EFFECT OF PLANT EXTRACTS AND CULTURAL PRACTICES ON *CUCUMBER MOSAIC VIRUS* DISEASE OF CAPSICUM

BY

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*This is to certify that the thesis entitled "**EFFECT OF PLANT EXTRACTS AND CULTURAL PRACTICES ON CUCUMBER MOSAIC VIRUS DISEASE OF CAPSICUM**", Sher-e-Bangla Agricultural University (SAU), Dhaka-1207 in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN PLANT PATHOLOGY**, embodies the results of a piece of bonafide research work carried out by **Ismam Aurin, Registration no.: 15-06684,** 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.

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ABSTRACT

The present investigation was initiated to evaluate the effects of plant extracts (neem leaf extract, garlic bulb extract, mahogany bark extract) and cultural practices (aluminium foil as reflective mulch, coriander as intercrop, marigold as border crop) for the management of cucumber mosaic viral disease of capsicum. The experiment was carried out at the research field of Sher-e-Bangla Agricultural University from November 2020 to April 2021. During the investigation, visual symptom observation and serological test, DAS-ELISA, confirmed that *Cucumber mosaic virus* (CMV) had infected capsicum and various types of symptoms like a mosaic, shoestring leaf, vein banding and stunted growth were observed in the field. The highest CMV incidence and severity were found on T_0 (control), 75% and 45% respectively, whereas the lowest CMV incidence (8.33%) and severity (22.30%) was observed on T_4 (neem leaf extract). The tallest plants (35.75 cm) were found in T_5 (aluminium foil as reflective mulch) and the shortest plants were seen in T_0 (control), 30.33 cm. The maximum number of healthy leaves were observed in T_5 (aluminium foil as reflective mulch) whereas the lowest number healthy leaves were found in T_0 (control). The lowest infected leaves and minimum leaf area reduction (24.33%) was observed in T_4 (neem leaf extract) compared to T_0 (control). Yield attributes were highest in T_5 (aluminium foil as reflective mulch) which were the highest number of fruits per plant (13.33), heaviest fruits (62.10 g), highest fruit yield per plant (725.74 g), highest yield per plot (2.78 Kg), highest total yield (5.56 ton/ha) and lowest yield loss (17.68%) compared to T_0 (control). A significant decrease in the number of aphids per leaf was observed in T_4 (neem leaf extract) compared to T_0 (control) and other treatments. The correlation-regression analysis established that there was a positive correlation between CMV incidence and severity. Total yield negatively correlated with CMV incidence (%) and severity (%) with 74.2% and 95.7% yield reduction due to an increase of CMV incidence and severity, respectively.

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ABBREVIATIONS AND ACRONYMS

AEZ	:	Agro-Ecological Zone
BARI	:	Bangladesh Agricultural Research Institute
BBS	:	Bangladesh Bureau of Statistics
CV%	:	Percentage of Coefficient of Variance
et al.	:	And others
M.S.	:	Master of Science
No.	:	Number
p^H	:	Negative logarithm of hydrogen ion concentration
pNPP	:	Para-Nitrophenylphosphate
IU	:	International Unit
ppm	:	Parts per Million
USDA	:	United States Department of Agriculture

CHAPTER I

INTRODUCTION

Capsicum (*Capsicum annuum* L.) belongs to the Solanaceae family and originated in Mexico (Hunziker *et al*., 1950). Capsicum is ecologically a perennial shrub in tropical areas but is usually grown as an herbaceous annual crop in temperate regions. The species "*Capsicum annuum*" usually refers to non-pungent sweet pepper, more commonly known as capsicum or bell pepper (OECD, 2006). The vegetable is considered an excellent source of Vitamin C and other antioxidants containing 39 calories, 1.5g of protein, 9g of carbohydrates, and 0.5g of fat (GMF, 2008).

Capsicum has been cultivated on a small-scale around peri-urban areas primarily to supply some city markets in Bangladesh (Hasanuzzaman *et al*., 2007). Capsicum production in Bangladesh was only 11 Metric Ton (MT) in 8 acres at 2018-2019 which rose to 176.5 MT in 54.02 acres at 2020-2021. Although initially capsicum was cultivated only in Sylhet district in 2018-2019, later other districts like Bhola, Bogura, Chuadanga, Dhaka, Sherpur and Rangamati picked up the trend (BBS, 2021). Due to the raising demand for capsicum, Bangladesh Agricultural Research Institute (BARI) released two varieties, BARI Mistimorich-1 and BARI Mistimorch-2, in 2009 and 2015 respectively.

Farmers have suffered many constraints in capsicum production including flower dropping, poor fruit setting and susceptibility to virus diseases (Hasanuzzaman *et al*., 2007). Berke *et al*. (2005) and Arogundade *et al*. (2015) said capsicum is susceptible to several pathogens, fungal, bacterial and viruses which causes severe yield loss. However, *Cucumber mosaic virus* (CMV) is a notable one among the 35 different viruses that can infect peppers (Green and Kim, 1991). CMV has an extensive host range and is transmitted by aphids in a non-persistent manner (Jiang *et al*., 2004).

CMV is the most predominant and destructive virus and has jeopardized chilli cultivation in many countries. The most prominent symptoms of CMV in peppers are mild mosaic and dull-coloured leaves, mottling, shoestring, fern leaf, vein banding, vein clearing, leaf deformation, stunted growth and reduced fruit size (Arogundade *et al*., 2019).

Reports on disease incidence and yield loss caused by virus diseases in capsicum and other Solanaceous crops are well documented. Myti *et al*. (2014), Rahman *et al*. (2015) and Rahman *et al*. (2016) recorded high CMV incidence in capsicum Bangladesh. Moreover, CMV reduced plant height, canopy diameter, fruit number per plant, fruit length and fruit weight which lowered the yield. *Pepper veinal mottle virus* (PVMV), *Pepper mild mottle virus* (PMMoV), *Pepper leaf curl virus* (PepLCV), CMV resulted in high disease incidence and caused significant yield loss in capsicum (Alegbejo and Abo, 2002; Martínez-Ochoa *et al*., 2003; Wu *et al*., 2006). CMV was able to cause 80% loss of marketable fruits per plant and fruit weight per plant with 100% disease incidence in capsicum (Avilla *et al*., 1997; Persley *et al*., 2011).

Chemical insecticides are considered uneconomical as they are generally ineffective in controlling non-persistent viruses (Kenyon *et al*., 2014). As a result, management options for virus infection in *Capsicum* spp. includes integration of several approaches such as the use of protected nurseries, cultivation of disease-resistant varieties and ensuring adequate photo-sanitary conditions after transplanting (Arogundade *et al*. 2020). Mitiku *et al*. (2013) said intercropping maize with pepper led to a lower incidence of potyviruses and unmarketable yields, and higher total and marketable yields in pepper fields compared to mono-cropping. Arogundade *et al*. (2019) noticed white plastic mulch had the lowest disease incidence of 34.43% in sweet pepper. Shabbir *et al*. (2020) observed neem extracts resulted in the lowest CMV incidence (15.49%) in cucumbers.

The lack of virus-resistant or tolerant cultivars, the presence of viruses and their vectors around the year and the cultivation of crops in numerous small plots over a large area with little isolation led to difficult control of plant viruses in Bangladesh (Gonsalves and Garnsey, 1989). As alternative and eco-friendly management of plant viruses have gained increased importance in agricultural practices, there is a lack of reports on using plant extracts or cultural practices for managing mosaic diseases in capsicum in Bangladesh.

Hence, the present investigation was initiated to evaluate the effects of some plant extracts and some cultural practices for the management of the *Cucumber mosaic virus* (CMV) of capsicum in field condition and lab condition.

Considering the above mentioned facts, the present experiment was undertaken to achieve the following objectives:

1. To identify *Cucumber mosaic virus* (CMV) by visual observation and serological test; and

2. To find out the effective strategies for management of *Cucumber mosaic virus* disease of capsicum by using plant extracts and cultural practices; and

3. To determine their effects on growth and yield attributes of capsicum

CHAPTER II

REVIEW OF LITERATURE

Capsicum (*Capsicum annuum* L.) is a newly introduced crop in Bangladesh. It is in demand as a vegetable because of its colourful appearance and highly nutritious fruits. However, virus diseases has become a massive threat to their production. This chapter presents the available literature on the virus diseases of capsicum and the different methods of managing virus diseases.

2.1. Origin and Distribution of Capsicum

The genus *Capsicum* belongs to the family Solanaceae and includes approximately 32 species and 5 of which are domesticated widely, namely, *Capsicum annuum*, C. *baccatum*, C. *chinense*, C. *frutescens*, and C. *pubescens*, with different terms depending on the region of cultivation. However, it is considered that C. *annuum* is the species with higher economic importance and is the only native of Mexico (Hunziker *et al*., 1950).

In general, most researchers agree that the cultivated *Capsicum annuum* var. *annuum* originated from the wild C. *annuum* var. g*labriusculum* (Mangelsdorf *et al*., 1965). McClung de Tapia (1992) said the oldest macro-botanical remains identified as domesticated C. *annuum* retrieved from preceramic strata of dry caves in two state of Mexico. Pickersgill (1997) agreed the genus *Capsicum* originated from South and Central America where it is still under cultivation. Columbus introduced several pungent forms of C*apsicum*, most of which were members of the species C. *annuum* to Europe after he travelled to America. From there, it was enthusiastically and rapidly incorporated into many cultures (Walsh and Hoot, 2001).

In Asia, capsicum was introduced to the Philippines, Japan and China by 1888. Currently, China is the largest producer of capsicum, followed by Mexico and Indonesia, Spain, Turkey, and the United States (Biswas *et al*., 2018). In China, pepper has the largest cultivated area, with an annual planting area of 2.13 million hectares and output of more than 25 million tons (Wang *et al*., 2019).

2.2. Nutritional Status of Capsicum

The nutritive value of capsicum is high as it contains 1.29 mg protein, 11 mg calcium, 870 IU vitamin A, 17.5 mg ascorbic acid, 0.6 mg thiamin, 0.03 mg riboflavin and 0.55 mg niacin per 100 g edible of fruit (Joshi and Singh, 1975). Capsicum is an excellent source of vitamin C and vitamin A followed by vitamin B6, folic acid, beta-carotene, and fibre and it also contains lycopene which is believed as important for reducing the risk of certain cancers (GMF, 2008).

Interestingly, capsicum is the only member of the genus *Capsicum* that does not produce ‘capsaicin’, a lipophilic chemical that can cause a strong burning sensation when it comes in contact with mucous membranes. The species “*Capsicum annuum*” usually refers to non-pungent sweet pepper, more commonly known as capsicum or bell pepper (OECD, 2006). Sweet pepper cultivars produce non-pungent capsaicinoids which is why their Scoville Heat Unit (SHU) is 0 while chillies with a slight bite may have 100 to 500 SHU (Macho *et al*., 2003; Nadeem, 2011).

According to USDA, one cup of chopped, raw red bell pepper (149g) provides 39 calories, 1.5g of protein, 9g of carbohydrates, and 0.5g of fat (Appendix V). Red bell peppers are an excellent source of vitamins A and C. Raw green bell peppers have slightly fewer carbs and less fibre than their red counterparts, with 6.9g of carbohydrate and 2.5g of fibre per cup.

2.3. Production Status of Capsicum in Bangladesh

Capsicum is ecologically a perennial shrub in tropical areas but is usually grown as a herbaceous annual crop in temperate regions (OECD, 2006).

Hasanuzzaman *et al*. (2007) said capsicum was cultivated on a small-scale around peri-urban areas primarily to supply some city markets in Bangladesh. Due to the diversity of colour and abundance of nutritional benefits, capsicum recently gained popularity in Bangladesh.

Capsicum production in Bangladesh was nonexistent till 2017 and it began in 2018-2019 when only 8 acres were under cultivation. Capsicum production rose from 11 MT in 2018-19 to 176.5 MT in 2020-21. Although initially capsicum was cultivated only in Sylhet district in 2018-19, later, other districts like Bhola, Bogura, Chuadanga, Dhaka, Sherpur and Rangamati picked up the trend (BBS, 2021). Due to the rising demand for capsicum, Bangladesh Agricultural Research Institute (BARI) released two varieties, BARI Mistimorich-1 and BARI Mistimorch-2, in 2009 and 2015 respectively.

2.4. Common Diseases of Capsicum

Hull and Davies (1992) reported that the low yield of chilli crops mainly occurred due to biotic and abiotic factors.

Suzuki and Mori (2003) observed pepper was more susceptible to biotic factors including fungi, bacteria and viruses.

Anthracnose, cercospora leaf spot, phytophthora blight, phytophthora root rot, bacterial wilt, bacterial spot, bacterial blight, fusarium wilt, root-knot nematodes, aphids, thrips, viruses like *Chili veinal mottle virus* (ChiVMV), *Chili leaf curl virus* (CLCV), *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY), Tobamoviruses like *Tobacco mosaic virus* (TMV), T*omato mosaic virus* (ToMV) and *Pepper mild mottle virus* (PMMV) are the some of the major biotic constraints in chilli and pepper production worldwide (Berke *et al*., 2005; Ridzuan *et al*., 2018).

Hasanuzzaman *et al.* (2007) shared farmers suffered many constraints in capsicum production including flower dropping, poor fruit setting and susceptibility to virus diseases. However, virus infection is an alarming threat to cultivated pepper.

Arogundade *et al*. (2014) found several pathogens attacked *Capsicum* sp. and high yield loss was caused by viruses, most of which are vectored by whitefly and aphids.

2.5. Major Viruses in Capsicum

Green and Kim (1991) found 35 different virus species able to infect peppers which are *Pepper mild mottle virus* (PMMoV), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Tomato yellow leaf curl virus* (TYLCV), *Chilli veinal mottle virus* (ChVMV), *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV) etc. as the most frequent pepper-infecting viruses.

Green (1992) said *Chilli leaf curl virus* (ChLCV), CMV and ChVMV were the most destructive affecting chilli cultivation and CMV was particularly severe in chilli in Asia.

Crescenzy (1993) said CMV infected a greater variety of vegetables, ornamentals, weeds, and other plants than other viruses.

Alegbejo (2002) reported the viruses infected capsicum individually or in combination and caused considerable yield reduction.

Pernezny *et al*. (2003) found five begomoviruses of capsicum in America and only one of them, ChLCV was from Asia. However, these viruses did not induce serious diseases in peppers.

Diaz-Perez (2010) said TYLCV infected tomato as the primary host though it can infect sweet pepper, chilli, tobacco, peppers etc. As well.

Kumar *et al*. (2011) observed TMV and ToMV both infected tobacco, tomato and peppers.

Kenyon *et al*. (2014) said many Begomovirus species from pepper in Asia caused diseases in pepper in Bangladesh, China, India, Indonesia, Thailand, and Vietnam.

Despite all these virus diseases, CMV is the most predominant and destructive virus that poses a threatening hazard to chilli cultivation (Ramesh and Sreenivasulu, 2018).

2.6. *Cucumber mosaic virus* (CMV)

Doolittle (1916) first described the *Cucumber mosaic virus* (CMV) in detail 1916 on cucumber and other cucurbits. CMV belongs to the genus Cucumovirus in the family Bromoviridae. CMV is a sense, single-stranded RNA virus with isometric particles (28-30 nm diameter) and has a tripartite genome (RNA1, RNA2 and RNA3) that encodes five open reading frames (ORFs) (Brunt, 1996; Palukaitis and Garcia-Arenal, 2003).

CMV has an extensive host range and is transmitted by aphids in a non-persistent manner (Jiang *et al*., 2004). Meena and Manivel (2019) said CMV has a wide host range and infects more than 1287 plant species.

2.7. Symptoms of *Cucumber mosaic virus* (CMV) in Capsicum

Green and Kim (1991) observed some common symptoms of *Cucumber mosaic virus* (CMV) infection in peppers including mottle, mosaic, vein clearing, yellow discoloration, shoe-stringing, severe stunting, reduced flower formation and fruits were small, malformed, bumpy and patchily discoloured with depressed spots or necrotic lesions, leading to significantly reduced fruit yield and quality.

Zitter and Murphy (2009) found CMV infection in young plants caused slightly wrinkled or bumpy and pale green early leaves, later, ringspot developed as well. Newer leaves developed a chlorotic mosaic pattern when they emerged and later encompassed the entire leaf.

CMV infection in young peppers were more severe symptoms, while older plants were asymptomatic (Kenyon *et al*., 2014).

Rahman *et al*. (2016) noticed mild mosaic, mottling, shoestring, fern leaf, vein bending, vein clearing and leaf deformation as prominent symptoms of CMV in the naturally infected chilli plants. Growth retardation resulted in the stunting of plants was observed.

Olobashola *et al*. (2017) monitored narrow leaves that dropped prematurely in susceptible varieties of capsicum as an expression of CMV infection. However, late-infected plants may show foliar mottling or no symptoms.

Kapoor *et al*. (2018) and Arogundade *et al*. (2019) said all strains of CMV-infected peppers expressed their symptoms differently depending on the age of the plant at the time of infection. Although the symptoms vary, the most prominent ones are mild mosaic and dull-coloured leaves, mottling, shoestring, fern leaf, vein banding, vein clearing, leaf deformation, stunted growth and reduced fruit size.

2.8. Transmission of *Cucumber mosaic virus* (CMV) in Capsicum

Cucumber mosaic virus (CMV) is transmitted by the mechanical inoculation of plant sap and aphids are the most important means of natural transmission (Edwardson and Christie, 1986).

Palukaitis *et al*. (1992) said that the transmission of CMV was mainly by *Myzus persicae* and *Aphis gossypii*, but it can be transmitted by other species of aphids as well.

Weed also serve as a repository for CMV and a primary source of inoculum for the development of disease epidemics (Grube *et al*., 2000).

Jiang *et al*. (2004) said CMV has an extensive host range and is transmitted by aphids in a non-persistent manner. Cerkauskas (2004) said CMV is not transmitted through pepper seed.

2.9. Disease Incidence of Virus Diseases in Capsicum

Soh *et al*. (1977) said the cultivar, the management practices, the environment and the infection stage were some of the deciding factors for the severity of the viral diseases of chilli.

Alegbejo and Uvah (1986) observed *Pepper veinal mottle virus* (PVMV) incidence in experimental fields (74.5% and 50%) and on farmers' fields (80-100%).

Bhagathi and Goswani (1992) found there was a positive correlation between disease incidence and the population of the vector as usually viral infections were controlled by preventing their vectors from attacking the host plants. Butler and Henneberry (1992) described that the reduction of insect infestation resulted in minimum disease incidence.

Martínez-Ochoa *et al*. (2003) reported disease incidence ranged from 20-80% *Pepper mild mottle virus* (PMMoV).

In Korea, 32 pepper production regions were affected by CMV (33.2%), *Pepper mottle virus* (PepMoV) (18.5%) and PMMoV (13.9%), and a total of 26.2% of the reported cases were caused by multiple infections of these three viruses (Kim, 2005).

Shah *et al*. (2008) said *Chilli veinal mottle virus* (ChVMV) was a major prevalent virus with an incidence range of 50%.

Persley *et al*. (2011) said the incidences of CMV and *Tomato spotted wilt virus* (TSWV) in capsicum were up to 100% and 80% respectively.

Myti *et al*. (2014) found the presence of CMV, *Potato virus Y* (PVY) and ChVMV in chilli in Bangladesh where the disease incidence was 11.74% to 55.90% depending on the locations in Sylhet and Rangpur districts. Asare-Bediako *et al*. (2014) suggested that controlling the incidence and severity of the *Okra leaf curl virus* (OLCV) on okra successfully increased yield.

Rahman *et al*. (2015) said CMV incidence ranged from 16.75-25% in different capsicum varieties where maximum reduction in plant height (50.89%), canopy diameter (51.48%), fruit number per plant (80.18 %), fruit length (45.81%) and fruit weight (60.97%) was recorded at an early stage of CMV infection.

Rahman *et al*. (2016) recorded 3-21.21% CMV incidence in chilli depending on different districts of Bangladesh. The highest canopy diameter/spreading reduction (36.84%) was recorded in Balujhuri and the lowest canopy diameter reduction (8.88%) were recorded in variety Comilla-2 because of CMV infection.

Spheia *et al*. (2017) said an increase in disease incidence coincided with the build-up of the aphid population.

Tanvi *et al*. (2020) found CMV incidence of 9.30-71.3% in Himachal Pradesh in India.

Deloko *et al*. (2022) said the prevalence of CMV in capsicum was 78.33% in Cameroon.

2.10. Yield Loss Caused by Viruses in Capsicum

Joshi and Dubey (1973) found 41% yield loss in chilli peppers by *Cucumber mosaic virus* (CMV).

More than 60% yield losses by CMV have been reported in peppers by Florini and Zitter (1987).

Singh and Cheema (1989) said 60-100% yield loss occurred in the early infection of CMV.

Avilla *et al*. (1997) observed early inoculation of CMV to capsicum caused up to 80% loss of marketable fruits per plant and marketable fruit weight per plant.

Alegbejo and Abo (2002) reported 54.5-64.3% yield loss of sweet pepper due to *Pepper veinal mottle virus* (PVMV).

Martínez-Ochoa *et al*. (2003) recorded 50-100% yield loss for *Pepper mild mottle virus* (PMMoV).

Kumar *et al*. (2006) said that *Pepper leaf curl virus* (PepLCV) caused severe epidemics in India and different countries of Africa, Moreover, PepLCV caused 100% crop loss in causing Indian farmers to withdraw from chilli cultivation. Guldur and Caglar (2006) reported 60-95% disease incidence for the PMMoV virus which resulted in 75-95% yield loss.

Wu *et al*. (2006) said CMV resulted in marketable yield losses of 20-40% in most of the pepper-growing regions in China.

Fajinmi and Odebode (2010) found a significant negative correlation between disease incidence, severity and the fruit yield of pepper.

Fajinmi *et al*. (2012) supported that the increase in PVMV disease incidence and severity significantly lowered the fruit yield of pepper.

Rahman *et al*. (2016) found CMV infection reduced the number of fruits (49.63-89.29%), length of fruits (14.03-44.14%), and weight of fruits (10.56-36.96%) in chilli which caused remarkable yield loss.

Rahmatullah (2018) found a negative relation between CMV disease severity (%) and yield (in kg) per treatment indicating that with the increase in disease severity (%) caused the yield of pumpkin to decrease.

2.11. Management Strategies for Viruses in Capsicum

Jayarajan and Doraiswamy (1986) said there is no safe and economical chemical (viricide) for application to virus-infected plants.

Gonsalves and Garnsey (1989) observed the lack of virus-resistant or tolerant cultivars, the presence of viruses and their vectors around the year and the cultivation of crops in numerous small plots over a large area with little isolation led to difficult control of plant viruses.

Green and Kim (1991) suggested organic manures, organic and other types of mulches, aluminium foil strips, insect traps, mineral oil sprays, skimmed milk sprays, whitewashes and the cultivation of non-susceptible barrier crops to lower virus infection in growers’ fields .

Kenyon *et al*. (2014) said insecticides would be ineffective to control non-persistently transmitted viruses because transmission would occur during probing by the vector and before insecticides are activated.

Arogundade *et al*. (2020) proposed management options for virus infection in *Capsicum* spp. by the integration of several approaches. These include the use of protected nurseries, cultivation of disease-resistant varieties and ensuring adequate phytosanitary conditions after transplanting.

Patel *et al*. (2021) claimed mulch can be effectively used in integrated pest management, as it reduced or eliminated the need for insecticide applications. Hence, the cultural methods when combined with other management practices help in containing the disease through vector control.

2.12. Effect of Different Plant Extracts on Virus Diseases of Capsicum

Schmutterer (1990) said neem derivatives would not disturb ecosystems and consequently would not cause outbreaks of new pests whereas insecticides would.

Neem extract, neem oil and seed kernel extract effectively repelled vectors (whitefly) and decreased the incidence of *Okra yellow vein mosaic virus* (OYVMV) and also increased okra yield (Ali *et al*., 2005; Pun *et al*., 2005; Ali *et al*., 2014).

Before the discovery of synthetic pesticides plant or plant-based products were the only pest-managing agents available to farmers around the world (Georges *et al*., 2008).

Madhusudhan *et al*. (2011) observed *Bougainvillea spectabilis* extract was most effective in reducing the tobamovirus concentration in bell pepper and tomato.

Moyin-Jesu (2011) found modified neem leaf extract decreased insect population, number of damaged leaves and number of holes per plant in maize by 33%, 70% and 30% respectively compared to the sole neem leaf extract.

Khan *et al*. (2011) found neem extract and garlic extract significantly reduced *Cucumber mosaic virus* (CMV) infection by 23.85% and 24.88% respectively and aphid population (2.83 and 2.94 respectively).

Asare-Bediako *et al*. (2014) found neem extracts lowered *Okra leaf curl virus* (OLCV) incidence and severity in Okra and there was a negative correlation between fruit yield and OLCV severity.

Kusumawati *et al*. (2015) observed higher concentration of *Mirabilis jalapa* leaf extract resulted in lower *Cucumber mosaic virus* (CMV) incidence (31-45%) and a longer incubation period in tomato.

Iftikhar *et al*. (2017) concluded that garlic extract (5% conc.) showed effective results in the reduction of *Chilli leaf curl virus* (ChLCV) disease incidence (48.29-50.83%) and whitefly population (6.82-7.74) on chilli varieties.

Mehmood *et al*. (2018) recommended using neem leaf and garlic clove extract to manage *Tomato yellow leaf curl virus* (TYLCV) and reduce whitefly in tomatoes. Jahanzaib *et al*. (2018) found neem extract performed better in controlling the whitefly population and TYLCV incidence (31.46%) in tomatoes.

Abbas *et al*. (2020) found neem extracts significantly reduced the disease incidence (61-65.2%%) of *Cotton leaf curl virus* compared to other plant extracts. Shabbir *et al*. (2020) observed neem extracts resulted in the lowest CMV incidence (15.49%) in cucumbers.

2.13. Effect of Reflective Mulch on Virus Diseases of Capsicum

Johnson *et al*. (1967) found mulching with laminated aluminium foil reduced the spread of the *Cucumber mosaic virus* (CMV) and repelled aphids in gladiolus.

Aluminium-painted plastic mulch delayed the onset of CMV incidence by as much as by 3 weeks and increased yield in squash and cantaloupes (George and Kring, 1971; Brown *et al.,* 1996; Stapleton and Summers, 2002).

McLean *et al*. (1982) witnessed reflective mulch reduced *Watermelon mosaic virus* (WMV) in watermelons by 21%, 30% and 72% in different trials.

Basky (1984) observed significantly lower (83%) virus incidence and higher (98.50%) healthy cucumbers on reflective mulches with reduction in the landing rate of aphids.

Greenough *et al*. (1990) found reduced incidence of *Potato virus Y* (PVY), CMV and *Tomato spotted wilt virus* (TSWV) in peppers and other Solanaceous crops when aluminium painted mulch was used.

Brown *et al.* (1993) established that the silver reflective mulch was superior to the other coloured plastic mulches in reducing aphid populations.

Greer and Dole (2003) claimed the reduction of aphid-transmitted virus diseases and increased yields due to lower incidence of the insect-transmitted virus as one advantage of reflective mulches.

Diaz-Perez (2010) observed growth attributes, marketable and total yields of capsicum were higher on silver mulches.

Kapoor (2012) witnessed minimum disease incidence (20.42%) and maximum yield per plot (6.5 kg/plot) was recorded in silver-coloured polyester films.

Reflective mulch combined with insecticide and yellow trap resulted in a minimum mean aphid population (2.33 per leaf/ plant), lower disease incidence (3%) of bell pepper mosaic virus with maximum fruit yield (5.5 kg/plant) than control was revealed by Spehia *et al*. (2017).

Arogundade *et al*. (2019) observed white plastic mulch had the lowest disease incidence of 34.43% in sweet pepper.

Niemann *et al*. (2021) and Patel *et al*. (2021) found reflective silver mulch helped in suppressing the whitefly population by lowering the number of whitefly eggs, nymphs and adults per leaf which lead to a reduction of the associated disease incidence and higher yield.

2.14. Effect of Intercrop and Border Crop on Virus Diseases of Capsicum

Sastry *et al*. (1977) said virus spread was minimized by growing border crops (maize) before transplanting vegetable crops which prevents incoming viruliferous whiteflies from entering the crop.

Alegbejo and Uvah (1986) observed intercropping maize and sorghum with peppers served as a barrier against the aphids and reduced the incidence of *Pepper veinal mosaic virus* (PVMV). High, tall, border crops would act simply as mechanical barriers impeding the aphid colonization of the protected crop (Alegbejo and Uvah, 1987).

Ahmed *et al*. (1996) found intercropping tomato with coriander worked as a whitefly repellent and could an effective against *Tomato yellow leaf curl virus*.

Difonzo *et al*. (1996) hypothesized that infective aphids which landed on the border crops, lost their virus charge while probing.

Fereres (2000) explained that viruliferous alate aphids would land on border crops where they would lost the virus while probing to identify a suitable host. Thus the border crops acted as natural 'sinks' for non-persistent viruses but did not reduce the number of aphids landing in the protected crop. He found the incidence of *Cucumber mosaic virus* (13%) was reduced in pepper plots surrounded by a sorghum border. Smith and Mcsorley (2000) implied aphids might invest less time in damaging main crops by alighting on and probing various intercrops.

Elena (2001) found a 20% reduction in the population of *Myzus persicae* in peppers grown with maize as a border.

Rizk (2005) recorded that the whitefly population on different tomato strains was significantly lower compared to when intercropped with coriander.

Hilje and Stansly (2008) reported that whitefly abundance and begomovirus incidence were low on tomatoes when intercropped with coriander.

Fajinmi and Odebode (2010) concluded intercropping peppers with tall companion crops reduced PVMV incidence and severity to 17% and 15% respectively compared with the sole pepper cropping.

Kapoor (2012) found maize lowered disease incidence when it was planted as an only border and as both border and intercrop (30.81% and 28.32% respectively).

Mitiku *et al*. (2013) said intercropping maize with pepper led to a lower incidence of potyviruses and unmarketable yields, and higher total and marketable yields in pepper fields compared to mono-cropping.

Sujayanand *et al*. (2016) told coriander and marigolds likely acted to repel aphids which resulted in smaller populations of aphids than the sole crops.

Rahmatullah (2018) found CMV incidence and severity both were the lowest when intercrop (coriander) was implemented which was 21.10% and 11.11%, respectively.

CHAPTER III

MATERIALS AND METHODS

This chapter includes a brief description of the experimental site, soil type, climatic condition, experiment period, experimental design, land preparation, planting materials, crop growing procedure, intercultural operations, treatments and data collection along with statistical analysis. Required materials and methodology are described below under the following headings this chapter.

3.1. Experimental Site

The experiment was conducted at the Agronomy research field of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207. The plot number was 31. The experimental site was situated at latitude 23˚46´ North and longitude 90˚23´ East with an elevation of 8.45 meters from the sea level (Appendix I).

3.2. Soil Type

The area belongs to the Agro-Ecological Zone of the Madhupur tract (AEZ-28) where the pH and CEC are 5.45-5.61 and 25.28, respectively. The soil texture of the study site was silty clay loam, non-calcareous, dark grey soil of the Tejgaon soil series. Soil compositions of the experimental plots were collected from the Soil Resources Development Institute (SRDI), Farmgate, Dhaka (Appendix II).

3.3. Climatic Condition

The experimental site was located in the sub-tropical monsoon climate, which is characterized by heavy rainfall during the Kharif season (May-September) and scanty in the Rabi season (October-March). The average maximum temperature during the period of investigation was 37.8889ºC (98.6ºF) and the average minimum temperature was 13.8889ºC (57.00002ºF). Details of the meteorological data in respect of temperature, rainfall and relative humidity during the period of the experiment were collected from Bangladesh Meteorological Department, Agargaon, Dhaka (Appendix III).

3.4. Experiment Period

The present study was conducted during the period from November 2020 to April 2021.

3.5. Design of Experiment

The experiment was conducted in a Randomized complete block design (RCBD) with 3 replications. Individual plot size was 2.5m x 2.0m and plot to plot distance was 1 m. Each plot was prepared followed by a good tillage.

3.6. Field Preparation

The experimental field was properly ploughed to obtain a good tilth on November 2020. Manures and fertilizers were applied as per the recommendation by Azad *et al*. (2019) (Appendix IV). Cowdung (237 kg/m^2) was applied during final land preparation, Urea, Triple Super Phosphate (TSP), Murate of Potash (MP), Gypsum and Zinc Sulphate were applied at the rate of 5.93, 8.30, 5.93, 2.61 kg/m^2 and 118.50 g/m^2, respectively.

At the time of final land preparation, total cow dung, TSP, Zinc Sulphate and gypsum were applied as basal dose along with 1/3 of urea and 1/3 of MP in the pit during transplanting. The rest of the urea and MP were applied in two splits to at 25 DAT and 50 DAT respectively.

3.7. Seedling Collection and Transplanting

The one-month-old capsicum seedlings were collected from the Department of Agricultural Extension (DAE) and transplanted in the main field 15 days after land preparation maintaining the plant-to-plant distance of 60 cm x 60 cm. After field preparation, the seedlings were transplanted and light irrigation was applied (Appendix VI).

3.8. Intercultural Operations

Necessary intercultural operations were done through the cropping season for proper growth and development of the experimental plants. Subsequent irrigation and fertilization were applied to all the plots when necessary. Weeding, gap filling, drainage and other intercultural operations were done in the plot whenever required. No herbicides, insecticides or pesticides were used in any plots.

3.9. Harvesting

Fruits were harvested based on firmness and data on fruit yield and yield contributing characters were recorded.

3.10. Treatments of the Experiment

In this study, a total of six treatments and a control plot was arranged. Different plant extracts (neem leaf extract, garlic bulb extract and mahogany bark extract) and cultural methods (aluminium foil as reflective mulch, border crop and intercrop) were used to influence viruses and their vectors in capsicum (Plate 1). In control plots, clean water was sprayed only.

The treatments are as follows:

T_0: Control

T_1: Coriander as intercrop

T_2: Garlic bulb extract

T_3: Marigold as border crop

T_4: Neem leaf extract

T_5: Aluminum foil as reflective mulch

T_6: Mahogany bark extract

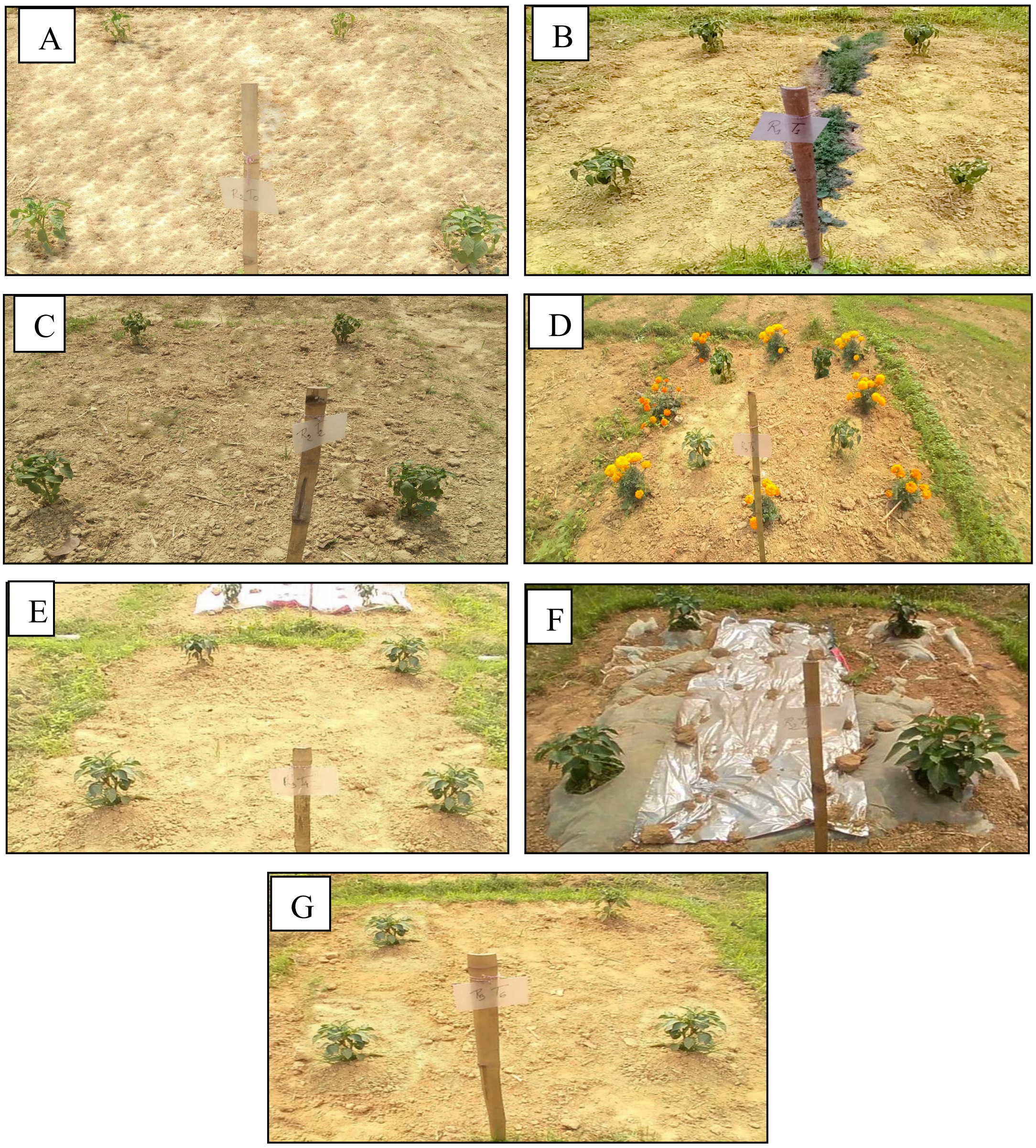


Plate 1. Different treatments applied in field:

(A) T_0 (Control);

(B) T_1 (Coriander as border crop);

(C) T_2 (Garlic bulb extract);

(D) T_3 (Marigold as border crop);

(E) T_4 (Neem leaf extract);

(F) T_5 (Aluminum foil as reflective mulch); and

(G) T_6 (Mahogany bark extract)

3.11. Application of the Treatments

3.11.1. Sowing of Seeds of Coriander as Intercrop

Coriander seed of Lal Teer company was bought from a nearby seed market and was sown on the allotted plots. Seeds were sown in a single line between the lines of capsicum plants and incorporated with light tillage and watering to enhance germination.

3.11.2. Preparation of Plant Extracts

Plant extracts were prepared in the Plant pathology lab of Sher-e-Bangla Agricultural University as described by Asare-Bediako *et al*. (2014) and shown in Plate 2.

Neem leaf and mahogany bark were collected from the trees located nearby the experimental fields and garlic bulbs were bought from local stores (Plate 2A). The collected materials were then blended into powder form in Plant Pathology Laboratory of Sher-e-Bangla Agricultural University. 100 g blended power for individual extracts was weighted in an electric balance (Plate 2B) which was steeped in 1 litre of tap water for 24 hrs. (Plate 2C). After 24 hours, the mixture was strained using cheesecloth to obtain the aqueous extracts (Plate 2D).

After preparation, each of the prepared aqueous plant extracts was sprayed on the allotted plots with capsicum plants by the use of a knapsack sprayer or hand sprayer, starting from 15 DAT with 7 days intervals till flowering occurred (Appendix VII). Before spraying, approximately 10 ml of liquid soap was added to each prepared plant extract to enhance their attachment to the leaf surfaces (Asare-Bediako *et al*., 2014). The spraying was done after 4 pm while the sun was low in the sky to avoid the possibility of the sun rays disintegrating the active ingredients in the aqueous extracts after application.

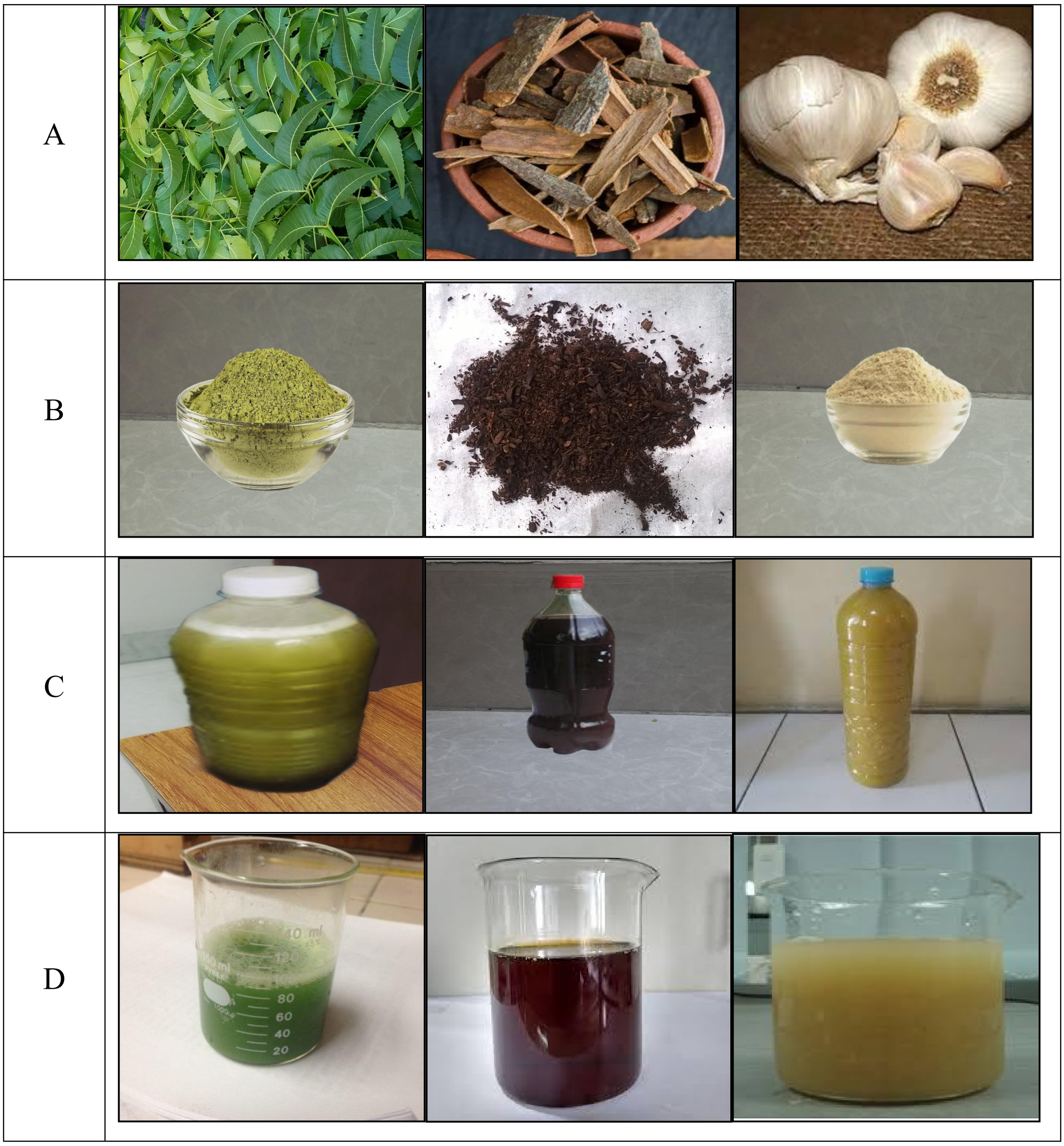


Plate 2. Preparation of plant extracts in lab:

(A) Collection of raw materials;

(B) Blended raw materials (100 gm);

(C) Blended powders steeped in water for 24 hrs; and

(D) Filtered extracts

3.11.3. Transplanting of Border Crops

The border crops (marigold) seedlings were bought from the nearby nurseries and transplanted in the allotted plots nearly two weeks before transplanting capsicum to the main field to allow the marigold to become well established and of substantial height, as suggested by Fereres (2000).

3.11.4. Application of Aluminium Foil Reflective Mulch

Polythene mulch and reflective aluminium foils were bought from Krishi Market. After the final land preparation, the polythene mulch was applied with the aluminium foil on top on the allotted plots 2-3 days prior to transplanting. The entire plots were covered and the edges of the mulch were buried. Necessary holes were made to the mulch in order to transplant the capsicum seedlings in those particular plots (Patel *et al*., 2021).

3.12. Observation of the Symptoms

The plants were inspected weekly in the morning (7 am to 9 am) to note the appearance and development of the virus symptoms related to virus diseases starting from 30 days after transplanting (DAT) to harvest. The capsicum plants which remained asymptomatic until the last harvest were determined as healthy plants. Based on appearance of the symptoms, six samples were collected randomly for further inspection.

3.13. Parameters Assessed

The data on the plant height, the total number of healthy and infected leaves, percentage of disease incidence, severity and leaf area reduction, the number of insect vectors per plant and yield parameters of capsicum plants were collected. The parameters were as follows:

- Plant height (cm)
- Number of healthy leaves per plant
- Number of infected leaves per plant
- % Leaf area reduction (LAR)
- % Disease incidence
- % Disease severity
- Number of aphids per leaf
- Number of fruits per plant
- Weight of individual fruits (g)
- Fruit Yield per plant (g)
- Yield per plot (Kg)
- Total yield (ton/ha)
- Yield Loss (%)

3.14. Data Collection and Calculation

Data collection was started 30 days after transplanting (30 DAT) the seedlings and all the data were collected once in a 15 days interval till 75 DAT. Data over the parameters were taken in the following ways:

3.14.1. Plant Height (cm)

Plant height was measured from all the plants in plots and recorded from 30, 45, 60 and 75 days after transplanting (DAT) to observe the growth rate of the plants in centimeters (cm). Calculating the mean plant height, the average height was recorded.

3.14.2. Number of Healthy Leaves Per Plant

Number of healthy leaves of selected infected plants from each plot at 30, 45, 60 and 75 days after transplanting (DAT) was recorded. Calculating the average number of healthy leaves, the average number was recorded.

3.14.3. Number of Infected Leaves Per Plant

Number of infected leaves of selected infected plants from each plot at 30, 45, 60 and 75 days after transplanting (DAT) was recorded. Calculating the average number of infected leaves, the average number was recorded.

3.14.4. % Leaf Area Reduction (LAR)

Leaf area reduction was recorded from each plot at 30, 45, 60 and 75 days after transplanting (DAT). The reduction of leaf area has been calculated using the following formula by Rahman *et al*. (2016):

$$R = \frac{Y - Y_1}{Y} \times 100$$

Where R= Percent reduction of growth (leaf) contributing character;

Y= Growth (leaf area) contributing characters of healthy plants

Y_1= Growth (leaf area) contributing characters of infected plants

3.14.5. % Disease Incidence

The disease incidence was calculated and expressed in percentage on the basis of infected plants per plot. The percent disease incidence (%DI) was calculated individually for each plot using the following formula mentioned by Rahman *et al*. (2016):

$$\%DI = \frac{X_1}{X_2} \times 100$$

Where, X_1= No. of infected plants, X_2= Total no. of plants

3.14.6. % Disease Severity

The disease severity was calculated and expressed in percentage on the basis of infected plants per plot. The disease severity index was calculated individually using the formulas mentioned by Galanihe *et al*. (2004):

$$\%DS = \Sigma(P \times Q)/ (M \times N)] \times 100$$

Where P = severity score,

Q = Number of infected plants having the same score,

M = Total number of plants observed,

N = Maximum rating scale number

Here, the disease severity was calculated based on a disease scoring scale described by Arif and Hassan (2002):

1 = Healthy plant;

2 = Slight mosaic;

3 = Moderate mosaic;

4 = Severe mosaic (leaf distortion and stunting); and

5 = Severe mosaic (stunting and plant death)

3.14.7. Number of aphids per Leaf

The presence of insect population was observed manually in each infected plant of each plot. The number of aphids per leaf from selected plants from each plot in treatment combinations was counted at 30, 45, 60 and 75 days after transplanting (DAT) from 6 am to 7 am. The mean values were recorded.

3.14.8. Number of Fruits per Plant

The number of fruits harvested from each plant in each plot was counted during harvesting. The mean number of fruits of selected plants for each plot as per treatment combination was recorded.

3.14.9. Average Weight of Fruits (g)

The weight of individual fruits was measured using an electric balance machine during harvest. The mean weight of individual fruits of selected plants was recorded in grams (g).

3.14.10. Fruit Yield Per Plant (g)

The weight of total fruits per plant was measured using an electric balance machine after harvest. The mean value was recorded in grams (g).

3.14.11. Yield per Plot (Kg)

The mean weight of fruits per plot was measured by totalling fruit yield from each unit plot after completing harvesting. An electric balance was used to take the fruit weight per plot which was recorded in kilograms.

3.14.12. Total Yield (ton/ha)

The total yield of each plots of treatment combination was calculated using the following formula:

$$Yield\,(ton/ha) = \frac{\text{Fruit yield per plot}(Kg)x10000m^2}{\text{Plot area }(\text{m}^2)x1000Kg}$$

3.14.13. Yield Loss (%)

Yield loss (%) was recorded after harvesting from each plot by calculating with the following formula by Rahman *et al*. (2016):

$$R = \frac{Y - Y_1}{Y} \times 100$$

Where R= Yield loss;

Y= Yield of healthy plants

Y_1= Yield of infected plants

3.15. Identification of *Cucumber Mosaic Virus* Using DAS-ELISA

Virus identification was done by using a standard double-antibody sandwiched enzyme-linked immunosorbent assay (DAS-ELISA) as described by Clark and Adams (1977) using polyclonal antisera raised against CMV (Bioreba Ag, Switzerland) and process in shown in Plant 3.

The test was carried out in the Plant Pathology laboratory of Bangladesh Agriculture Research Institute (BARI), Gazipur, Bangladesh. Six leaf sample was collected randomly from infected plants during observation of symptoms and dried and stored in silica gel for further investigation.

The dried leaf samples were homogenized (dilution 1:50 g/v) in extraction buffer 8.0 g NaCl, 0.2 g KH2PO4, 1.1 g Na2HPO4, 0.2 g KCl/L, pH 7.4) containing 0.05% v/v Tween 20, and 2% w/v polyvinylpyrrolidone. The microtiter plate was coated with CMV antiserum (Plate 3A). The IgG antibodies were diluted in the coating buffer (Na2CO3+NaHCO3+NaN2) at a recommended dilution of 1:1000 according to the manufacturer‘s instructions. A hundred microliters of the dilution were distributed in the wells and incubated at 37°C for 3 hrs (Plate 3B).

The microplates were washed three times with the phosphate buffer saline-Tween 20 (PBS-T) (Plate 3C). The homogenized leaf samples were added to the well and incubated overnight at 4°C (Plate 3D).

Next day, after washing the plates three times with PBS-T, they were incubated with the enzyme conjugate (alkaline phosphatase conjugate, diluted at 1/1000 in PBS-T+BSA+NaN2) at 37°C for 2 hrs. Then after washing the plates three times with PBS-T, they were incubated for one hour at room temperature with freshly prepared phosphate substrate solution (100µL per well). The substrate was a p-nitrophenyl phosphate (pNPP) tablet (Sigma-Aldrich Co. LLC) applied at 1.0 mg Ml-1 in 9.87% diethanolamine, pH 9.8 (Plate 3G). The optical density (OD) values were measured with ELISA reader EAR00 FW at 405 nm wavelength (Plate 3H).

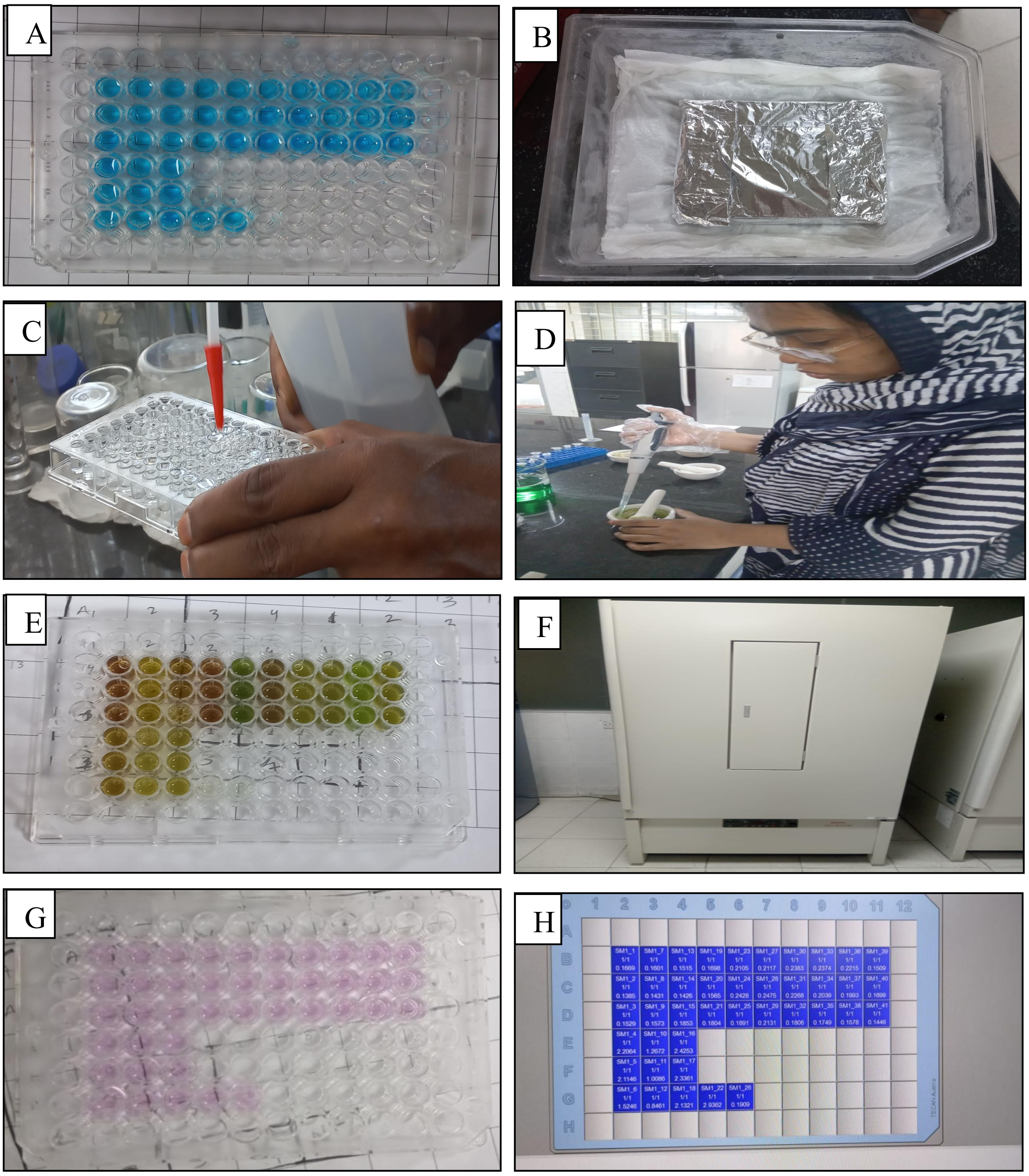


Plate 3. Different steps of DAS-ELISA Test:

(A) Microtiter plate coated with CMV-antiserum;

(B) Incubation for 3 hrs.;

(C) Washing of plate with PBS-T;

(D) Preparation of homogenous leaf samples with extraction buffer;

(E) Homogenized leaf samples in each well;

(F) Overnight incubation; (G) Addition of pNPP in each well;

(H) Final result by ELISA reader

3.16. Statistical Analysis

The collected data were subjected to analyses of variance (ANOVA) and the means were separated with the least significant difference (LSD) method at a 5% level of probability. The statistical package Statistix 10 software was used for the proper interpretation of the data. Tables, bar diagrams, linear graphs and photographs were used to present the data as and when required. The correlation of different parameters was performed to find out the influence.

CHAPTER IV

RESULTS AND DISCUSSION

The present chapter comprises the presentation and explanation of the results obtained from the experiment on the effect of different plant extracts and cultural practices on virus diseases of capsicum. The results have been presented and possible interpretations have been given under the following headings in this chapter.

4.1. Identification of the Virus

Identification of the virus was done on the basis of visual observation of the appeared symptoms of infected capsicum plants in field. For confirmation of CMV which were identified during field observation, later serological test, DAS-ELISA, was done. The results of visual observation and serological test are shown in Plate 4 and Table 1, respectively.

4.1.1. Visual Observation

A variety of symptoms were observed in the leaves of infected capsicum plants. These symptoms includes shoestring-like leaves, vein banding, leaf mosaic and stunted growth (Plate 4).

Shoestring leaves appeared as the deformation and constriction of the leaf blades leading to the formation of fern leaves or shoe string-like structures (Plate 4A). In some infected plants, growth (plant height and leaf area) was reduced (Plate 4B).

Some of the infected leaves were presented with yellow-green patches of mosaic which is a common symptom of mosaic virus infection in plants.

Mosaic symptoms were mostly observed in growing leaves compared to young leaves (Plate 4C). In case of some infected leave, the veins were yellowish and more prominent in size compared to the healthy leaves in infected capsicum plants (Plate 4D).

The majority of the observed symptoms were consistent with symptoms found in *Cucumber mosaic virus* infection in capsicum, chilli and peppers supported by different literatures.

Based on the results of field observation, the present study indicate that capsicum plants were infected with *Cucumber mosaic virus* (CMV).

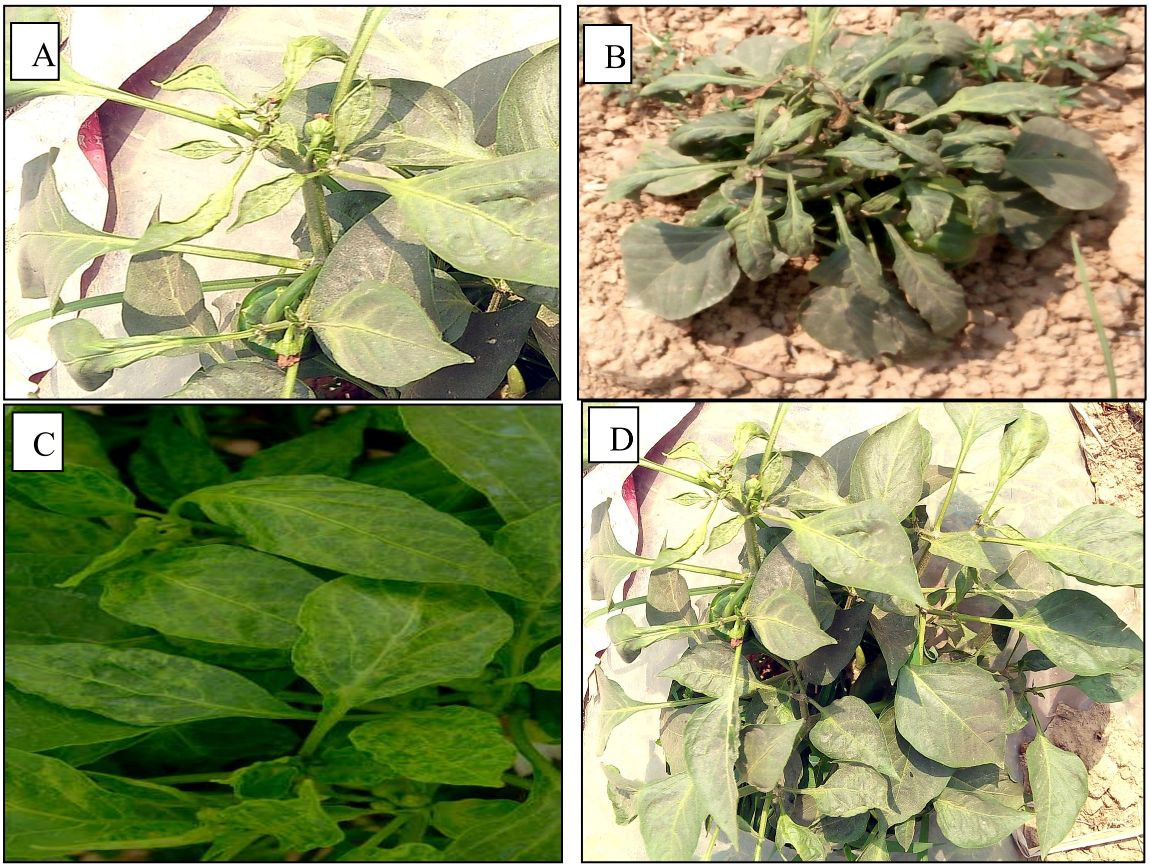


Plate 4. Symptoms of *Cucumber mosaic virus* observed in capsicum:
(A) Shoestring leaves; (B) Stunted growth;
(C) Mosaic; and (D) Vein banding

4.1.2. Identification of *Cucumber mosaic virus* (CMV) by DAS-ELISA

For confirmation of CMV, serological test (DAS-ELISA) was done by using only one antiserum (CMV). The reactions, optical density (OD) values and symptoms of the samples of infected capsicum leaves are presented in Table 1.

During DAS-ELISA test of 6 infected capsicum leaves was performed using *Cucumber mosaic virus* (CMV) antiserum. The samples were collected randomly from infected capsicum plants which were mosaic, leaf deformation, shoe-string leaves, stunt growth and vein banding.

Among the leaf samples, all six of them showed positive reaction against CMV antiserum used for detection of virus. The OD values of the collected leaf samples were 0.21-2.30 where OD value of positive control was 2.93 and OD value of negative control was 0.19. Therefore, the OD value of collected leaf samples were higher than the negative control.

Based on the results of DAS-ELISA, the present study ifrndicate that capsicum plants were infected with *Cucumber mosaic virus* (CMV).

Table 1. Reactions and optical density (OD) values of symptoms of *Cucumber mosaic virus* (CMV) by DAS-ELISA

Sample No.	Reaction	Mean OD Value	Symptoms
Positive control		2.93	
Negative control		0.19	
Sample 1	+	0.21	Mosaic, stunt growth
Sample 2	+	0.22	Stunted growth, mosaic
Sample 3	+	0.22	Leaf deformation, vein banding
Sample 4	+	1.94	Severe mosaic, stunt growth
Sample 5	+	1.04	Shoe string leaf
Sample 6	+	2.30	Mosaic, vein clearing, shoe string leaf

4.2. Effect of Plant Extracts and Cultural Practices on *Cucumber mosaic virus* (CMV) Incidence (%) in Capsicum

The effects of different treatments on the *Cucumber mosaic virus* (CMV) incidence was observed in capsicum and significant variations of disease incidence (%) was found among different treatments. The results are presented in Table 2.

A range of 8.33-75.0% of CMV incidence was observed among different treatments and control where the highest CMV incidence was 75.0% and the lowest CMV incidence was 8.33%.

At 30 DAT, the highest CMV incidence was observed in T_0 (control), 33.33%. T_1 (coriander as intercrop), T_3 (marigold as border crop) and T_5 (aluminium foil as reflective mulch) resulted in 8.33% which was the lowest CMV incidence at 30 DAT.

At 45 DAT, T_0 (control) and T_1 (coriander as intercrop) recorded the highest CMV incidence which was 33.33% whereas T_2 (garlic bulb extract), T_3 (marigold as border crop), T_5 (aluminium foil as reflective mulch) and T_6 (mahogany bark extract) resulted in the lowest CMV incidence (8.33%).

At 60 DAT, the highest CMV incidence (50%) was recorded in T_0 (control) and T_1 (coriander as intercrop), followed by T_3 (marigold as border crop) and T_6 (mahogany bark extract) which was the second highest incidence (25.0%). CMV incidence of 16.33% was observed in T_2 (garlic bulb extract) and T_5 (aluminium foil as reflective mulch). The lowest CMV disease incidence, 8.33%, was found in T_4 (neem leaf extract).

At 75 DAT, T_0 (control) resulted in the highest CMV incidence (75.0%). T_5 (aluminium foil as reflective mulch), and T_6 (mahogany bark extract) expressed a statistically similar CMV incidence of 25.0%.

T_2 (garlic bulb extract), T_1 (coriander as intercrop) and T_3 (marigold as border crop) observed a CMV incidence of 41.67%, 50% and 58.33% respectively. The lowest CMV incidence was found in T_4 (neem leaf extract) (8.33%).

Table 2. Effect of different treatments on *Cucumber mosaic virus* (CMV) disease incidence (%) in capsicum

Treatments	*Cucumber mosaic virus* (CMV) incidence (%)							
	30 DAT		45 DAT		60 DAT		75 DAT	
T_0	33.33	a	33.33	a	50.00	a	75.00	a
T_1	8.33	b	33.33	a	50.00	a	50.00	c
T_2	0.00	c	8.33	b	16.33	c	41.67	d
T_3	8.33	b	8.33	b	25.00	b	58.33	b
T_4	0.00	c	0.00	c	8.33	d	8.33	f
T_5	8.33	b	8.33	b	16.33	c	25.00	e
T_6	0.00	c	8.33	b	25.00	b	25.00	e
LSD (0.05)	0.27		0.85		0.23		1.13	
CV (%)	3.93		7.31		1.01		3.42	

Means followed by same letters not significantly different at 5% level of significance
DAT: Days After Transplanting; LSD: Least Significant Difference

T_0= Control, T_1= Coriander as intercrop, T_2= Garlic bulb extract, T_3= Marigold as border crop, T_4= Neem leaf extract, T_5= Aluminium foil as reflective mulch, T_6= Mahogany bark extract

4.3. Effect of Plant Extracts and Cultural Practices on *Cucumber mosaic virus* (CMV) severity (%) in Capsicum

The effect of different treatments on the *Cucumber mosaic virus* (CMV) severity (%) was observed based on symptoms in capsicum plants and the results are presented in Figure 1.

Significant variations in CMV severity were observed among the different treatments in capsicum ranging from 20-45% where the lowest CMV severity was 20% and the highest CMV severity was 45%.

At 30 DAT, the highest CMV severity, 26.67%, was found in T_0 (control). T_1 (coriander as intercrop), T_3 (marigold as border crop), and T_5 (aluminium foil as reflective mulch) recorded 21.67% CMV severity. The lowest CMV severity, 20.0%, was observed in T_2 (garlic bulb extract), T_4 (neem leaf extract) and T_6 (mahogany bark extract).

At 45 DAT, the highest CMV severity was recorded in T_0 (control) (28.33%), followed by T_1 (coriander as intercrop) (26.67%). T_2 (garlic bulb extract), T_3 (marigold as border crop), T_5 (aluminium foil as reflective mulch) and T_6 (mahogany bark extract) recorded statistically similar CMV severity, 21.67%. T_4 (neem leaf extract) showed the lowest CMV severity (20%).

At 60 DAT, the highest CMV severity (41.67%) was observed in T_0 (control). The second highest CMV severity (31.67%) was recorded in T_1 (coriander as intercrop), followed by T_6 (mahogany bark extract) (28.33%). T_3 (marigold as border crop) (25.0%) and T_5 (aluminium foil as reflective mulch) (23.33%) resulted in statistically similar CMV severity. The lowest CMV severity (21.67%) was observed in both T_2 (garlic bark extract) and T_4 (neem leaf extract).

At 75 DAT, the highest CMV severity, 45.0%, was observed in T_0 (control). T_1 (coriander as intercrop), T_2 (garlic bulb extract) and T_3 (marigold as border crop) resulted in the CMV severity of 38.33%, 35.0% and 28.33% respectively.

The disease severity of T_6 (mahogany bark extract) and T_5 (aluminium foil as reflective mulch) were statistically similar which were 26.67% and 25.0% respectively. The lowest CMV severity, 21.67%, was recorded in T_4 (neem leaf extract).

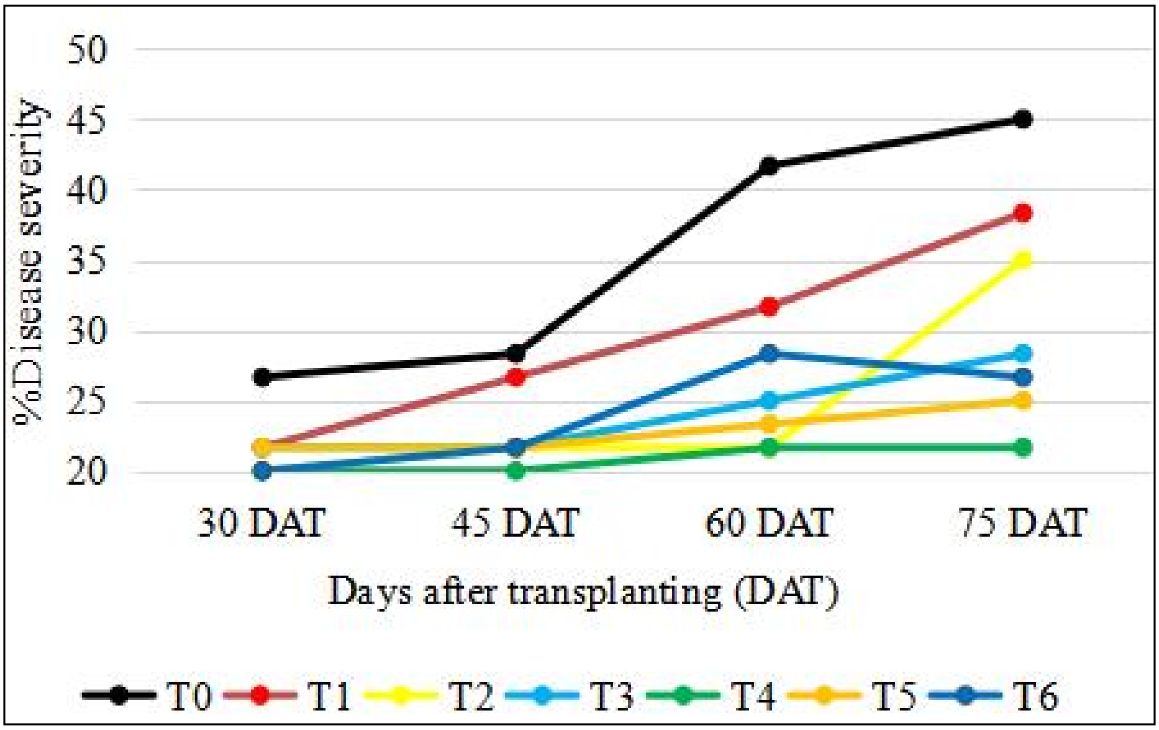


Figure 1. Effect of different treatments on *Cucumber mosaic virus* (CMV) disease severity (%) in capsicum

T_0= Control, T_1= Coriander as intercrop, T_2= Garlic bulb extract, T_3= Marigold as border crop, T_4= Neem leaf extract, T_5= Aluminium foil as reflective mulch, T_6= Mahogany bark extract

4.4. Effect of Plant Extracts and Cultural Practices on Growth Contributing Characteristics in Capsicum

The effects of plant extracts and cultural practices on the growth contributing factors were observed in capsicum plants. Growth contributing characters such as plant height (cm), the number of healthy and infected leaves and leaf area reduction (%) per plant were recorded and significant differences were observed among the treatments in capsicum and the results are presented in Table 3 and Figure 2.

4.4.1. Effect of plant extracts and cultural practices on plant height (cm) in capsicum

The effects of plant extracts and cultural practices on plant height (cm) of capsicum is presented in Table 3 and significant variations were observed.

At 30 DAT, the tallest plants were observed in T_4 (neem leaf extract), 15.25 cm, followed by T_5 (aluminium foil as reflective mulch), T_3 (marigold ad border crop) and T_1 (coriander as intercrop) (15.17, 15.14, 15.0 cm respectively) which were statistically similar. In T_2 (garlic bulb extract), plant height was measured at 14.67 cm, followed by T_6 (mahogany bark extract) (14.17 cm). The shortest plants were found in T_0 (control) at 13.17 cm.

At 45 DAT, T_3 (marigold border crop) and T_5 (aluminium foil as reflective mulch) produced statistically similar plant heights, 23.17 and 23.25 cm respectively, which were the tallest plants. T_4 (neem leaf extract) resulted in 22.58 cm plants in height. Plant height of T_1 (coriander as intercrop) and T_2 (garlic bulb extract) were observed at 21.92 and 20.92 cm respectively. T_6 (mahogany bark extract) and T_0 (control) produced statistically similar plant heights, 19.67 and 19.08 cm respectively, which was the shortest length at 45 DAT.

At 60 DAT, T_3 (marigold as border crop), T_4 (neem leaf extract) and T_5 (aluminium foil as reflective mulch) resulted in the tallest and statistically similar plant height (28.58, 28.66 and 28.08 cm respectively). The remaining treatments, T_6 (mahogany bark extract), T_1 (coriander as intercrop), T_2 (garlic bulb extract) and T_0 (control) also produced statistically similar plant heights which were recorded as 25.75, 25.50, 24.57 and 25.33 cm individually, where T_2 (garlic bulb extract) resulted in the shortest plants.

At 75 DAT, T_5 (aluminium foil as reflective mulch) produced the tallest plant (35.75 cm), followed by T_4 (neem leaf extract) (34.81 cm), T_3 (marigold as border crop) (33.38 cm), T_1 (coriander as intercrop) (31.83 cm), T_6 (mahogany bark extract) (30.50 cm) and T_0 (control) (30.33 cm). The shortest plant height, 29.75 cm, was seen in T_2 (garlic bulb extract).

Table 3. Effect of different treatments on plant height (cm) in capsicum

Treatments	Plant height (cm)							
	30 DAT		45 DAT		60 DAT		75 DAT	
T_0	13.17	c	19.08	d	25.33	b	30.33	ef
T_1	15.17	a	21.92	b	25.50	b	31.83	d
T_2	14.67	ab	20.92	c	24.57	b	29.75	f
T_3	15.00	a	23.17	a	28.58	a	33.38	c
T_4	15.25	a	22.58	ab	28.66	a	34.81	b
T_5	15.14	a	23.25	a	28.08	a	35.75	a
T_6	14.17	b	19.67	d	25.75	b	30.50	e
LSD (0.05)	0.31		0.32		0.65		0.32	
CV (%)	2.57		1.85		3.01		1.2	

Means followed by same letters not significantly different at 5% level of significance

DAT: Days After Transplanting; LSD: Least Significant Difference

T_0= Control, T_1= Coriander as intercrop, T_2= Garlic bulb extract, T_3= Marigold as border crop, T_4= Neem leaf extract, T_5= Aluminium foil as reflective mulch, T_6= Mahogany bark extract

4.4.2. Effect of Plant Extracts and Cultural Practices on Number of Healthy and Infected Leaves and Leaf Area Reduction (%) in Capsicum

The effect of different treatments on the number of healthy and infected leaves and leaf area reduction (%) (% LAR) were recorded. Significant variations in the number of healthy and infected leaves was found and % LAR was significantly varied in infected plants and the results are shown in Figure 2.

At 30 DAT, the maximum healthy leaves was recorded in T_5 (aluminium foil as reflective mulch) which was 29.67 per plant. In T_2 (garlic bulb extract), T_3 (marigold as border crop) and T_4 (neem leaf extract), 27.33, 27.00 and 28.00 healthy leaves were found respectively which were statistically similar. T_6 (mahogany bark extract), T_1 (coriander as intercrop) and T_0 (control) also had a statistically similar number of healthy leaves (26.67, 26 and 25.33 respectively) where T_0 (control) produced the lowest number of healthy leaves. In contradiction, the highest number of infected leaves was observed in T_0 (control) (8.33), followed by T_3 (marigold as border crop) (5.5), T_5 (aluminium foil as reflective mulch) (5), and T_1 (coriander as intercrop) (4) which was the lowest number of infected leaves per plant. The highest %LAR was recorded in T_0 (control) (40.92), followed by T_1 (coriander as intercrop) (37.52) and T_3 (marigold as border crop) (22.88). The least %LAR was observed in T_5 (aluminium foil as reflective mulch) (17.75) (Figure 2A).

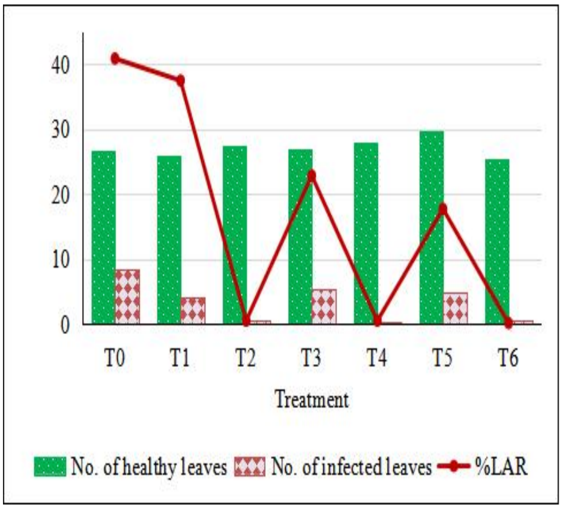
At 45 DAT, the highest number of healthy leaves was 43.67 leaves per plant and recorded in T_5 (aluminium foil as reflective mulch). T_0 (control), T_1 (coriander as intercrop), T_2 (garlic bulb extract), T_3 (marigold as border crop), T_4 (neem leaf extract) and T_6 (mahogany bark extract) resulted in statistically similar numbers of healthy leaves (37.00, 36.33, 38.33, 39.00, 38.67 and 37.33 respectively) where the lowest number of healthy leaves was noticed in T_0 (control). On the other hand, T_0 (control) and T_1 (coriander as intercrop) resulted in a statistically similar number of infected leaves, 15.67 and 15.50 respectively where the highest number of infected leaves was observed in T_0 (control).

T_5 (aluminium foil as reflective mulch) and T_3 (marigold as border crop) resulted in 9.33 and 7.16 infected leaves per plant respectively. T_2 (garlic bulb extract) and T_6 (mahogany bark extract) produced statistically similar (6.33 and 6.00 infected leaves per plant respectively). The highest %LAR was recorded in T_0 (control) (50.28), followed by T_1 (coriander as intercrop) and T_3 (marigold as border crop) resulting in similar % LAR which was 40.76 and 40.49. T_2 (garlic bulb extract) and T_6 (mahogany bark extract) resulted in a 36.62% and 39.09% reduction in leaf area. The least % LAR was observed in T_5 (aluminium foil as reflective mulch) (21.43) (Figure 2B).

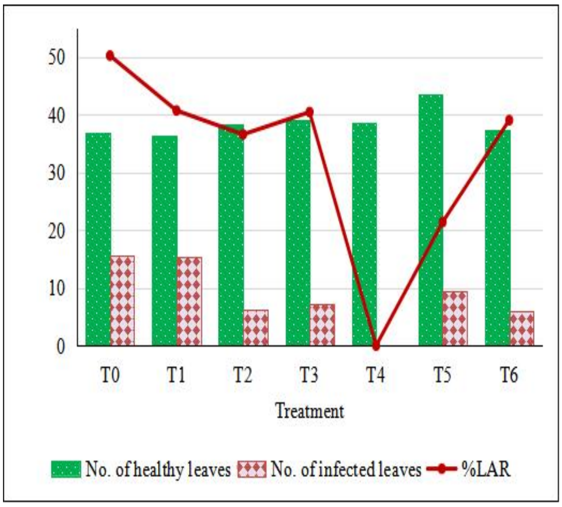
At 60 DAT, T_0 (control), T_5 (aluminium foil as reflective mulch), T_1 (coriander as intercrop), T_2 (garlic bulb extract), T_3 (marigold as border crop), T_4 (neem leaf extract) and T_6 (mahogany bark extract) resulted in 60.67, 51, 49.33, 51, 52, 52.33 and 50.67 healthy leaves per plant where the highest number of healthy leaves was observed in T_5 (aluminium foil as reflective mulch) and the lowest number of healthy leaves was observed in T_0 (control). In comparison, the highest number of infected leaves was observed in T_0 (control) (19.50), followed by T_1 (coriander as intercrop) (16.67), T_6 (mahogany bark extract) (13), T_5 (aluminium foil as reflective mulch) (11.17), T_2 (garlic bulb extract) (10.33), T_3 (marigold as border crop) (9.33). T_4 (neem leaf extract) showed the lowest number of infected leaves (4). Similar leaf area reduction (% LAR) was observed in T_0 (control) (56.89%) and T_1 (coriander as intercrop) (56.38%) where T_0 (control) was the highest leaf area reduction. T_3 (marigold as border crop) resulted in 47.09% leaf area reduction followed by T_2 (garlic bulb extract) and T_6 (mahogany bark extract) which was 45.36% and 45.68% respectively. T_5 (aluminium foil as reflective mulch) resulted in 40.01% leaf area reduction followed by the least % LAR, 24.33, in T_4 (neem leaf extract) (Figure 3C).

At 75 DAT, the highest number of healthy leaves was recorded in T5 (aluminium foil as reflective mulch) which was 79.00 leaves per plant. T_0 (control), T_1 (coriander as intercrop), T_2 (garlic bulb extract), T_3 (marigold as border crop), T_4 (neem leaf extract) and T_6 (mahogany bark extract) resulted in 62.33, 61, 64, 63.33, 64.33 and 63 per plant respectively. The lowest number of healthy leaves was recorded in T_0 (control). On the contrary, the highest number of infected leaves was observed in T_0 (control) (24.67) followed by T_1 (coriander as intercrop) (22). On the other hand, T_5 (aluminium foil as reflective mulch) (15) and T6 (mahogany bark extract) (15.50) were statistically similar in the number of infected leaves. Similarly, T_2 (garlic bulb extract) (11.33) and T_3 (marigold as border crop) (10.67) were statistically similar in result. T_4 (neem leaf extract) showed the least number of infected leaves which was 7 per plant. Similar leaf area reduction (% LAR) was observed in T_0 (control) (71.60%) and T_1 (coriander as intercrop) (71.06%) where T_0 (control) was the highest leaf area reduction. T_2 (garlic bulb extract), T_3 (marigold as border crop), T_6 (mahogany bark extract) and T_5 (aluminium foil as reflective mulch) resulted in 65.24%, 62.21%, 59.90% and 55.42% of leaf area reduction. T_4 (neem leaf extract) resulted in the least %LAR (36.40%) (Figure 2D).

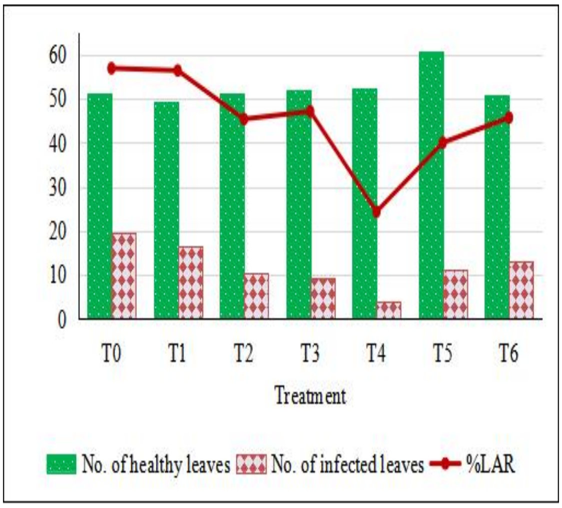
(A) At 30 DAT



(B) At 45 DAT



(C) At 60 DAT



(D) At 75 DAT

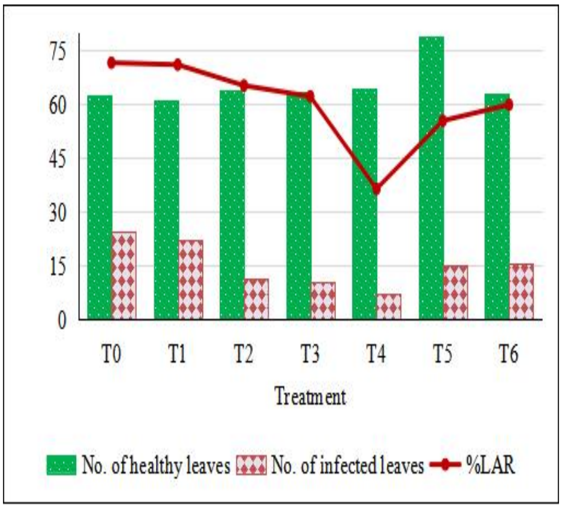


Figure 2 (A-D). Effect of different treatments on number of leaves (healthy and infected) and leaf area reduction (%) in capsicum

DAT: Days After Transplanting; % LAR: % Leaf Area Reduction

T_0= Control, T_1= Coriander as intercrop, T_2= Garlic bulb extract, T_3= Marigold as border crop, T_4= Neem leaf extract, T_5= Reflective mulch (aluminium foil), T_6= Mahogany bark extract

4.5. Effect of Plant Extracts and Cultural Practices on Yield and Yield Contributing Characters of Capsicum

Effect of plant extracts and cultural practices on the yield and yield contributing characteristics of capsicum was observed. Variations in the number of fruits per plant, average weight of fruits (g), fruit yield per plant (g), yield per plot (Kg), total yield (ton/ha) and yield loss (%) were recorded. The results of yield contributing characters are shown in Table 4, 5 & 6.

4.5.1. Effect of Plant Extracts and Cultural Practices on Number of Fruits Per Plant and Average Weight of Fruits (g) of Capsicum

Significant variations in the number of fruits per plant and the average weight of fruits (g) were observed among the different treatments (Table 4).

The highest number of fruits was recorded in T_5 (aluminium foil as reflective mulch) which was 13.33. T_4 (neem leaf extract) produced 12.33 fruits and T_3 (marigold as border crop) and T_6 (mahogany bark extract) produced a statistically similar number of fruits, 11.50 and 11.67 respectively. T_2 (garlic bulb extract) and T_1 (coriander as intercrop) resulted in 10.33 and 9.25 fruits per plant. The lowest number of fruits was found in T_0 (control) produced t (7.17).

In the case of the average weight of fruits, the heaviest fruits were recorded in T_5 (aluminium foil as reflective mulch) which was 62.10 g, followed by T_6 (mahogany bark extract), T_2 (garlic bulb extract) and T_3 (marigold as border crop) which produced statistically similar fruit weights, which were 59.56, 57.87 and 56.0 g respectively. The weight of individual fruits was 55.18 g in T_4 (neem leaf extract). In T_1 (coriander as intercrop), the recorded weight of fruit was 53.76 g and the lightest fruits were recorded in T_0 (control) (49.91 g).

Table 4. Effect of different treatments on number of fruits per plant and average weight of fruits (g) of capsicum

Treatments	No. of fruits per plant		Average weight of fruits (g)	
T_0	7.17	e	49.91	d
T_1	9.25	d	53.76	cd
T_2	10.33	bc	57.87	a-c
T_3	11.50	b	56.00	bc
T_4	12.33	ab	55.18	c
T_5	13.33	a	62.10	a
T_6	11.67	b	59.56	ab
LSD (0.05)	0.70		1.99	
CV (%)	8.13		4.32	

Means followed by same letters not significantly different at 5% level of significance LSD: Least Significant Difference

T_0= Control, T_1= Coriander as intercrop, T_2= Garlic bulb extract, T_3= Marigold as border crop, T_4= Neem leaf extract, T_5= Reflective mulch (aluminium foil), T_6= Mahogany bark extract

4.5.2. Effect of Plant Extracts and Cultural Practices on Fruit Yield per Plant (g) and Yield per Plot (Kg)

Significant variations in fruit yield per plant (g) and yield per plot (g) were observed among the different treatments and the results are shown in Table 5.

The highest fruit yield per plant, 725.74g, was found in T_5 (aluminium foil as reflective mulch), followed by T_4 (neem leaf extract), T_6 (mahogany bark extract), T_3 (marigold as border crop), T_2 (garlic bulb extract) and T_1 (coriander as intercrop) which were 689.15g, 614.20g, 592.17g, 558.34g and 476.19g respectively. The lowest fruit yield per plant was recorded in T_0 (control) which was 337.10g.

In the case of yield per plot, the highest yield per plot (2.78 Kg) was recorded in T_5 (aluminium foil as reflective mulch) which was statistically similar to T_4 (neem leaf extract), 2.78 kg per plot, followed by T_6 (mahogany bark extract) (2.50 kg). T_2 (garlic bulb extract) and T_3 (marigold as border crop) resulted in similar yields, 2.20 and 2.38 kg per plot. Finally, T_1 (coriander as intercrop) and T_0 (control) resulted in the lowest harvest which was 1.83 and 1.35 kg per plot respectively.

Table 5. Effect of different treatments on fruit yield per plant (g) and yield per plot (Kg) of capsicum

Treatments	Fruit yield per plant (g)	Yield per plot (Kg)
T_0	337.10 g	1.35 e
T_1	476.19 f	1.83 d
T_2	558.34 e	2.20 c
T_3	592.17 d	2.38 bc
T_4	689.15 b	2.74 a
T_5	725.74 a	2.78 a
T_6	614.20 c	2.50 ab
LSD (0.05)	8.76	0.13
CV (%)	1.88	7.20

Means followed by same letters not significantly different at 5% level of significance

LSD: Least Significant Difference

T_0= Control, T_1= Coriander as intercrop, T_2= Garlic bulb extract, T_3= Marigold as border crop, T_4= Neem leaf extract, T_5= Reflective mulch (aluminium foil), T_6= Mahogany bark extract

4.5.3. Effect of Plant Extracts and Cultural Practices on Total Yield (ton/ha) and Yield Loss (%)

Significant variations in total yield (ton/ha) and yield loss (%) were observed among different treatments and the results are shown in Table 6.

T_5 (aluminium foil as reflective mulch) resulted in the highest yield (5.56 ton/ha), followed by T_4 (neem leaf extract) (5.48 ton/ha) and T_6 (mahogany bark extract) (5.00 ton/ha) where all were statistically similar. T_3 (marigold as border crop) and T_2 (garlic bulb extract) yielded statistically similar produces which were 4.76 and 4.40 tons/ha respectively. T_1 (coriander as intercrop) resulted in 3.66 tons/ha. T_0 (control) produced the lowest yield (2.70 tons/ha).

The highest yield loss was observed in T_0 (control), 66.30% followed by T_1 (coriander as intercrop) (56.42%). T_2 (garlic bulb extract) (44.56%). T_3 (marigold as border crop) and T_6 (mahogany bark extract) resulted in 31.42% and 25.46%. The lowest yield loss was observed in T_4 (neem leaf extract) and T_5 (aluminium foil as reflective mulch) which were 18.28% and 17.68% respectively.

Table 6. Effect of different treatments on total yield (ton/ha) and yield loss (%) of capsicum

Treatments	Total yield (ton/ha)		Yield loss (%)	
T_0	2.70	d	66.30	a
T_1	3.66	c	56.42	b
T_2	4.40	ab	44.56	c
T_3	4.76	ab	31.42	d
T_4	5.48	a	18.28	f
T_5	5.56	a	17.68	f
T_6	5.00	a	25.46	e
LSD (0.05)	0.58		0.94	

Means followed by same letters not significantly different at 5% level of significance

LSD: Least Significant Difference

T_0= Control, T_1= Coriander as intercrop, T_2= Garlic bulb extract, T_3= Marigold as border crop, T_4= Neem leaf extract, T_5= Reflective mulch (aluminium foil), T_6= Mahogany bark extract

4.6. Effect of Plant Extracts and Cultural Practices on Number of Aphids per Leaf in Capsicum

Significant variations in the number of ahids per leaf was observed among the treatments and the results are presented in Figure 3.

At 30 DAT, the highest number of aphids per leaf was observed in T_0 (control), 16.33 aphids per leaf, followed by T_1 (coriander as intercrop) and T_3 (marigold as border crop) which were statistically similar (15 and 11 aphids per leaf respectively). The number of aphids found on T_4 (neem leaf extract), T_5 (aluminium foil as reflective mulch) and T_6 (mahogany bark extract) was statistically similar as well (8.17, 7.67 and 8 aphids per leaf respectively). T_2 (garlic bulb extract) resulted in the lowest number of aphids per leaf (6.25).

At 45 DAT, the highest number of aphids per leaf was observed in T_0 (control), 20.25 aphids per leaf, followed by T_1 (coriander as intercrop) and T_3 (marigold as border crop) which resulted in 17.5 and 8.33 aphids per leaf respectively. T_5 (aluminium foil as reflective mulch), T_6 (mahogany bark extract) and T_2 (garlic bulb extract) were statistically similar which were 7.33, 7.33 and 6.67 aphids per leaf. The lowest number of aphids was monitored in T_4 (neem leaf extract), 6 aphids per leaf.

At 60 DAT, the highest number of aphids per leaf was observed in T_0 (control), 21 aphids per leaf. T_1 (coriander as intercrop) and T_5 (aluminium foil as reflective mulch) resulted in 15.17 and 11.50 aphids per leaf respectively. T_2 (garlic bulb extract) and T_3 (marigold as border crop) were statistically similar in aphids per leaf which were 8.33 in both treatments. T_6 (mahogany bark extract) and T_4 (neem leaf extract) found 5.67 and 4.25 aphids per leaf respectively where T_4 (neem leaf extract) resulted in the lowest number of aphids per leaf.

At 75 DAT, the highest number of aphids per leaf was observed in T_0 (control), 21.33 aphids per leaf. 20.67 aphids per leaf were found on T_1 (coriander as intercrop).

T_2 (garlic bulb extract) and T_3 (marigold as border crop) resulted in a statistically similar number of aphids per leaf which were 12.33 and 12.17 respectively whereas 9.33 aphids per leaf were seen on T_6 (mahogany bark extract). finally, a statistically similar number of aphids per leaf was observed in T_4 (neem leaf extract) and T_5 (aluminium foil as reflective mulch) which were 6.67 and 7.17 per leaf.

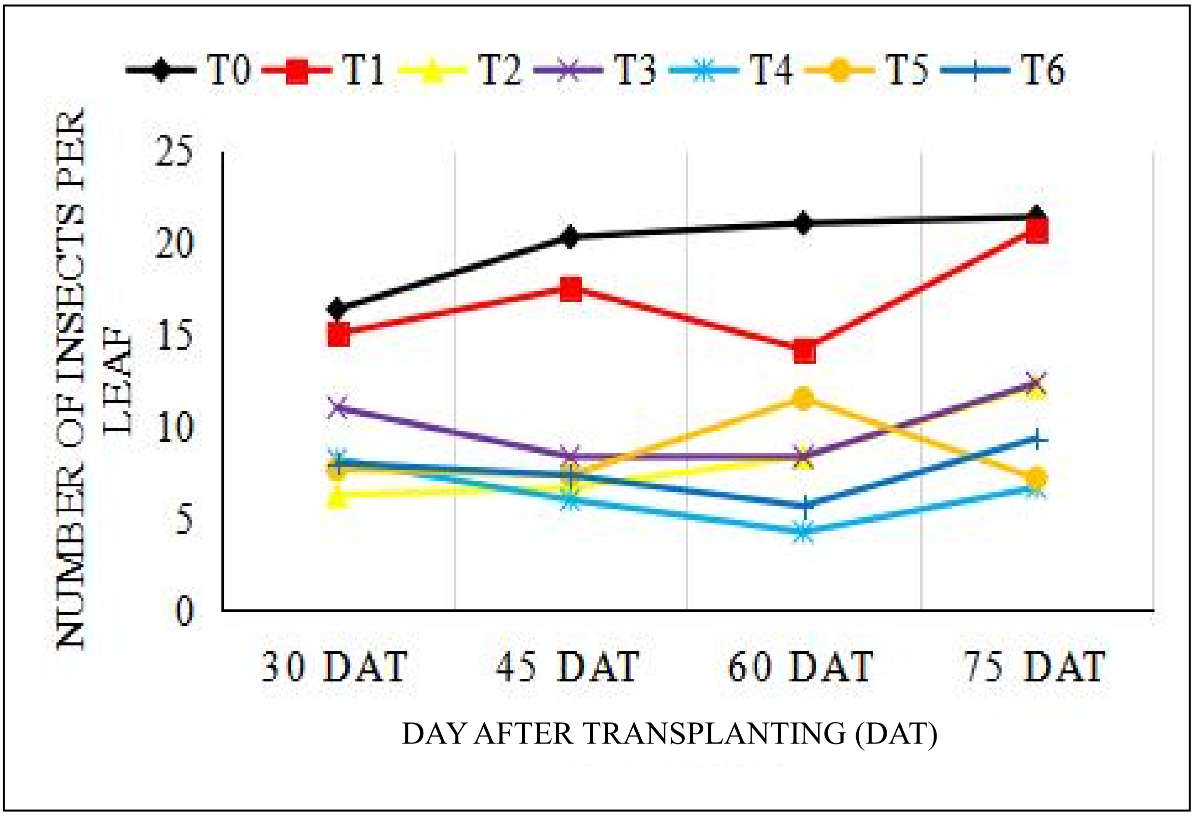


Figure 3. Effect of different treatments on the number of aphids per leaf in capsicum

Means followed by same letters not significantly different at 5% level of significance

T_0= Control, T_1= Coriander as intercrop, T_2= Garlic bulb extract, T_3= Marigold as border crop, T_4= Neem leaf extract, T_5= Reflective mulch (aluminium foil), T_6= Mahogany bark extract

4.7. Relationships among Disease Incidence (%), Disease Severity (%) and Total Yield (ton/ha)

The correlation and regression analysis among *Cucumber mosaic virus* (CMV) incidence (%), CMV severity (%) and total yield (ton/ha) was done to understand their relationship (Table 7, Figure 4, Figure 5 and Figure 6).

The correlation analysis revealed that there was a correlation between the CMV incidence (%) and severity (%) with the total yield (ton/ha) of capsicum (Table 7).

The total yield (ton/ha) was negatively correlated with the CMV incidence (%) and severity (%) (r = -0.86 and -0.98 respectively) whereas CMV incidence (%) and severity (%) was positively correlated (r= 0.85). These implied that as the CMV severity (%) increased when CMV incidence (%) increased and on the other hand, as CMV incidence (%) and severity increased, the total yield (ton/ha) of capsicum decreased.

The regression analysis revealed that there was a significant increase in CMV severity (%) when CMV incidence (%) increased. An unit increase in disease incidence (%) caused a significant increment (R^2 = 0.731) in CMV severity (%) which was 73.1% (Figure 4).

The regression analysis of CMV incidence (%) and total yield (ton/ha) further revealed that there was a significant reduction of total yield (ton/ha) of capsicum when CMV incidence (%) increased. Any unit increase in CMV incidence (%) caused a significant reduction (R^2= 0.743) in the total yield (ton/ha) of capsicum which was 74.3% (Figure 5).

The regression analysis of of total yield (ton/ha) and CMV severity (%) also revealed that when CMV severity (%) increased, there was reduction of total yield (ton/ha) of capsicum. The increase in CMV severity (%) caused a significant reduction (R^2 = 0.957) in the total yield (ton/ha) of capsicum which was 95.7% (Figure 6).

Table 7. Relationship between disease incidence (%), disease severity (%) and total yield (ton/ha)

	Disease incidence (%)	Disease severity (%)	Yield (ton/ha)
Disease incidence (%)	-	-	-
Disease severity (%)	0.85	-	-
Yield (ton/ha)	- 0.86	- 0.98	-

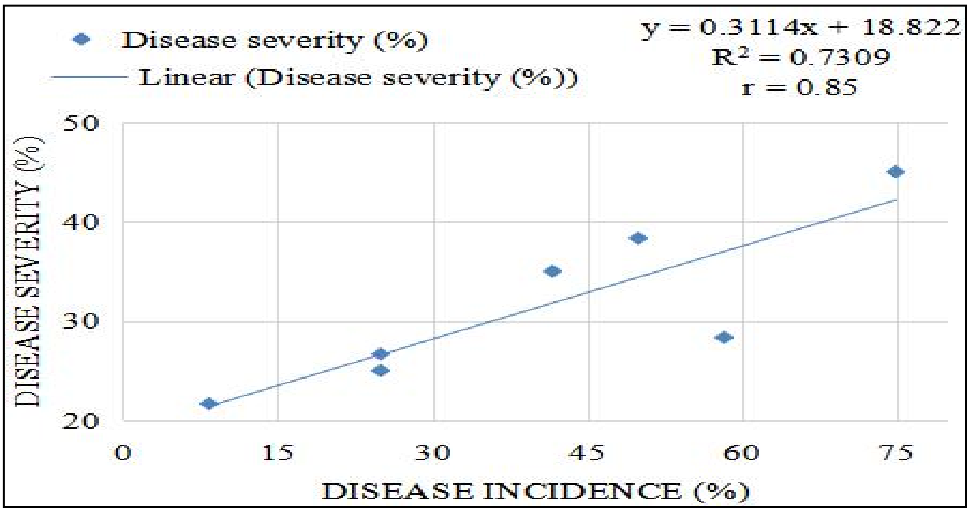


Figure 4. Linear curve estimation showing increase in disease severity as disease incidence increase

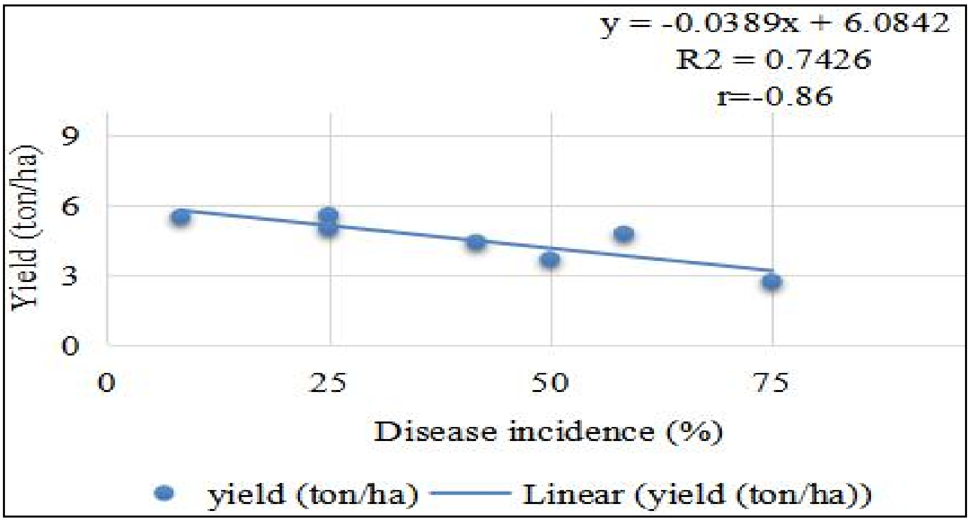


Figure 5. Linear curve estimation showing reduction in the total yield as the disease incidence increased

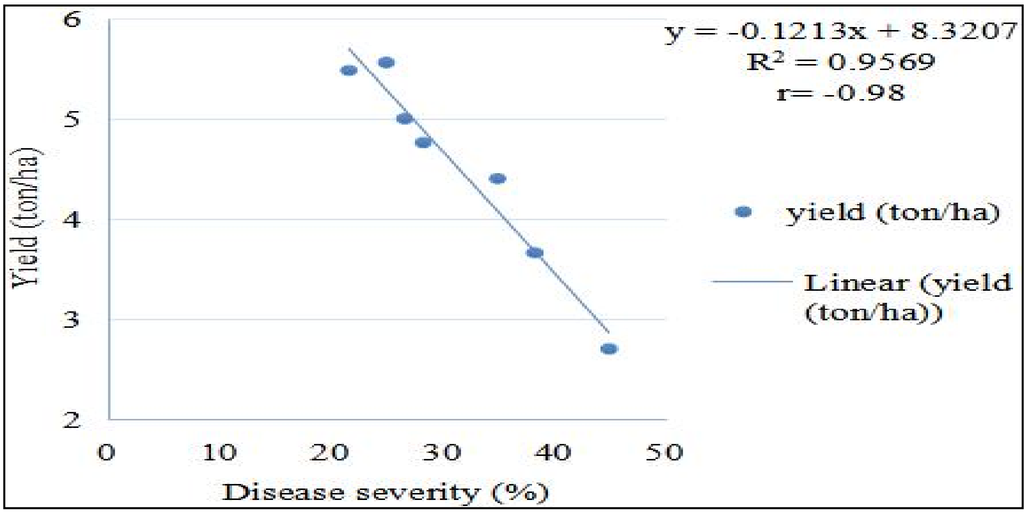


Figure 6. Linear curve estimation showing reduction in the total yield as the disease severity increased

DISCUSSION

Capsicum is considered as an excellent source of Vitamin C and other antioxidants (GMF, 2008). Nearly 176.5 MT of capsicum is produced in Bangladesh in 2020-2021 (BBS, 2021). Capsicum is susceptible to a large number of pathogens, including viruses vectored by whitefly and aphids, which causes severe yield loss. These viral diseases can reduce both the quality and quantity of fruit and so decrease the yield. In case of non-persistent viruses, insecticides can be ineffective. Moreover, the lack of virus-resistant or tolerant cultivars, the presence of viruses and their vectors and the cultivation of crops without any isolation make the control of plant viruses more difficult (Gonsalves and Garnsey, 1989; Kenyon *et al*. 2014; Arogundade *et al*., 2015). Recently, eco-friendly management practices, such as the use of plant extracts and cultural practices etc. to manage virus diseases of plants have gained popularity among farmers.

This study was aimed to evaluate the effects of different plant extracts and cultural practices on *Cucumber mosaic virus* diseases of capsicum. The study was conducted at the research farm of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 from November 2020 to April 2021. The experiment evaluated the effects of some plant extracts (neem leaf extract, garlic bulb extract, mahogany bark extract) and some cultural practices (marigold as border crop, coriander as intercrop and aluminium foil as reflective mulch) on *Cucumber mosaic virus* diseases of capsicum.

Visual observation of symptoms during field experiment was done and DAS-ELISA test was conducted on the collected samples of the infected plants to identify the virus. The effects of neem leaf extract, garlic bulb extract, mahogany bark extract, marigold as border crop, coriander as intercrop and aluminium foil as reflective mulch were observed on disease incidence (%), disease severity (%), growth contributing characteristics, namely, plant height (cm), number of healthy and infected leaves per plant, leaf reduction area (%), number of aphids per leaf at

30, 45, 60 and 75 days after transplanting (DAT) whereas yield contributing characteristics, namely, number of fruits per plant, weight of individual fruit (g), yield per plant (g), yield per plot (kg), total yield (ton/ha), yield loss (%) were recorded after harvest. Relationships among different parameters were later established as well.

Infected capsicum leaves were presented with mosaic, shoestring-like leaves, vein banding and stunted growth. These symptoms have been associated with *Cucumber mosaic virus* (CMV) infection in peppers and chillis by Zitter and Murphy (2009), Rahman *et al*. (2016), Kapoor *et al*. (2018) and Arogundade *et al*. (2019). DAS-ELISA test on the collected samples further confirmed that the crops were infected with the CMV as all the collected samples showed positive reaction with the CMV anitserum.

The average CMV disease incidence ranged from 8.33-75% among different treatments and control. The highest CMV incidence was 75% on T_0 (control) while the lowest CMV incidence, 8.33%, was found on T_4 (neem leaf extract). The average CMV severity ranged from 20-45% among different treatments and control. The highest CMV severity was 45% on T_0 (control) while the lowest severity was recorded on T_4 (neem extract). Kusumawati *et al*. (2015), Shabbir *et al*. (2020) found similar results. Moreover, these results are in line with Khan *et al*. (2003; 2011) who found significant reduction in virus disease by using neem extract. The reason behind disease reduction was the repellent efficacy of neem extract against the sucking insect pests (Butler *et al*., 1991). The repellent behaviour of plant extracts were also observed in studies of (Butler and Henneberry, 1992) who described that reduced insect infestation resulted in minimum disease incidence.

In the case of growth contributing characters, plant height (cm) varied from 13.17 cm to 35.75 cm from 30 DAT to 75 DAT among different treatments and control. The tallest plants were found in T_5 (aluminium foil as reflective mulch)

(15.14-35.75 cm) and the shortest plants were found in T_0 (control) (13.17-30.33 cm). Among different treatments and control, the average number of healthy leaves ranged from 29.67 to 79.00 per plant for 30 DAT to 75 DAT. The maximum number of healthy leaves (79.0 per plant) was observed in T_5 (aluminium foil as reflective mulch) whereas the minimum number of healthy leaves (29.67) was observed in T_0 (control). On the other hand, the average number of infected leaves varied from 8.33 to 24.67 per plant. T_0 (control) resulted in the highest number of infected leaves, 24.67 per plant and the lowest number of infected leaves T_4 (neem leaf extract), 8.33 per plant. The leaf area reduction ranged from 17.75-71.60% among different treatments and control. The maximum leaf area reduction, 40.92-75.60% was observed in T_0 (control) and the minimum leaf area reduction, 24.33-36.40%, was observed in T_4 (neem leaf extract) from 30 DAT to 75 DAT. The results were similar to the findings of Diaz-Perez (2010), Moyin-Jesu (2011), Rahman *et al*. (2016) and Arogundade *et al*. (2019) where use of reflective mulch resulted in higher growth contributing characteristices.

In the case of yield characteristics, the number of fruits ranged from 7.17 to 13.33 per plant among different treatments and control. The most number of fruits per plant (13.33) was observed in T_5 (aluminium foil as reflective mulch) and the least amount of fruits per plant, 7.17, was observed in T_0 (control). The average weight of fruits ranged from 49.91 g to 62.10 g among different treatments and control. The heaviest fruits were seen in T_5 (aluminium foil as reflective mulch) and the lightest fruits were seen in T_0 (control). Fruit yield per plant among different treatments and control ranged from 337.10-725.74 g. The highest yield per plant (725.74 g) was observed in T_5 (aluminium foil as reflective mulch) and the lowest yield per plant (337.10 g) was observed in T_0 (control). Yield per plot among different treatments and control ranged from 1.35-2.78 Kg. The highest yield per plot (2.78 Kg) was observed in T_5 (aluminium foil as reflective mulch) and the lowest yield per plot (1.35 Kg) was observed in T_0 (control). Total yield ranged from 2.70-5.56 tons/ha among different treatments and control. The highest total yield (5.56 ton/ha) was observed in T_5 (aluminium foil as reflective mulch) and the lowest total yield (2.70 ton/ha) was observed in T_0 (control). Yield loss (%) among different treatments and control ranged from 17.68-66.30%. The highest yield loss was observed in T_0 (control) (66.30%) and the lowest yield loss was found in T_5 (aluminium foil as reflective mulch) (17.68%). George and Kring (1971) and Greer and Dole (2003) said using reflective mulches increased yield by delaying and lowering virus incidence. Porter and Etzel (1982) indicated the photosynthetically active radiation (PAR) being reflected into the plant canopy by reflective (aluminium-painted mulch) as the reason for the increased yield of capsicum. Monette and Stewart (1987), Diaz-Perez (2010), Kapoor (2012) and Ashrafuzzaman *et al*. (2011) supported mulching produced more fruits per plant and greater fruit weight which contributed toward higher yield.

The number of aphids varied from 6.25-21.33 per leaf among different plant extracts and cultural practices. The highest number of aphids were observed in T_0 (control) and the lowest number of aphids were observed in T_4 (neem extract) (4.25-7.67 per leaf) from 30 DAT to 75 DAT. Arif *et al*. (2009) found that plant extracts are less toxic way to control the sucking insect pests. Ali *et al*. (2005; 2014), Pun *et al*., 2005; Khan *et al*. (2011), Jahanzaib *et al*. (2018) found neem leaf extracts repelled aphids vectors (aphids, whiteflies).

Strong positive correlations were found among CMV incidence (%) and CMV severity (%). As CMV incidence (%) increased CMV severity (%) increased as well. There were negative correlations between total yield with CMV incidence and CMV severity. Total yield (ton/ha) decreased as CMV incidence (%) and CMV severity (%) increased. As CMV incidence (%) increased, total yield (ton/ha) was reduced by 74.2% and for any addition in CMV severity, yield reduction was by 95.7%. Fajinmi and Odebode (2010) and Asare-Bediako *et al*. (2014) found similar relations between disease incidence (%), severity (%) and yield (ton/ha) where significant negative correlation between disease incidence, severity and the fruit yield of pepper.

Neem leaf extract resulted in the lowest disease incidence (%) and severity (%) as it repelled the insect vectors (aphids). Neem extracts contain azadirachtin, nimbin, nimbidin, nimbolide and limonoids which were effective in repelling sucking aphids and thus the CMV incidence and severity were lower due to antimicrobial effects. In addition, azadirachtin has anti-viral, anti-fungal, antibacterial, antifeedant and anti-ovipositional properties which repel virus-carrying vectors and so reduced the incidence and severity (Gurjar *et al*., 2012; Alzohairy, 2016).

Reflective mulch (aluminium foil) was able to repel aphids contributing to healthier plants as the reduction of aphid-transmitted virus diseases as increased yields Greer and Dole (2003). Furthermore, the mulch surface reflected and influenced the amount and quality of light that reflected upwardly to the plants. In addition, mulch influenced and modified soil temperature and weed population reducing the competition among the crops (Porter and Etzel, 1982). Moreover, Diaz-Perez (2010) observed growth attributes, marketable and total yields of capsicum were higher on silver mulches. As a result, reflective mulch (aluminium foil) promoted the growth of capsicum and resulted in higher yield as well.

CHAPTER V
SUMMERRY AND CONCLUSION

Capsicum is a popular crop among both producers and consumers. However, the yield of capsicum is threatened by virus diseases. 80-100% yield loss in capsicum can be contributed to virus diseases and some of these viruses have caused epidemics in the past. Unfortunately, chemical pesticides are ineffective against some plant viruses. Moreover, virus disease management in Bangladesh is challenging. Therefore the present study was initiated to evaluate the effectiveness of some plant extracts and cultural practices on virus diseases of capsicum. The study was conducted at the research farm of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 during the period from November 2020 to April 2021.

Three types of plant extracts, neem leaf extract, garlic bulb extract, mahogany bark extract and three cultural practices, aluminium foil as reflective mulch, coriander as intercrop and marigold as border crop were used to evaluate the effectiveness on viruses of capsicum in field conditions. Symptoms of virus infection were observed and samples were tested based to identify the virus. Data were collected on the disease incidence, disease severity, growth and yield contributing characters such as plant height (cm), number of healthy leaves per plant, number of infected leaves per plant, leaf area reduction (%), number of fruits per plant, average fruit weight (g), fruit yield per plant (g), yield per plot (kg), total yield (ton/ha) and yield loss (%) under different treatments.

Mosaic, shoestring leaf, vein banding and clearing along with stunted plants were observed as symptoms of mosaic virus infection in capsicum. Serological test on collected samples revealed the capsicum-infecting virus was the *Cucumber mosaic virus* (CMV).

CMV incidence and severity were the highest in T_0 (control), 75% and 45% respectively, whereas T_4 (neem leaf extract) resulted in the lowest CMV incidence and severity, 8.33% and 22.3% respectively.

Growth and yield contributing factors were highest in T_5 reflective mulch (aluminium foil) compared to T_0 (control). The highest number of fruits per plant (13.33), heaviest fruits (62.10 g), highest yield per plant (725.74 g), highest yield per plot (2.78 Kg), highest total yield (5.56 ton/ha) and lowest yield loss (17.68%) were observed in T_5 (aluminium foil as reflective mulch), along with tallest plant (35.75 cm) and the highest number of healthy leaves (79.00). However, the lowest number of infected leaves and the minimum leaf area reduction were observed in T_4 (neem leaf extract) compared to T_0 (control)

The correlation and regression analysis revealed that there was a positive relationship between CMV incidence and severity. Moreover, there was a negative correlation between CMV incidence and severity with total yield. Higher incidence and severity resulted in lower yield. A 74.2% and 95.7% reduction of total yield were observed due to increase of CMV incidence and severity respectively.

Based on overall consideration, neem leaf extract and reflective mulch (aluminium foil) performed better to manage CMV incidence and severity and increased yield. In view of the results, the present study may be concluded as-

- ✓ Visual observation of symptoms included leaf mosaic, shoe string leaf, vein banding and stunted plants and a serological test identified the capsicum-infecting virus as *Cucumber mosaic virus* (CMV).
- ✓ The highest CMV incidence (75%) and the highest CMV severity (45%) was in T_0 (control) whereas T_4 (neem leaf extract) significantly lowered CMV incidence and severity to 8.33% and 22.3% respectively.

- ✓ Growth and yield attributes were higher in T_5 (aluminium foil as reflective mulch). T_5 (aluminium foil as reflective mulch) resulted in the tallest plants, highest number of healthy leaves (79.00 per plant), the highest number of fruits per plant (13.33), heaviest fruits (62.10 g), highest fruit yield per plant (725.74 g), highest yield per plot (2.78 Kg), the highest total yield (5.56 ton/ha) and the lowest yield loss (17.68%) compared to T_0 (control).
- ✓ The lowest number of infected leaves and leaf area reduction (%) was observed in T_4 (neem leaf extract) compared to T_0 (control).
- ✓ There was a positive correlation was found between disease incidence and disease severity. On the other hand, the yield was negatively correlated with the CMV incidence and severity. A 74.2% and 95.7% reduction of yield was observed due to increase of disease incidence and severity respectively.

CHAPTER VI

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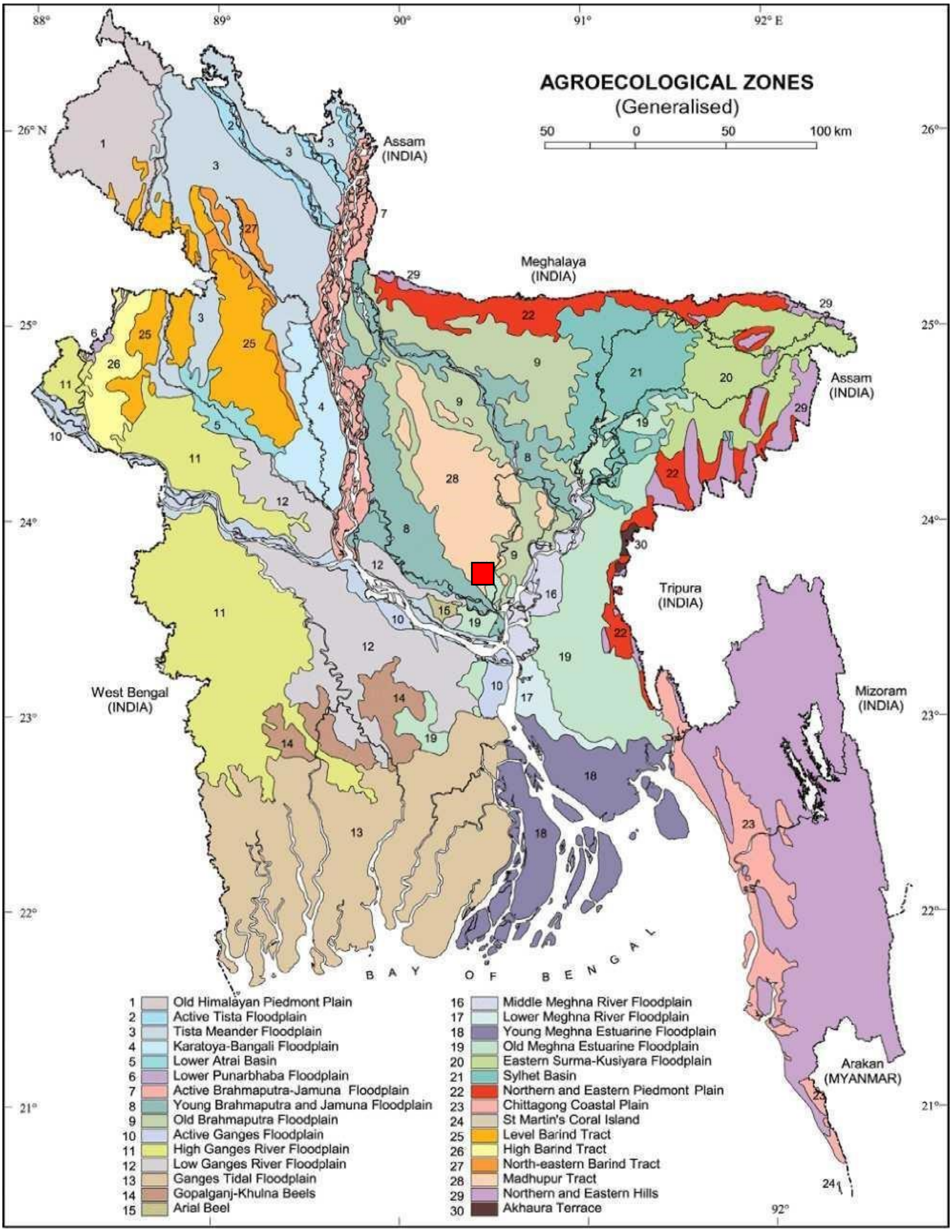
APPENDICES

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



Appendix II: The characteristics of soil of the experimental site

Morphological characteristics

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

Chemical composition

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

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

Appendix III: Monthly records of meteorological observation at the period of experiment (November, 2020 to April, 2021)

Name of the Month	Temparature (°C)		Avg. Relative Humidity (%)
	Maximum	Minimum	
November, 2020	31	16	66
Decmeber, 2020	31	15	66
January, 2021	30	15	61
February, 2021	38	22	59
March, 2021	37	24	60
April, 2021	36	25	64

Source: https://www.timeanddate.com/weather/bangladesh/dhaka/

Appendix IV: Recommended and applied doses of fertilizers and manure

Fertilizers	Total Amount		Basal dose (Kg/m^2)	@25DAT (Kg/m^2)	@50DAT (Kg/m^2)
	Recommended (Kg/ha)	Applied (Kg/m^2)			
Cowdung	10 ton	237	237	-	-
Urea	250	5.93	1/97	1.97	1.97
TSP	350	8.30	-	-	-
MP	250	5.93	1.97	1.97	1.97
Gypsum	110	2.61	2.61	-	-
Zinc Sulphate	5	118.5 g/m^2	118.5 g/m^2	-	-

Source: Azad, A., Wahab, M., Shaha, M., Nesa, J., Rahman, M., Rahman, M. and Al-amin, M. (2019). KRISHI PROJUKTI HATBOI (Handbook on Agro-Technology), 8th ed. Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh.

Appendix V: Nutritional status of capsicum

Name of component	Amount
Water	93.9 g
Energy	20 kcal/84 kJ
Protein	0.86 g
Total lipid (fat)	0.17 g
Ash	0.43 g
Carbohydrate	4.64 g
Fiber	1.70 g
Sugars	2.40 g
Calcium	10 mg
Magnesium	10 mg
Phosphorus	20 mg
Potassium	175 mg
Sodium	3 mg
Vitamin C, total ascorbic acid	80.4 mg
Thiamin	0.057 mg
Riboflavin	0.028 mg
Vitamin B-6	0.224 mg
Folate	10 µm
Vitamin A	18 µg/ 370IU
Vitamin E	0.37 mg
Vitamin K	7.4 µg
Fatty acids	0.058 g
Cholesterol	0 g
Others	Trace amount

Source: USDA

Appendix VI: One Month of Capsicum Seedlings and Transplanting


Capsicum seedling


Transplanting

Appendix VII: Application of Plant extracts on capsicum plants

Appendix VIII: Field Preparation and Field View