflecting mulch) and minimum no. of leaves per plant was recorded in T_0 (control plot). The area of healthy leaf varied from 604.29 cm^2 to 920.57 cm^2. The highest (920.57 cm^2) area of healthy leaf was found in T_4 (reflecting mulch) followed by T_5 (Neem leaf extracts, 816.93 cm^2) which was statistically identical with each other. The lowest (604.29 cm^2) area of healthy leaf was found in T_0 (Control). The area of diseased leaf varied from 239.33 cm^2 to 368 cm^2. The highest (368 cm^2) area of healthy leaf was found in T_4 (reflecting mulch) and the lowest (239.33 cm^2) area of diseased leaf was found in T_0 (Control). The leaf area reduction varied from 25.23% to 74.35% among different treatments.

The maximum (74.35%) leaf area reduction was found in T_0 (control plot) and minimum value (25.23%) was found in T_4 (reflecting mulch). The results of the study were more or less similar with the findings of Sharma *et al*. (2017) in watermelon.

In case of yield contributing characters, average no. of fruit, average length of fruit, average diameter of fruit was highest in T_4 (reflecting mulch) and lowest in T_0 (control plot). Average fruit weight plant^{-1} ranged from 1.27 to 6.98 kg. The highest fruit weight plant^{-1} (6.98 kg) was recorded in T_4 (reflecting mulch) while the lowest fruit weight per plant was recorded in T_0 (control plot). From the above results it can be concluded that average fruit weight plant^{-1} was more in T_4 (reflecting mulch) than the other treatments also in case of fruit weight per plot. Fruit yield (ton/ha) varied from 15.20 to 83.80 ton/ha whereas the highest fruit yield was found in T_4 (reflecting mulch) and the lowest fruit yield was recorded in T_0 (control plot). Results of the present study showed that avoidable yield loss (%) was also maximum in T_4 (reflecting mulch) than the other treatments over T_0 (control plot). The results indicated that the yield of squash was positively influenced by number of leaves, number of fruits and fruit weight. The results of the study were more or less similar with the findings of Sharma *et al*. (2017) while experimented with different biopesticides in watermelon. Wyman (1979) also found more or less similar result that the yield of summer squash increased while using aluminum and white plastic mulch.

In insect's infestation, the number of aphid population plant^{-1} at 60 DAS differed from 7.33 to 21.00. Maximum (21.00) number of aphid plant^{-1} was observed in T_0 (control plot). On the contrary, minimum (7.33) aphid plant^{-1} was observed in treatment T_4 (reflecting mulch) followed by T_5 (Neem leaf extracts, 9.66).

The relationship between CMV disease incidence (%) of squash and insect population was investigated. During experiment a strong positive correlation (r= 0.953) was found between disease incidence (%) of viral infection of squash and insect population and which was supported by Aboul-Ata *et al*. (2000). Verma *et al*. (1989) stated that the disease incidence was directly related to the population density of the vector developed.

In the present study the increasing of insect population was also found to be positively correlated with the spread of viral infection in the field which was almost similar with Rahman *et al*., (2010); Gupta (2000).

The relationship between disease severity (%) of viral infection of squash and insect population was investigated. During experiment a strong positive correlation (r=0.9733) was found between disease severity (%) of viral infection of squash and insect population and which was supported by Aboul-Ata (2000).

The present study also revealed the relationship between the insect population and yield of squash. There was a negative correlation between the insect population and yield of squash which was supported by Gupta (2000) and Greenough (1990).

There also a negative correlation (r = -0.7649) between the incidence of viral disease and yield of squash was found and which was in accordance with Gupta's (2000) findings.

The present study also found that yield (ton/ha) of squash was negatively correlated with CMV disease incidence (%) and disease severity (%). The correlation value (r) of disease incidence (%) and yield (ton/ha) was -0.8972 and disease severity (%) and yield (ton/ha) was -0.7990 which was supported by the study of Aliyu (2010); Asare *et al*. (2014) and Smith *et al*. (1964).

The relationship between disease severity (%); leaf area reduction (%) and average yield (ton/ha) was observed. The value of average yield (ton/ha) decreased with the increase of the value of disease severity (%) and leaf area reduction (%). The value of average yield (ton/ha) increased with the decrease of the value of disease severity (%) and leaf area reduction (%) which was supported by Delmiglio and Pearson (2006).

The relationship between disease severity (%) and number of fruits plant^{-1} was also investigated. It was found that the number of fruits plant^{-1} decreased with the increase of disease severity (%) and the number of fruits plant^{-1} increased with the decrease of disease severity (%). The result was supported by De Palma *et al*. (2019).

The present study showed the possible capability of different cultural practices and plant extracts to reduce the disease incidence and severity of CMV disease in squash. From the overall management study, reflecting mulch (aluminium foil) followed by neem leaf extract found the best against the CMV disease in squash. This may be for the cause of mulches protect the crop during the early growing season from insect herbivores and delay the onset of insect-vectored viruses (Chalfant *et al*., 1977 and; Wyman, 1979) and neem leaf exhibited a potency of reducing virus was highly significant because of its higher azadirachtin and nimbin contents (Wisler, 1998 and; Ahmed, 2015) in extracts.

CHAPTER V

SUMMERY AND CONCLUSION

Virus disease is a serious problem of squash cultivation in the field. Due to this, the production squash is hampered and yield loss can be high. No resistant variety of squash is presently available in Bangladesh and there is also a lack of effective method to manage the viral disease. Therefore, the present study was initiated to develop an effective management strategy by using different cultural practices and plant extracts against viral diseases in squash.

Two types of mulches viz reflecting mulch (aluminum foil), black polythene mulch; intercropping by coriander, border cropping by wheat; three type of plant extract viz neem leaf, papaya leaf and mahogany bark were used for evaluating their efficacy to manage the viral diseases in field condition. Data were collected on the disease incidence and severity and its impact on growth and yield contributing characters such as leaves plant^{-1}, healthy leaf area (cm^2), diseased leaf area (cm^2), reduction of leaf area (%), fruits plant^{-1}, fruit weigh plant^{-1}, fruit weight plot^{-1}, insect population plant^{-1}, yield (ton/ha), avoidable yield loss (%) of fruit under different treatments.

Different symptoms like mosaic, curling, crinkling, stunting etc. were observed visually and serological test was done to confirm the virus diseases in squash. Five samples were found positive against the antiserum of CMV among eight samples. Remaining of the samples, giving negative result may be due affected by other viruses which antiserum were not used.

The results of the study revealed that the application of reflecting mulch (T_4) significantly reduced the disease incidence and severity. The incidence in case of other treatments did not differ significantly between each other but they were significantly differed from control (T_0).

Considering average disease incidence and severity, the lowest incidence and severity was recorded in T_4 (reflecting mulch) while the highest incidence and severity was recorded in T_0 (Control plot).

The prevalence and spread of disease in squash was positively correlated with the aphid population which indicated that the increase of aphid population in the squash field increased the number of viral infected plants during the study period. However, the disease incidence and disease severity were increased with the increase of aphid population in the field and the relationship was positive and significant. Disease incidence (%) and disease severity (%) were negatively correlated with the yield of the squash. There was also a negative correlation between the insect population and yield of squash. The average yield (ton/ha) was inversely proportional to disease severity (%) and leaf area reduction (%). Similarly, the number of fruits plant^{-1} was inversely proportional to the (%) disease severity.

The correlation and regression analysis revealed that the percent reduction of growth and yield contributing characters due to viral infection had pronounced effect on yield of squash and significant effect was observed in all cases.

The results of the study on all growth and yield contributing characters including the virus incidence suggested that, none of the treatments had impressive level of reduction against viral infection in squash. Although T_4 (reflecting mulch) performed better as compared to other treatments on all over consideration. However, it needs further investigations and observations before final recommendation.

In view of the results the present study may be concluded as-

- The virus disease was confirmed by biological method and DAS-ELIZA test. Among the eight samples, five samples showed positive result when treated with the antiserum of CMV. The samples showing negative result might be affected by other viruses which antiserum were not used.
- CMV was prevalent on squash plant though the infection was varied with treatments. The maximum prevalence (disease incidence and severity were 83.33 % and 45.83 %, respectively) was found in T_0 (Control plot) and the minimum prevalence (average disease incidence and severity were 22.22 % and 5.56 %, respectively) was observed in T_4 (light reflecting mulch).
- There were significant reductions of different growth and yield contributing characters of squash plant. The (%) disease incidence and severity in the field was also positively correlated with the insect population in squash field. The yield was found negatively and appreciably correlated with the insect population, disease incidence and severity.
- Considering the disease incidence and severity, growth and yield contributing characters among the treatment light reflecting mulch and neem leaf extracts gave better results than the other treatments.

CHAPTER VI

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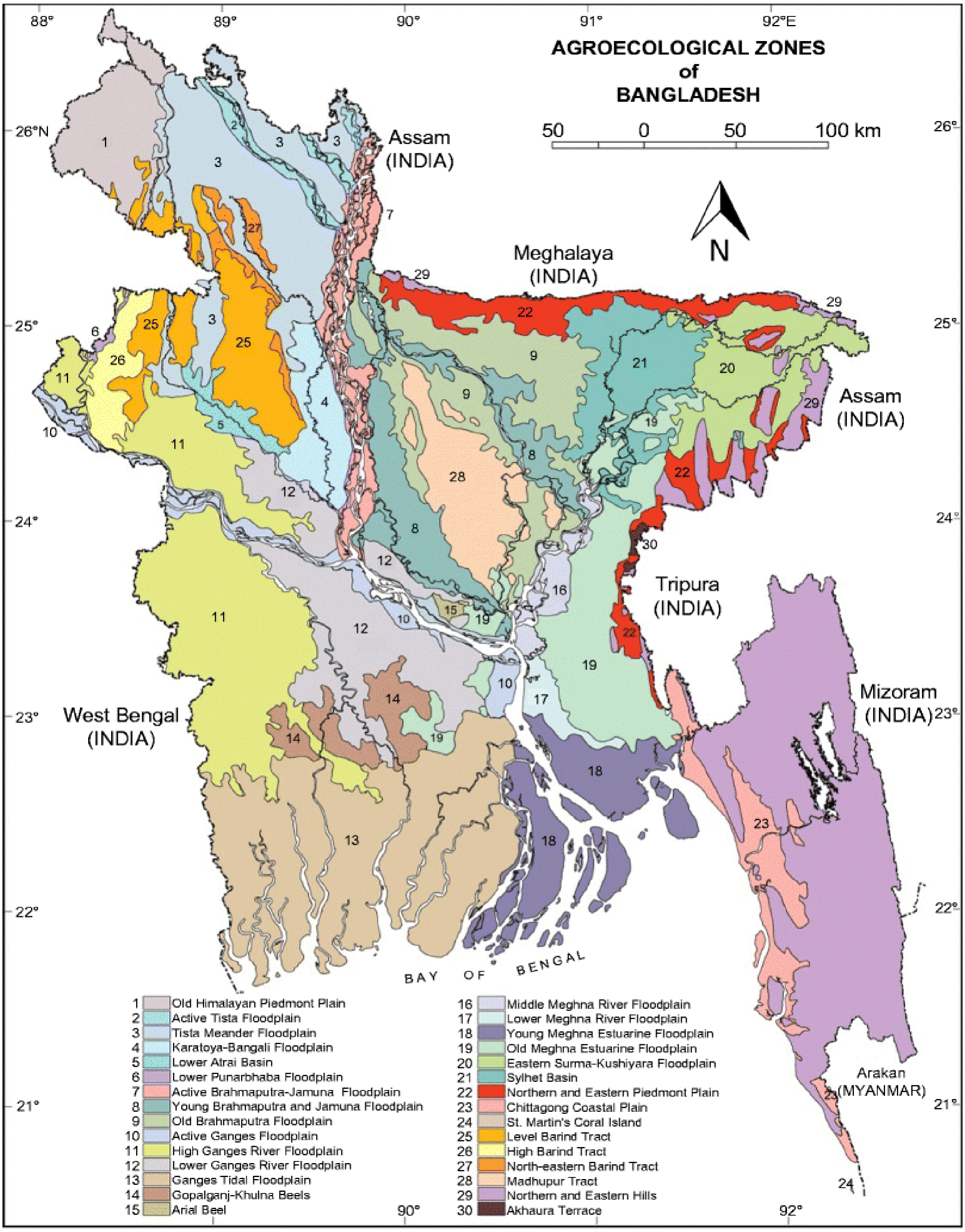
CHAPTER VII

APPENDICES

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



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

Morphological characteristics of soil of the experimental site

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 leveled
Flood level	Above flood level
Drainage	Well drained
Texture	Loamy

Chemical composition

Constituents	**Amount**
Depth	0-15 cm
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 (December, 2020 to March, 2021)

Months	**Temperature (ºC)**		**Relative humidity (%)**
	Maximum	**Minimum**	
November, 2020	34	19	71
December, 2020	20.4	17.3	66
January, 2021	26	15.3	53
February, 2021	29.6	17.4	45
March, 2021	34	21.3	48

Source: Time and date.com/weather/Bangladesh, Dhaka