ELISA-Based Screening of Tomato Varieties against *Tomato Yellow Leaf Curl Virus* (*TYLCV*)

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CERTIFICATE

This is to certify that the thesis entitled **ELISA-Based Screening of Tomato Varieties against *Tomato Yellow Leaf Curl Virus (TYLCV)*** submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology, embodies the results of a piece of bonafide research work carried out by **Registration No. 12-04865** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

Dated: 28/02/2019
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Dedicated to

My

Beloved Parents

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The Author

ELISA-BASED SCREENING OF TOMATO VARIETIES AGAINST *TOMATO YELLOW LEAF CURL VIRUS (TYLCV)*

ABSTRACT

A pot experiment was conducted in net house under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. The purpose of the study was to evaluate the incidence and severity level of the *Tomato Yellow Leaf Curl Virus* (*TYLCV*) and to screen the resistance/tolerance of selected tomato varieties against *TYLCV* through serological test (DAS-ELISA). DAS-ELISA test was performed at tissue culture laboratory of Bangladesh Council of Scientific and Industrial Research (BCSIR).The experiment was carried out during the period October 2017 to April 2018. In total ten tomato varieties viz. BARI Tomato-2, BARI Tomato-8, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-5, BARI Hybrid Tomato-7, BARI Hybrid Tomato-9, Ratan and Sorno Komol were selected to conduct this study. Among the selected ten tomato varieties, the highest disease incidence (100%) was recorded in 5 (five) varieties viz. BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-7 and Sorno Komol and there was no incidence was found in BARI Hybrid Tomato-5. The highest disease severity was recorded in BARI Hybrid Tomato-7 (70%) and there was no severity was found in BARI Hybrid Tomato-5. Although the variety BARI Hybrid Tomato-5 was not shown any kind of typical symptoms in the net house conditions when investigated on the basis of biological properties but it was found infected in DAS-ELISA test. From the above mentioned results, it can be concluded that symptomology is not the reliable method for all virus identification/detection. Different growth parameters, growth and yield attributes were also studied in this piece of research work and it was revealed that morphology, physiology, growth and yield contributing factors are significantly affected by *TYLCV* infection in different varieties.

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CHAPTER 1

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is a solanaceous self-pollinated vegetable crop. It is the second most important vegetable crop next to potato in the world (Choudhury, 1979). The cultivated types of tomato belong to *Lycopersicon esculentum* and are originated from South American Andes. Due to high adaptability of tomato plant to wide range of soil and climate, it is widely grown in our country (Ahamed, 1995).

It is one of the most important, popular and nutritious vegetables that grown in Bangladesh and other countries. Present world production of tomato is about 170.8 million tons and total tomato growing area is 4.9 million hectares (FAOSTAT, 2016). In Bangladesh, the recent statistics shows that tomato was grown in 67535 acres of land and the total production was approximately 368121 metric tons during the year 2015-2016 and the average yield of tomato was 5451 kg/acre in winter season (BBS, 2016). It is used both as salad and to prepare curry. It is also used to make soups, pickles, conserves, ketchup's, juices, sauces etc. It is widely grown in both winter and summer season around all parts of the country (Haque*et al*., 1999). It also contains a large quantity of water, calcium and niacin all of which have great importance in the metabolic activities of human. It is also a good source of vitamin A, C, E and minerals (potassium, calcium, phosphorus, iron and zinc) that are very good for body and protect the body against diseases (Taylor, 1987). It is an excellent source of lycopene, carotenoids and polyphenolic compounds which are a powerful source of antioxidant and reduces the risk of prostate cancer (Hossain *et al.*, 2004). It is even present when tomatoes are cooked. It also has medicinal value. The pulp and the juice are easily digestible and blood purifier (Frasher *et al.*, 1991). It can be referred as poor man's Orange.

The best tomato growing areas in Bangladesh are Chittagong, Cumilla and Rajshahi. As a cash crop, it has great demand in the International market (Solieman *et al.*, 2013). Tomato is an important condiment in most diets and a

very cheap source of vitamins. The yield of tomato in our country is not satisfactory in comparison with other tomato growing countries (Aditya *et al.*, 1999*).*

Tomato production in our neighboring country India was 7873 kg/acre (Indian Horticulture Database, 2017) where as our production is only 5451 kg/acre. In comparison with this our production is very low. Although the total cultivated area and production of tomato in our country have increased gradually over the last few years but the productivity is still too low (6.46t ha^{-1}) compared to the average of the world yield (34.86 t ha^{-1}) as per FAO (2016).

As India and our environment is almost similar with some exception the variation comes mainly due to pest and diseases. There are several diseases occurs in tomato like viral, fungal, bacterial and nemic disease. Globally tomato is susceptible to more than 200 diseases, among them 40 are caused by viruses (Martelli and Quacquarelli, 1982; Lukyanenko and Kalloo, 1991). However, the incidence and economic impact of virus infections in tomato varies greatly upon different factors like country, cropping method and the virus itself (Martelli and Quacquarelli, 1982).

In our country 16 different tomato viruses are identified. Among the viral diseases *Tomato Yellow Leaf Curl Virus* (*TYLCV*) is the most devastating one. In our country it can cause up to 100% yield loss. *TYLCV* is also wide spread in many Mediterranean, Middle Eastern, American, African, and Asian countries. *TYLCV* is an ssDNA plant virus, which belongs to the family Geminiviridae of the genus Begomovirus (Czosnek and Laterrot, 1997). This viral disease is transmitted by whiteflies (*Bemisia tabaci*) and by grafting but it is mechanically non- transmitted. The disease was first reported in Israel and Jordan Valley in the early 1960s and is now economically significant in many countries (Jones *et al.*, 1993). The causal agent was described in 1964 and named as *Tomato yellow leaf curl virus* (*TYLCV*) by (Cohen and Harpaz, 1964). Since then *TYLCV* has been reported from all over the tropics, subtropics, the Mediterranean, the Caribbean's and the Americas (Czosnek and Laterrot, 1997, and Nakhla *et al.*, 1994). *TYLCV* threatens both commercial

tomato productions in the fields and home garden which could be able to infect plants at any stage of plant growth (Gupta, 2000).

In Bangladesh, *TYLCV* incidence was first reported by Akanda in 1991, based on symptomatology. Symptoms of *TYLCV* include stunted plant growth, chlorotic yellowing of leaves, and distortion of leaflets in a cupped down and inward shape or upward curling of the leaflet margins (Cohen and Lapidot, 2007). The impact of *TYLCV* on tomato production is very severe. If plants are infected at an early stage, they do not bear fruit and their growth becomes severely stunted resulting 100% yield loss.

Since the discovery of *TYLCV* many efforts have been made to characterize the virus systematically to manage the disease through manipulation of sowing dates, growing seedlings in net house, application of insecticides and so on (Paul, 2002; Rahman, 2003; Gupta, 2000; Azam, 2001; Akhter, 2003; and Sultana, 2001). Although the information provides a number of information about *TYLCV* and its management in Bangladesh but none of the efforts could provide conclusive information about *TYLCV*. The frequent development of disease epidemic and very high yield loss leading to a total crop failure which have drawn attention of the scientists to develop effective management program against *TYLCV* for profitable tomato production in many countries. Various strategies have been taken to manage the disease but all are in vain.

So far, there are many methods are reported for plant viruses identification/detection for example biological properties, physiological properties/in-vitro properties, intrinsic properties, Serological test and modern molecular techniques. The purpose of this study was to detect and characterize the *TYLCV* on the basis of Biological properties and Serological test (ELISA). For that purpose, *TYLCV* was detected through symptomological test and transmission method. Symptomatology based identification is possible but it needs good skill and experience as because similar symptoms may be caused by various growing conditions and other viruses. ELISA is the best method to detect most of the plant viruses specially DNA viruses. In this study, we detected the *TYLCV* through DAS-ELISA test (Webter *et al*., 2004).

Objectives:

The specific objectives of this study are given below:

-To identify the *Tomato Yellow Leaf Curl Virus (TYLCV)* on the basis of biological properties

-To evaluate the incidence and severity level of *TYLCV* against the selected tomato varieties.

-To screen the level of resistance of varieties against *TYLCV* through ELISA test

CHAPTER-2

REVIEW OF LITERATURE

Tomato (*Lycopersicon esculentum* Mill.) is an important and most widely grown vegetable crop in Bangladesh. Tomato production in Bangladesh is under constant threat of Tomato yellow leaf curl disease caused by *Tomato Yellow Leaf Curl Virus* (*TYLCV*). A lot of work has been done on various aspects of *TYLCV* in Bangladesh and abroad and is reviewed as under:

2.1. About Tomato

Tomato originated from the Andean region, an area now located in parts of Chile, Colombia, Bolivia, Ecuador and Peru (Bai & Lindhout, 2007). Because tomato was first domesticated by the Mayas and the Aztecs (Barndt, 2008), Mesoamerica is considered as the birthplace for cultivated tomato. The word *tomatl* existed in the native Mexican language nahuatl to describe plants bearing globose and juicy fruit (Blanca *et al*., 2012).

Tomato was introduced to Europe most probably from Mexico (Blanca *et al*., 2012) in the 16th century by Spanish conquistadors. Due to its resemblance with toxic *Solanum* species like belladonna and mandrake, the tomato was long used for ornamental purposes only appearing in cookbooks by the beginning of the 17th century. From Spain, the tomato reached Italy and England, whence British subsequently "exported" tomato to Asia, Middle East and North America (McCue, 1952; Bergougnoux, 2014).

Tomatoes are adapted to a wide range of environmental conditions, but in temperate areas low temperatures and short growing seasons can limit growth. Tomatoes prefer slightly acidic soils with a pH of 6.0 to 6.8. The tomato plant requires significant quantities of water, but not in excess, since tomato roots will not function under water-logged (anaerobic) conditions. (Cox and Tilth, 2009).

Sufficient moisture must be maintained to establish the plant and carry it through to fruit production. When the moisture level surrounding the roots is too high, epinasty, poor growth, late flowering, fewer flowers and lower fruit set occurs. Fruit disorders such as cracking and blossom-end-rot are common when water availability is inconsistent. Even under moderate water stress, photosynthesis is slowed because the movement of gases through the stomata is restricted and the movement of water up the xylem is slowed (Benton, 2008).

2.2. Tomato Morphology

Tomato was classified by the Swedish botanist Carl Linnaeus in 1753 in the genus *Solanum* with the species ephitet *lycopersicum*. It belongs to the family Solanaceae, which contains over 3000 plant species, including many economically important plants such as potato, eggplant, peppers, petunia and tobacco. With 1250–1700 species, *Solanum* is the largest genus in the Solanaceae family. Tomato is botanically classified as the cultivated tomato *S. lycopersicum* and its twelve wild species. Wild tomato species have very small fruit while the modern cultivated tomatoes have a large variation in fruit size, ranging from less than 20 g for cherry tomato up to 500 g for the beef tomato (Bergougnoux, 2014).

Although usually cultivated as an annual crop, tomato is a perennial plant. It has bipinnate leaves, hairy stems and flowers with usually 5–7 petals (Blanca *et al*., 2012). Tomato is diploid (Nesbitt & Tanksley, 2002) and its genome size is approximately 900 Mb, comprising 12 chromosomes and 34,727 protein-coding genes (The Tomato Genome Consortium, 2012).

Tomato is cultivated for its fleshy fruit (Blanca *et al*., 2012). Botanically, tomato is a fruit berry, and not a vegetable (Bergougnoux, 2014). The fruit is a specialized organ that results from the development of the ovary after successful flower pollination and fertilization. It provides a suitable environment for seed maturation and dispersal (Chevalier *et al*., 2011). The fleshy fruit corresponds to the ovary and is composed of an epidermis, a thick pericarp (composed of exocarp, mesocarp and endocarp) and the placental tissues, which surround the seeds. The pericarp is the outer wall of the

gynoecium, and is composed of at least two carpels, which determine the number of fruit locules (Bergougnoux, 2014).

2.3. About *TYLCV*

The virus belongs to genus Begomovirus and has a single-stranded DNA (ssDNA). The genomes are encapsidated in about 20X30 nm geminate particles (Goodman, 1977).

Among the viruses infecting tomato, TYLCV has the highest economical impact (Czosnek, 2007) and it is considered as one of the most devastating plant viruses worldwide (Hanssen *et al*., 2010; Péréfarres *et al*., 2012). Currently, 10 different begomovirus species and their strains are associated with tomato yellow leaf curl disease (TYLCD) (Brown *et al*., 2015). Among them, TYLCV is the most dominant species and it is divided into different strains, among which the Israel (TYLCV) and mild (TYLCV-Mld) strains are most prevalent (Hanssen *et al*., 2010; Lefeuvre *et al*., 2010; Navas-Castillo *et al*., 2011).

Symptoms of TYLCD were first observed in the Jordan Valley in 1929 (Cohen & Lapidot, 2007). It took about 30 years before the virus was first described and found to be circulative and persistent in the insect vector (Cohen & Harpaz, 1964). During the 1970´s, the first electron micrographs (EM) were produced showing the novel geminate particle morphology of geminiviruses (Goodman, 1981) and it was discovered that the virions of begomoviruses contain a genome of ssDNA (Goodman, 1977). EM observations of thin sections of TYLCV-infected tomato leaves also indicated that geminate particles are located in the nuclei of phloem parenchyma cells (Russo *et al*., 1980; Cherif & Russo, 1983). In the following decade, TYLCV virions were isolated and purified (Czosnek *et al*., 1988) and in 1991, the genome sequence of TYLCV was published (Navot *et al*., 1991).

TYLCV has a wide host range with more than 30 plant species in over 12 families, including vegetables and ornamentals as well as wild plants and

weeds. The reservoirs for TYLCV vary among regions and because infection of other hosts than tomato can be symptomless, reservoirs may not be obvious (Polston & Lapidot, 2007). In tomato, TYLCV can cause yield losses of up to 100% and induce symptoms such as upward curling, reduction and yellowing of leaves as well as flower abortion and overall reduction in growth (Díaz-Pendón *et al*., 2010; Navas-Castillo *et al*., 2011).

Tomato leaf curl virus (*TYLCV*) is a group of whitefly-transmitted geminiviruses (Cohen and Harpaz, 1964; Czosnek *et al*., 1988), causing an extensive yield loss to tomato crops in many tropical and subtropical regions worldwide (Czosnek and Laterrot, 1997).

2.4. Disease symptoms

Tomato leaf symptoms include chlorotic margins, small leaves that are cupped, thick rubbery. The majority (90%) of flowers abscises after infection and therefore few fruits are formed. *TYLCV* is considered as a phloem limited virus (Ganif, 2003).

The various prominent symptoms of tomato leaf curl virus such as upward curling of leaf margins, stunting, reduction of leaf size, corrugated leaf, shortening of internodes and severe reduction in fruit yield, had been observed from Middle East (Makkouk and Laterrot, 1983).

The upward leaf curling and interveinal and marginal chlorosis in tomato plants due to tomato leaf curl virus is reported by (Zhang *et al*., 2008).

Avgelis *et al.,* 2001, first reported that *TYLCV* in Greece. They described the disease symptom as leaf curling, reduced leaf size, yellowing, shortened internodes and a bushy appearance. Mechanical inoculation was unproductive while transmission was obtained by grafting on to healthy tomato plants.

It was reported that symptoms of stunting, curling and yellowing of leaf margins, and marked reductions in the number of fruits were observed in some greenhouse-grown tomato cv. Naxos plants in the province of Bari in Apulia, Italy, were observed in the being an isolate of *TYLCV-Sar*. The

nucleotide sequence of the 580 bp amplicon shared 99.5% homology with a clone from a Sicilian isolate and 97.5% with a clone from a Sardinian isolate of *TYLCV-Sar*. This is the first report of *TYLCV* in Apulia, Italy (Sialer *et al.*, 2001).

2.5. Virus Identification

A survey was conducted to determine the incidence of *Cucumber mosaic virus* (CMV), *Beet curly top virus* (BCTV), *Tomato yellow leaf curl virus* (TYLCV), *Tomato chlorotic spot virus* (TcSV), *Potato virus Y* (PVY), *Potato virus S* (PVS), *Tomato spotted wilt virus* (TSWV), *Tomato ringspot virus* (TRSV), *Tomato aspermy virus* (TAV), *Arabis mosaic virus* (ArMV), *Tobacco streak virus* (TSV), *Tomato bushy stunt virus* (TBSV), *Tobacco mosaic virus* (TMV), and *Tomato mosaic virus* (ToMV) on tomato (*Solanum lycopersicum*) in the major horticultural crop growing areas in the southeast and central regions of Iran. Samples of symptomatic plants were analyzed for virus infection by enzyme-linked immunosorbent assay (ELISA) using specific polyclonal antibodies. ArMV and CMV were the most frequently found viruses, accounting for 25.6 and 23.4%, respectively, of the collected samples. BCTV, TSWV, TMV, PVY, ToMV, and TYLCV were detected in 6.1, 5.8, 5.6, 5, 4.8, and 1.6% of the samples, respectively. TBSV, TAV, TSV, PVS, and TRSV were not detected in any of the samples tested. Double and triple infections involving different combination of viruses were found in 13.9 and 1.7% of samples, respectively. This is the first report of PVY and ArMV as viruses naturally infecting tomato in Iran (Michael, 2009).

viruses are very tiny compared to other groups of plant pathogens like fungi and bacteria which can be visualized through microscopes but plant viruses are too small to observe using light microscopes and they can be seen only using a transmission electron microscope and are made of a coat protein and a types of nucleic acid, DNA or RNA based on the nucleic acid core carrying genetic information (Ellis et al., 2008).

Since Tobacco mosaic virus (TMV) was first recognized over a century ago, more than 1000 of plant viruses have been found (King et al., 2011; Scholthof, 2000). It has been known that like other plant pathogens including bacteria, fungi, and phytoplasma, plant viruses spread and cause major economic losses to many crops such as barley, Tomato, potato, rice, and wheat (Agrios, 2005; Ellis et al., 2008; Strange, 2005).

Symptoms of viral diseases include crinkling, browning of leaf tissues, mosaic, and necrosis. Sometimes, however, symptoms may not be visually detected because infection of plant viruses causes no symptoms (Bove et al., 1988; Vander Want and Dijkstra, 2006). In addition, plants can also display virus like symptoms when plants respond to unfavorable weather, nutritional imbalances, infection by other types of pathogens, damage caused by pests or abiotic agents and others (Vander Want and Dijkstra, 2006). Thus, viral disease diagnosis by symptoms is more difficult than other pathogens (Livenes *et al*., 2005). The diagnosis is the basis to manage plant diseases and to predict the crop loss by infection of plant pathogens (Vander Want and Dijkstra, 2006). Accurate diagnosis of virus diseases, is the first important step for crop management system (Aboul-Ata *et al*., 2011)

As the internationalization of the domestic agricultural market, virus diagnostics is very essential to use high quality seed as well as virus free seeds (Lievens et al., 2005; Wang et al., 2011). The methods for detection and identification of viruses are critical in virus disease management (Aboul-Ata et al., 2011). Therefore, detection methods should be more convenient, effective, specific and permitted the use for detecting plant pathogens (McCartney et al., 2003).

A lot of methods have been developed to detect plant viruses, such as microscopical observation, serological techniques, molecular methods and so on (Lopez et al., 2008; Makkouk and Kumari, 2006; Webster et al., 2004).

2.6. ELISA

Serological detection systems use specific antibody developed in animals in respond to antigens. Viruses can be detected if viral antigens are used to develope antibody. In fact, these kinds of techniques have been used for the routine diagnostic tool. Many serological methods have been reported including Enzyme-Linked Immunosorbent Assay (ELISA) reported by (Torrance, 1998).

Common ELISAs are performed in polystyrene plate capable of binding antibodies or proteins with association of the enzyme-substrate reaction (Corning Life Science, 2001; Luminex, 2010). In order to get an accurate and reproducible result, the enzyme-substrate reaction needs to be optimized timing and development conditions (Corning Life Science, 2001).

ELISA has been used as very popular assay to detect plant viruses within plant material, insect vectors, and seeds (Clark and Adams, 1977; Naidu and Hughes, 2001; Webster et *al.*, 2004). Level of infection is measured based on the optical density (the degree of coloration) of ELISA reaction (Corning Life Science, 2001; Webster et al., 2004).

Advantages of ELISA are that it is sensitive to a great number of samples which can be examined at the same time (Vemulapati et al., 2014) little amount of antibody for the detection of diseases, and the process can be semi-automated (Naidu and Hughes, 2001). Specific antiserum has been developed against the target virus (Torrance, 1998). It has been employed for the detection of a lot of viruses including,*Tomato Yellow Leaf Curl Virus* (*TYLCV*) *CMV*, Citrus tristeza virus (*CTV*), Potato leaf roll virus (*PLRV*), Potato *virus X* (*PVX*), and *Potato virus Y* (*PVY*) (El-Araby *et al*., 2009; Sun *et al*., 2001). Large amount of sample for ELISA is needed for capturing antigen of interest from the sample compared to sample requiring for molecular methods and it takes about 2 days for diagnosis (Lievens et al., 2005; Luminex, 2010).

Cucumber mosaic virus (*CMV*) was detected and characterized by bioassay, double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Sharma *et al.,* 2005) .Indirect enzyme linked immunosorbent assay (I-ELISA) is used for CMV detection in cucumber plants (El-Afifi *et al.,* 2007). It was reported that, positive reactions against CMV in leaves of *Echium candicans* in France were recorded by double antibody sandwich-ELISA to CMV specific polyclonal antibodies(Cardin and Moury, 2007). DAS-ELISA analysis revealed the presence of *Cucumber mosaic virus* (CMV) in the infected tomato plants (Aramburu *et al.*, 2007).

ELISA is a serological test that uses antiserum prepared against a particular virus. The antiserum contains antibodies generated in blood serum of rabbits inoculated with that particular virus' antigen, and can be made in a local and simple laboratory. This antiserum and alkaline buffers are used on microtiter plastic plates to test plant sap for that specific virus (Clark and Adams, 1977).

Double-Antigen-Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) used for immediate serological identification of viruses in a sample, based on viral protein differences (Clark and Adams, 1977).

DAS-ELISA is widely used. The reagents and chemicals required are readily available, and it gives adequate identification of viruses. DAS-ELISA was used to identify PVMV in sweet pepper (*Capsicum annuum*) in Cameroon. Like all other ELISAs, it is fairly cheap, especially if antisera can be produced locally and do not have to be bought from commercial companies (Nono-Womdim and Atibalentja, 1993).

2.7. *TYLCV* Identification

Tomato yellow leaf curl (TYLC) is one of the most devastating viral diseases of cultivated tomato (*Lycopersicon esculentum*) in tropical and subtropical regions worldwide, and losses of up to 100% are frequent. In many regions, TYLC is the main limiting factor in tomato production. The causal agents are a group of geminivirus species belonging to the

genus *Begomovirus* of the family *Geminiviridae*, all of them named *Tomato yellow leaf curl virus* (*TYLCV*). There has been almost 40 years of research on *TYLCV* epidemics and intensive research programmes have been conducted to find solutions to the severe problem caused by these viruses. (Moriones and Navas-Castillo, 2000).

The major tomato virus having monopartite single-stranded DNA is *Tomato yellow leaf curl virus* (*TYLCV*). Symptoms caused by this virus are chlorotic and leathery leaves, leaf curling, blistering, reduced leaf size, shortened internodes, chlorosis of leaf margins, rounding of leaflets, flower abscission and poor bearing (Cohen and Nitzany, 1966: Yassin *et al.*, 1982; Makkouk and Laterrot, 1983; Thomas, 1984).

There are three distinct TYLCVs based on nucleotide sequence comparisons. It is also considered that viruses of the genus *Begomovirus*, which have nucleotide sequence similarity levels below 90 % are distinct from each other (Padidam *et al*., 1995), although later on ICTV reported that this can only be concluded when complete genome sequences have been compared (Fauquet *et al*., 2003), and not on the basis of the intergenic region (IR) or coat protein gene alone. Similarity comparisons have previously been done on the basis of the intergenic region and partial sequences for other TYLCVs including isolates from Egypt and Israel, which are similar but different from isolates from Spain (GenBank No. L 277081) and Sicily (GenBank No. Z28390) (Noris *et al*. 1993).

2.8. Incidence and distribution of *TYLCV*

TYLCV was present in almost all fields of Belgaum, Dharward, Haveri districts of Karnataka with percent disease incidence of 4 to 100 % in rabi and 60 to 100 % during summer season. (Reddy *et al*., 2011).

TYLCV is quite general in the tropics. In Africa, it has been reported from South Africa, Senegal, Tanzania, Malawi, Zambia, Zimbabwe, Nigeria, Ivory Coast, Egypt and Sudan (Yassin *et al.*, 1982; AVRDC, 1987; Czosneck *et al.*1990; Nakhla *et al.*, 1993; Nono-Womdim *et al*, 1994; Chiang *et al*.,

1996). It is also widespread in the rest of the Old World and in the New World, e.g. in South East Asia and East Asia, the Americas and the Mediterranean (Green and Kallo, 1994; Chiang *et al.*, 1996; Polston and Anderson, 1997; Czosnek and Laterrot, 1997).

A survey of tomato and pepper viruses was conducted in Sudan during the last ten years. It covered Central, Northern, Eastern, Southeastern and Western regions of Sudan. The results revealed the presence of many mosaic - inducing virus and virus like agents. *Cucumber mosaic virus* (*CMV*), *tomato mosaic virus* (*ToMV*), *tobacco mosaic virus* (*TMV*), *Tomato yellow leaf curl virus* (*TYLCV*) and *potato virus Y* (*PVY*) were all found to infect both tomato and pepper (Elshafie *et al.*, 2005).

In the semi-tropical climatic zone of Egypt, indicated that at the beginning of Spring and early Summer (February - April), when tomato plants have just established, TYLCV incidence is very low (Moustafa, 1991). The latter becomes high towards the end of Summer (September – mid October), and then coincides with peak whitefly population density (Riley *et al.*, 1995).

This is followed by high TYLCV incidence and severe damage in the fall (Autumn) when production losses rise to 80% and almost all plants are infected. Similarly, Cohen and Antignus (1994) observed that in the Jordan Valley, the spread of TYLCV was significantly correlated with *B. tabaci* population size. As in Egypt, peak whitefly population occurred between the first week of September and Mid-October. In Tanzania, TYLCV symptoms and whitefly vector presence are reported to be most common during November to February (Nono-Womdim *et al*., 1996).

2.9. Transmission of *TYLCV*

Three-quarters of all known plant viruses are transmitted by insect vectors (Hogenhout *et al*., 2008). Until recently, TYLCV was known to be

transmitted only by *B. tabaci* or artificially via grafting, particle bombardment or agroinoculation using *Agrobacterium tumefaciens* (Stanley *et al*., 2001; Scholthof *et al*., 2011). Notably, TYLCV was recently reported to be also seed transmissable with floral infection and seed transmission rates of 20–100%. Importantly, virus was detected in the embryos of the seeds by PCR (Kil *et al*., 2016).

More than 230 plant virus and viroid diseases are transmitted through seeds (Sastry, 2013) and infected seeds can be the initial source of inoculum for subsequent vector mediated transmission (Ali and Kobayashi, 2010). In addition, seed transmission enables survival of viral inoculum between growing seasons and virus diseases may be disseminated worldwide through exchange of seeds having undetected infections (Sastry, 2013).

TYLCV is transmitted by a whitefly (*Bemisia tabaci* Gennadius) of the Family *Aleyrodidae* (Gerling and Mayer, 1995). *Bemisia tabaci* occurs in biotypes A and B. Biotype B is more common than A and is regarded by some as a separate species designated *B. argentifolii* (Bellows *et al.*, 1994). Others continue to regard it as a biotype of *B. tabaci*, even though there are many more biotypes, which include biotype Q (Demichelis *et al*., 2000).

In some circumstances, the incidence and rate of spread of TYLCV are directly proportional to the whitefly population present in the environment (Mansour *et al.*, 1992; Mehta *et al.*, 1994). Both adults and larvae can acquire the virus by feeding on infected plants with a minimum access and acquisition period (AAP) of 15 minutes. The virus has a latent period of 21-24 hours, and persists for 10 to 20 days in viruliferous *B. tabaci* adults (Cohen *et al.*, 1966).

Whiteflies are vectors of viruses causing many diseases in the tropics and subtropics. The whiteflies are a snow white insect measuring about 1mm in length (Bohmfalk *et al*., 2006). The adult whitefly starts laying eggs

immediately after emerging from the nymph. Eggs are laid underneath leaves to protect them from adverse weather conditions such as rainfall and direct solar radiation (Marks, 2006).

2.10. Screening of *TYLCV* in tomato

Twenty three tomato accessions were screened for resistance to *tomato yellow leaf curl virus* under field conditions and examined that accessions of the wild species *Lycopersicon pimpinellifolium*, *Lycopersicon hirsutum*, and *Lycopersicon peruvianum* showed variance in their response to infection, however *Lycopersicon chilense* showed highest degree of resistance against the disease (Zakay *et al*., 1991).

Twenty tomato genotypes were screened for resistance against *tomato yellow leaf curl virus* (*TYLCV*) in Madhya Pradesh, India and reported that the cultivars Hisar Anmol and Hisar Gaurav were resistant to tomato leaf curl disease (Rai *et al*., 2001).

Ten tomato cultivars were screened against *TYLCV* at 45 days after planting and observed that among all the cultivars Punjab Chhuhara showed higher degree of resistance against tomato leaf curl virus (Sajeed *et al*., 2002).

A total of 34 tomato genotypes were screened for resistance to *TYLCV* under glasshouse and field conditions and found that *Lycopersicon hirsutum* LA1777 and PI 390659 were best sources of resistance to the virus (Maruthi *et al*., 2003).

Total 22 cultivars of tomatos were screened against *TYLCV* in Faizabad and out of 22 cultivars screened, none of the cultivars was found resistant against the disease. However Hisar Anmol was found moderately resistant to the virus, while three cultivars were categorized as moderately susceptible and 18 were found susceptible to tomato leaf curl virus (Yadav and Awasthi, 2009).

The screening of tomato germplasm against *TYLCV* was done in Ghana. The researcher evaluated 30 accessions against the disease under field conditions at 30, 45 and 60 days after transplanting and found that no accession provided complete resistance to tomato leaf curl virus (Osei *et al*., 2012).

Thirty two tomato genotypes were screened for resistance against tomato leaf curl disease during rabi season at Institute of Agricultural Sciences, Banaras Hindu University, Varanasi and Vegetable research farm, Varanasi, Uttar Pradesh. It was observed that one wild accession, H-88-78-1 showed immune reaction against *TYLCV*, three genotypes viz., Hissar Lalima, TLBRH-6 and NS-515 showed resistant reaction and eight genotypes viz., Hissar Anmol, Kishi Vishesh, Kashi Amrit, Kashi Sharad, KS-17, KS-118, Avinash-2 and US-1008 were found moderately resistant against tomato leaf curl virus (Singh and Prajapati, 2014).

Twenty seven tomato varieties/lines were screened for the source of resistance against *tomato yellow leaf curl virus* (*TYLCV*) under field conditions and found that three varieties were highly susceptible, six were susceptible, four were moderately susceptible, six were moderately resistant and eight were resistant. No variety/line was highly resistant or immune against tomato leaf curl virus disease (Zeshan *et al*., 2016).

2.11. Yield loss

Water deficits improved the quality of fruits, increased soluble solids and acidity and that water stress throughout the growing season significantly reduced yield and fruit size, but plants stressed only during flowering showed fewer but bigger fruits than completely non-stressed plants (Nuruddin *et al*., 2003).

Virus is ranked as the second most important plant pathogens following fungi (Vidaver and Lambrecht, 2004). Economic loss has been estimated more than several billion dollars per year worldwide because of plant viruses (Hull, 2002; Plant Viruses, 2003). The crop damages owing to viral diseases are difficult to predict, because it depends on region, virus strain, host plant cultivar/variety, and time of infection (Strange, 2005).

The tomato yellow leaf curl (TYLC) is one of the most devastating viral disease of cultivated tomato (*Lycopersiconesculentum*) in tropical and

subtropical regions of worldwide causing the losses up to 100 per cent (Moriones and Navas, 2000).

It was reported that 96.90 % yield loss of tomato plant due to *TYLCV* in autum season (Ajlan et al., 2007).

Tomato yellow leaf curl virus is a geminivirus transmitted by whitefly *(Bemisia tabaci).* It causes most destructive disease of tomato throughout the Mediterranean region, the Middle East and the tropical regions of Africa and Central America. It is also reported from Japan, Australia and the USA. In many cases yield loss can be up to 90% reported by (Ganif, 2003).

It has been reported that water deficit stress increases the flower abortion, thus affects the fruits settings. The low marketable fruit yield obtained for some tomato varieties might be due to non-development of flowers. It was observed that only 50% of the flowers produced developed into fruits, thus sink size was a limiting factor to fruit production in tomato (Olaniyi *et al*., 2010).

CHAPTER 3

MATERIALS AND METHODS

Tomato leaf curl disease caused by *Tomato Yellow Leaf Curl Virus* (*TYLCV*) of the genus *Begomovirus* of the family Geminiviridae is one of the most devastating disease in many tropical and subtropical regions in the world and yield losses exceeds 90.00% when infection occurs at three to four weeks after transplanting. The present investigations were carried out under field as well as laboratory condition during 2017-18 to ascertain the incidence, severity and serological detection of *TYLCV* in the Depertment of Plant Pathology, Sher-e-Bangla Agricultral University, Dhaka. The material used and techniques adopted during the investigation are being summerized under here.

3.1. Experimental Site

The experiment was conducted at Net house of the Dept. of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh, during the period of October 2017 to April 2018. The experimental area was situated at 23°46' N latitude and 90°22'E longitude at an altitude of 8.6 meter above the sea level (Anon. 1988). (Appendix- I).

3.2. Soil Characteristics

The soil of the experiment was taken from a medium high land which belongs to the Modhupur tract, Agro Ecological Zone no 28. The soil texture was silt loam, Low level of nutrients, non-calcareous, acidic, brown or red soil of Tejgaon soil series with a pH 6.7. Before conducting the experiment Soil samples of the experimental pots were collected from the experimental field of Sher-e-Bangla Agricultural University (SAU) at a depth of a 0 to 30 cm and analyzed in the Soil Resources Development Institute (SRDI), Farmgate, Dhaka. (Appendices- II).

3.3. Climate

The climate of the Modhupur Tract varies slightly from north to south, the northern reaches being much cooler in winter. Average temperatures vary from 28^0 C to 32^0 C in summer, falling to 20^0 C in winter, with extreme lows of 10^0 C. Rainfall ranges between 1,000 mm and 1,500 mm annually, heavy rainfall in kharif season (May-September) and scanty in rabi season (October-March) . Severe storms are unusual but tornadoes have struck the southern areas. During the month of December, January and February there was no rainfall. During the period of investigation the average maximum temperature was 32^0 C and average minimum temperature was 20^0 C. Details of the meteorological data in respect of temperature, rainfall and relative humidity during the period of experiment were collected from Bangladesh Meteorological Department, Agargaon, Dhaka. (Appendices-III).

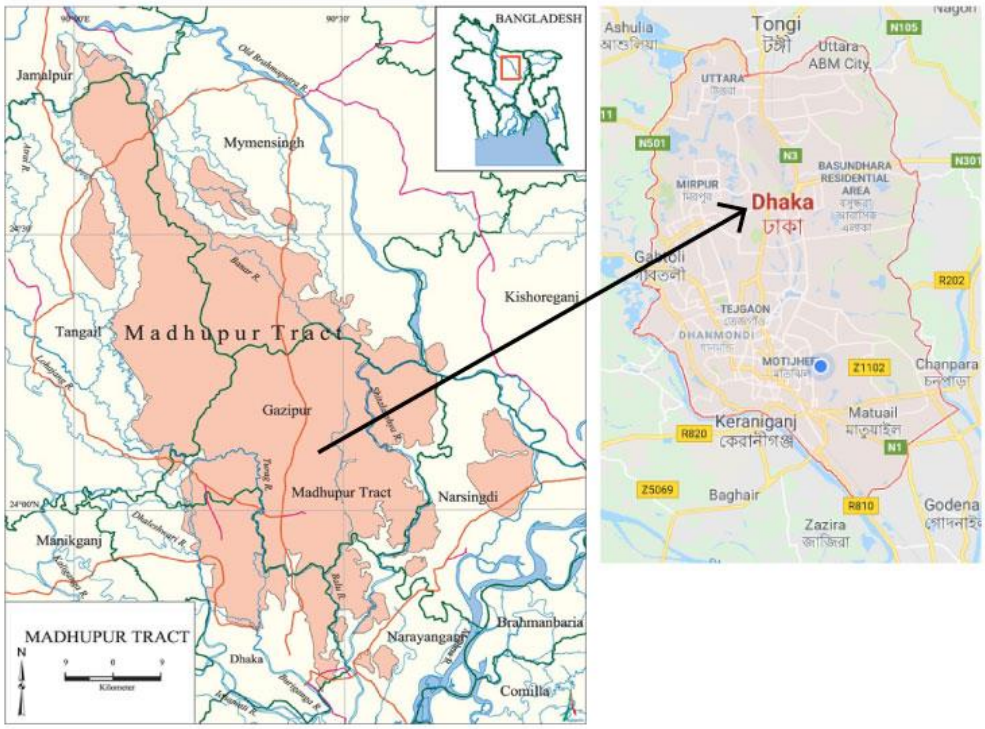


Figure 1: Madhupur tract, AEZ No-28

3.4. Planting materials

Total ten tomato varieties were selected to conduct the research. Seeds were collected mainly from Bangladesh Agricultural Research Institute (BARI), Gazipur. Two varieties called “Ratan” and “Sorno Komol” were collected from a local market named “Siddik Bazar”, Gulistan.

Table 1: Name and origin of 10 tomato varieties used in the present study

SI No	Name	Origin
1	BARI Tomato-2	BARI
2	BARI Tomato-8	BARI
3	BARI Tomato-11	BARI
4	BARI Tomato-14	BARI
5	BARI Tomato-15	BARI
6	BARI Hybrid Tomato-5	BARI
7	BARI Hybrid Tomato-7	BARI
8	BARI Hybrid Tomato-9	BARI
9	Ratan	Lal tir
10	Sorno Komol	Local

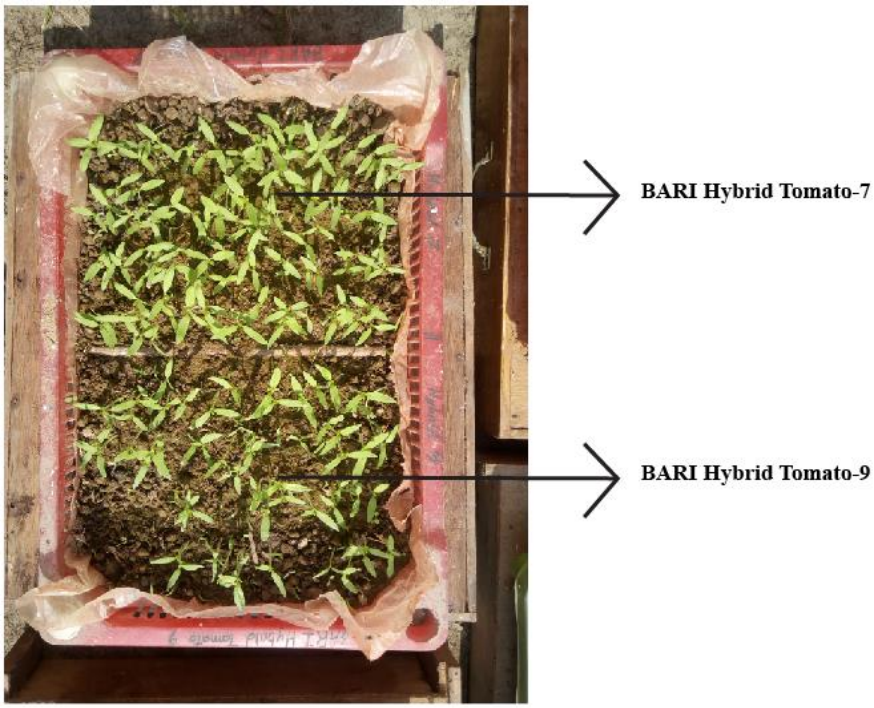
3.5. Experimental Design

The experiment was carried out in a complete randomized design (CRD) with three replications and each variety contains 3 pots and each pot contain one plant. The total number of unit pots were 30.

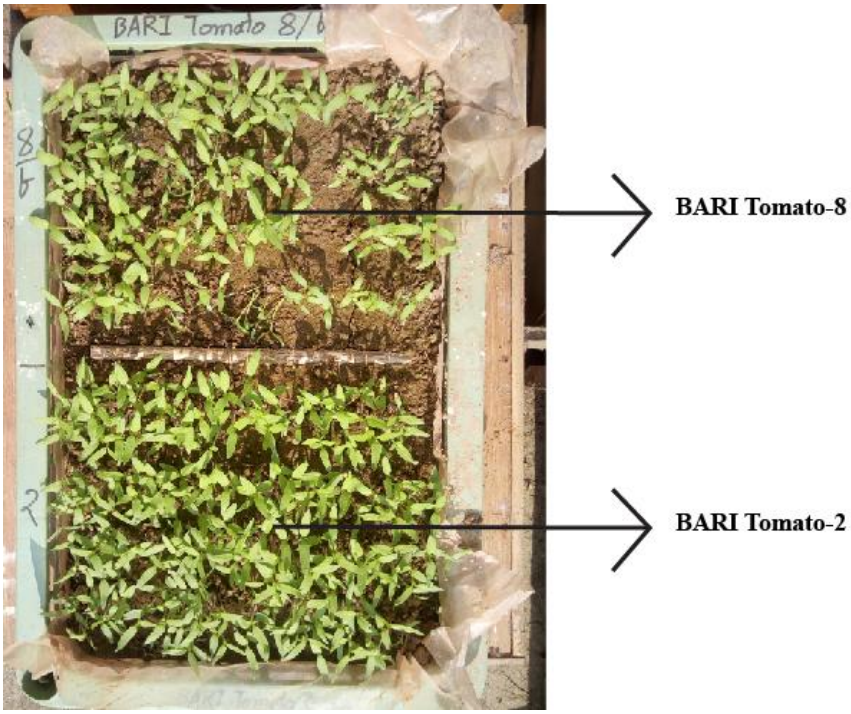
3.6. Seedlings preparation

For the seedlings preparation seeds were soaked overnight in distil water. Seedlings were grown in a tray. The soil was collected from the agronomy field of SAU. Soil was mixed with Furadan 5G and kept for one day by covering the whole soil with polythene sheet to sterilize the soil. Then it was mixed with

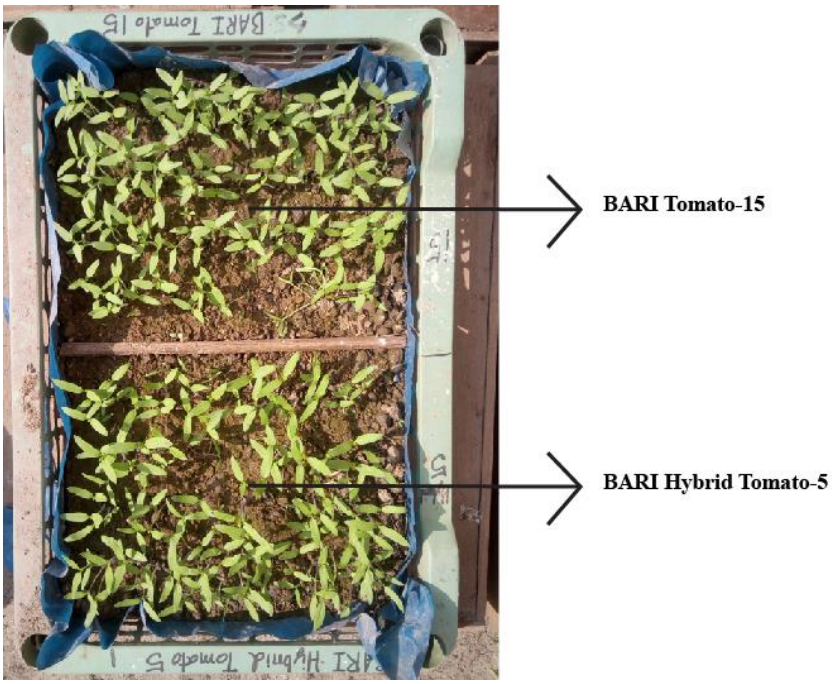
desired amount of fertilizers and Cowdung. Finally soil was poured in tray and the seeds were sown in individual row and proper care was taken for better germination and seedling development. Some seedlings were found damping off diseased then Cupper oxychloride (Semco) was treated in the tray @ 1g/L water (Figure 2: Tray 1-5).



Tray 1: Seedlings of BARI Hybrid Tomato-7 and BARI Hybrid Tomato-9

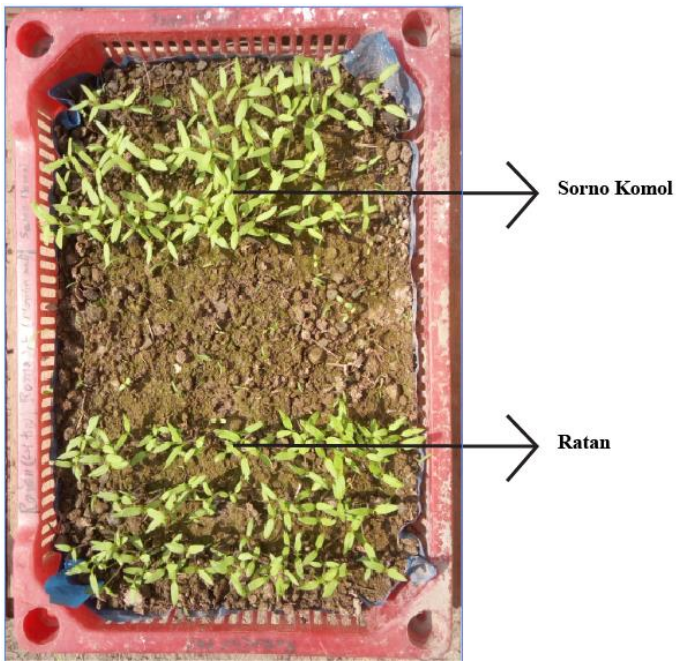


Tray 2: Seedlings of BARI Tomato-8 and BARI Tomato-2



Tray 3: Seedlings of BARI Tomato-15 and BARI Hybrid Tomato-5

Tray 4: Seedlings of BARI Tomato-14 and BARI Tomato-11



Tray 5: Seedlings of Sorno Komol and Ratan

Figure 2: Raising seedlings of tomato

3.7. Pot preparation and transplanting of Seedlings

At first soil was prepared in a ratio of 1:1:1 (Sand:Clay:Compost). The soil was treated with proper amount of Furadan 5G and kept under cover for 1 day. Then it is mixed with proper amount of fertilizer and cowdung. The pot was filled with the soil and seedlings from the tray were transferred in the pot (Figure 3).

Figure 3: Seedlings transplantation in pot

3.8. Intercultural operations

3.8.1. Gap filling

Gap filling was done after one week of transplanting. The seedlings were taken from the same source and a minor gap filling was done where it was necessary.

3.8.2. Weeding

Three hand weeding was done. First one was done at 20 DAT (Days after Transplantation) and second and third weeding were done at 40 and 60 DAT, respectively.

3.8.3. Manure and Fertilizer application:

During the final pot preparation approx 15 kg cowdung, 2 kg Urea, 1kg TSP (Triple Super phosphate) and 0.80 kg MoP (Murate of Potash) were mixed with soil. All the fertilizers were applied in basal dose except urea.

3.8.4. Irrigation water and drainage

Irrigation was done according to the need. The pots were irrigated by a watering cane. Excess water also drained out from pot after heavy rain.

3.9. Identification of *Tomato yellow leaf curl virus (TYLCV)*

Identification of the virus was done on the basis of the biological properties such as symptomology and transmission of insect vector by ELISA test. Visual observation was done by observing the typical symptoms of *TYLCV* infection like cupping, upward curling, marginal chlorosis of the leaf, smaller sized leaflets and stunting of plant (Sinistera *et al.*, 2000). The disease incidence of *TYLCV* was calculated on the basis of the appearance of typical symptoms of the virus. This was done by counting the plants observed everyday starting from the transplanting to harvesting date. The plants were inspected every alternate day morning to note the visual appearance and also to count the insect vectors.

3.10. Serology of virus

The ELISA test was done at BCSIR (Bangladesh Council of Scientific and Industrial Research) lab to identify the virus present in the plants.

3.11. Whitefly Association (Whitefly/Leaf)

The whitefly association study was done by direct visual method (Hirano *et al.*, 1993). For the study of whitefly association, in total three leaves were investigated from each of the plant at early morning. The whitefly was counted and number was recorded as per leaf so that whitefly association with each variety could be measured. The sampling on the infestation of whitefly was

taken only at fruiting stage at an interval 10 days. The sampling on the infestation of whitefly was taken at adult stage of the plant (Alam *et al*., 2016) (Figure 4).

Figure 4: Whitefly population at lower portion of tomato leaves

3.12. Disease incidence

Disease incidence, which measures the extent of proportion of a disease within a given field was estimated by using the following formula given by (Agrios 2005).

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

Disease was identified by visual basis, observing the typical symptoms of *TYLCV*. The disease incidence reaction was assessed by using the following disease rating scale given by Ali *et al*. (2005). The disease incidence rating scale is shown in table 2.

Table 2: Disease incidence rating scale of *TYLCV*

Rating Scale	Incidence Range (%)
0 Immune	0%
1 Highly Resistant	1-10%
2 Moderately Resistant	11-25%
3 Tolerant	26-50%
4 Moderately Susceptible	51-60%
5 Susceptible	61-70%
6 Highly Susceptible	71-100%

Source: Ali *et al.,* (2005)

3.13. Disease severity

Symptom development was evaluated according to the symptom severity scale described by Lapidot and Friedmann, 2002. Disease severity was calculated by the following formula (calculation given in appendix-IV) and following disease rating scale (Table 3).

$$\% \text{ Disease severity} = \frac{\text{Sum of total disease rating}}{\text{Total No of observation} \times \text{ Maximum grade in the scale}} \times 100$$

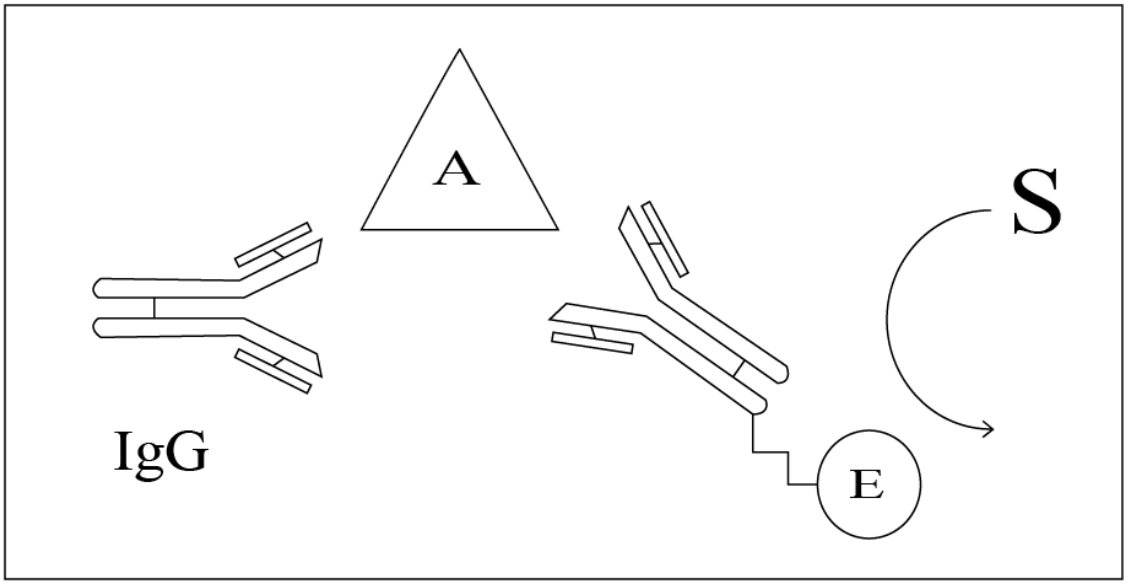
Table 3: Disease severity rating scale of *TYLCV*

Grading Scale	Symptoms
0	No visible symptoms, healthy plant.
1	Very slight yellowing of leaflet margins on apical leaf.
2	Some yellowing and minor curling of leaflet ends
3	A wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop
4	very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant growth stops

Source: Lapidot and Friedmann, (2002).

3.14. ELISA test

Tomato leaf samples of the selected varieties was taken to the tissue culture laboratory of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka for ELISA (Enzyme linked Immunosorbent Assay) test using DAS-ELISA protocol to authenticate the presence or absence of *Tomato Yellow Leaf Curl Virus* (*TYLCV*) in infected leaves of different tomato varieties.



The Double Antibody Sandwich ELISA (DAS) used antibodies (IgG) which are bound to the surface of a microtitre plate to capture the antigen (A) of interest. A specific antibody enzyme conjugate (E) was then used to detect the trapped antigen. The presence of enzyme (in this case alkaline phosphate) was detected by a colorimetric substrate (S) reaction.

3.14.1. ELISA kit collection

ELISA kits were collected from the local supplier. The brand name of the ELISA chemicals was “Life Science” and “Agdia”.

3.14.2. Reagents required for DAS-ELISA test:

Coating antibody (codes in the ADGEN catalogue ending in -01/-02

Conjugate (ADGEN codes ending in -03/-04)

Coating buffer (ADGEN codes 02-001/02-002)

Phosphate buffered saline + Tween 20 (ADGEN code 02-003)

Extraction Buffer (ADGEN codes 02-004 - 02-016 depending on antigen of interest)

Conjugate buffer (ADGEN codes 02-008/02-009)

pNNP tablets (ADGEN code 0-001/0-002)

Substrate buffer (ADGEN code 02-010/02-011)

Alternatively for your convenience ADGEN supply a DAS ELISA buffer pack (02-017/02-018) and prepared liquid substrates which are stable, convenient and easy to use (03-003/03-004) and for enhanced detection in your assay choose ADGEN blue liquid substrate system (03-005/03-006)

3.14.3. Recommended buffer for DAS-ELISA

3.14.3.1. Coating Buffer (Carbonate buffer)

Sodium carbonate	1.59g
Sodium hydrogen carbonate	2.93g

Up to 1 litre with dH_2O was made. The pH of this buffer is 9.6 and does not require to be adjusted.

3.14.3.2. Phosphate buffer saline (PBS) ×10

Sodium chloride	80g
Potassium diHydrogen orthophosphate	2g
Disodium Hydrogen orthophosphate	11.5g
Potassium chloride	2g

Made up to 1litre with dH_2O. The pH of this solution when diluted to 1×s is 7.2

3.14.3.3. Wash buffer (PBS+Tween 20)

Phosphate Buffer Saline	1 litre
Tween 20	0.5ml

3.14.3.4. General Extraction Buffer

Polyvinylpyrrolidone (PVV)	20g
Ovalbumin	2g
Sodium sulphite (anhydrous)	1.3g
Sodium azide	0.2g
Tween 20	0.5ml
Sodium Chloride	8g
Potassium diHydrogen orthophosphate	0.2g
Disodium Hydrogen orthophosphate	1.15g
Potassium chloride	0.2g

Made up to 1 litre with distilled/deionised water. This buffer can be difficult to get into solution and it is easier if the PVP is mixed into a "paste" with a small volume of water before adding the other components and the remainder of water.

3.14.3.5. Conjugate buffer

Bovine serum albumin	0.2g
PBST	0.2g

3.14.3.6. Substrate buffer (Diethanolamine buffer 1M)

Dietanolamine	90.39g
Dietanolamine-HCl	19.82g
Magnesium chloride	0.1g

Made up to 1litre with dH_2O. The pH of the buffer is 9.8 and it does not require to be adjusted. (The diethanolamine and dietanolamine-HCl are liquids

however; it is easier to weight them out than to measure their volumes as they are extremely viscous.)

pNPP was added to the above buffer at 1mg/ml to make up the substrate for alkaline phosphatase.

3.14.4. Protocol of ELISA test

1. Dilute coating antibody in coating buffer as recommended on the bottle label and add 100µl to the required number of wells for the test.

2. Wrap the plate tightly in cling film or place in a plastic box with some damp paper towels and close the box. Incubate the plate at 37^0C for 4 hours.

3. Wash the plate three times with phosphate buffered saline + Tween 20 (0.05) –PBST. To do this fill the wells of the plate with PBST and invert to remove the buffer. Repeat twice, pat the plate dry on paper towels.

4. Extract the samples by grinding 1g of tissue with10ml of general extraction buffer in a mortar and pestle for an alternative method of grinding. Then filter the sample through a layer of muslin (or similar fine cotton gauze). If this is not available then allow the plant material settle and use the supernatant in the test. In some cases the recommended ratio of sample to buffer may have to be reduced to allow a clear signal to be obtained if the plant material is not highly infected.

5. Add 100µl of each sample, positive and negative control to the coated wells. ADGEN recommended that all samples and controls are tested in duplicate. Remember, 1 ADGEN UNIT= 2 TEST WELLS.

ADGEN positive and negative controls are reconstituted by adding 2ml of distilled/deionised water and gently shaking. Any unused reconstituted control may be stored at -20^0C. However, the performance of the positive controls may decrease when stored in this manner.

6. Wrap the plate as described in (2) above and incubate at 4^0C overnight (at least 16 hours).

7. Wash the plate as described in (3) above.

8. Dilute the antibody-enzyme conjugate as recommended on the bottle label in conjugate buffer and add 100µl to each test well.

9. Wrap as in (2) above and incubate at 37^0C for 1hour.

10. Wash four times as described in (3) above. An extra wash is included at this stage to ensure that all unbound antibody-enzyme conjugate is removed from the wells.

11. Prepare the substrate just before use – add pNPP at 1mg/ml to substrate buffer (one 5g tablet in 5ml of buffer). Alternatively use one of the ADGEN liquid substrates. All of these substrates may change color when exposed to light and should be protected from light to prevent this occurring.

12. Add 100µl of prepared substrate to each test well.

13. Wrap the plate as in (2) above and incubate in the dark ar room temperature for 1 hour.

14. Read the absorbance using a spectrophotometer at 405nm (for pNPP and ADGEN Yellow) or 595-650nm (for ADGEN Blue). Alternatively positive and negative samples may be scored visually although this may not be as accurate as using a spectrophotometer. A positive sample may be determined as one which gives an absorbance value which is greater than the absorbance values of the negative control. A negative sample is one which gives an absorbance value which is the same as, or less than the negative control. Visually a positive sample will give a darker color than the negative control and a negative sample will give a similar or lighter color to the negative control.

3.15. Parameters assessed

All experimental plants were selected for data collection and mean data of the following parameters were recorded. The following parameters were assessed:

a. No of leaves/plant
b. No of infected leaves/plant
c. No of branches/plant
d. No of flowers/plant
e. No of fruits/plant
f. Fruits diameter
g. Individual fruit weight
h. Shoot length
i. Root length
j. Yield/plant

3.15.1. No of leaves/plant

The leaves of each plant were counted at an age of 45, 60 and 75 DAT. Only adult leaves were counted excluding the very young leaves and buds.

3.15.2. No of infected leaves/plant

The infected leaves of each plant were counted at an age of 45, 60 and 75 DAT. The infected leaves were identified by the typical symptoms.

3.15.3. No of branches/plant

The number of branch of each plant was counted at an age of 45, 60 and 75 DAT. As the branch was counted at adult age so there was no young branch calculated in the counting.

3.15.4. No of flowers/plant

The number of flowers of each plant was counted at an age of 45, 60 and 75 DAT. Only the healthy flowers were considered and the data was recorded.

3.15.5. No of fruits/plant

The number of fruits of each plant was counted and mean number of tomato fruits of each variety were recorded.

3.15.6. Fruits diameter

Mean diameter of collected tomatoes from each plant as per variety were measured by a slide calipers in centimeter (cm).

3.15.7. Individual fruit weight

Individual fruit weight was measured by a digital balance meter in gram (g). A mean weight was taken of collected fruits from each plant as per variety.

3.15.8. Shoot length

Shoot length of the plant of was measured by a meter scale from the ground to longest tip of the plant in centimeter (cm) at an last harvesting time stage.

3.15.9. Root length

Root length of the plant of was measured by a meter scale in centimeter (cm) while the plant was uprooted.

3.15.10. Yield/plant

Every time tomato was harvested followed by mearsing the weight and diameter and data was recorded. Total yield per plant was measured in kg and the diameter was measured in cm.

3.16. Statistical analysis of data

The data was analyzed by using the “Statistix 10” Software. The mean value was compared according to LSD range test at 5% level of significance. Tables, bar diagram, linear graphs and photographs were used to present the data as and when necessary for comparing different parameters.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter represents the experimental results. The evaluation of tomato varieties against *Tomato yellow leaf curl virus* (*TYLCV*) of some tomato varieties viz. BARI Tomato-2, BARI Tomato-8, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-5, BARI Hybrid Tomato-7, BARI Hybrid Tomato-9, Ratan, Sorno Komol under pot condition. Results were compiled based on ELISA test, % of disease incidence, % disease severity and morphological parameters.

4.1. *TYLCV* Disease Incidence of selected tomato varieties

The highest incidence i.e. 100% infected plants were found in 5 varieties of tomato viz, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-7 and Sorno Komol. On the other hand there was no disease incidence found in BARI Hybrid Tomato-5. The effect of different varieties on disease incidence (%) of *Tomato yellow leaf curl virus* was observed based on disease rating scale of *TYLCV* as shown in Table 2 in methodology section. The highly susceptible varieties were BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-7 and Sorno Komol. Among the varieties BARI Tomato-2, BARI Tomato-8, BARI Hybrid Tomato-9, Ratan were susceptible. BARI Hybrid Tomato-5 was found Immune among the selected tomato varieties on the basis of biological properties of disease incidence of *TYLCV* are presented in table 4.

Table 4: Disease Incidence of different tomato varieties against *Tomato Yellow Leaf Curl Virus* (*TYLCV*)

Variety	Disease Incidence (%)	Level of resistance/ susceptibility
BARI Tomato-2	66.67	Susceptible
BARI Tomato-8	66.67	Susceptible
BARI Tomato-11	100	Highly Susceptible
BARI Tomato-14	100	Highly Susceptible
BARI Tomato-15	100	Highly Susceptible
BARI Hybrid Tomato-5	0	Immune
BARI Hybrid Tomato-7	100	Highly Susceptible
BARI Hybrid Tomato-9	66.67	Susceptible
Ratan	66.67	Susceptible
Sorno Komol	100	Highly Susceptible

4.2. *TYLCV* Disease Severity of selected tomato varieties

The effect of different varieties on Disease severity (%) of *Tomato yellow leaf curl virus* was observed based on disease severity rating scale of *TYLCV* as shown in Table 3 in methodology section. The highest disease severity (70% and 62.5%) was found in BARI Hybrid Tomato-7 and Sorno Komol, respectively, on the basis of grading scale it was showed the very severe plant stunting and yellowing with severe leaf curling and cupping. There was no visible symptoms was found in BARI Hybrid Tomato-5. The disease severity of other varieties were showed lower to medium, BARI Tomato-2, BARI

Tomato-11, Ratan and BARI Tomato-8 was showed lower disease severity (18%, 23.81%, 26.25%, 28.91%, respectively), while BARI Tomato-15, BARI Hybrid Tomato-9, BARI Tomato-14 showed the moderate disease severity (35%, 35%, 37.5% respectively). According to the disease severity grading scale these varieties are showed very slightly yellowing, some yellowing with minor curling and a wide range of leaf yellowing, curling and cupping. Disease severity is shown graphically in figure 5.

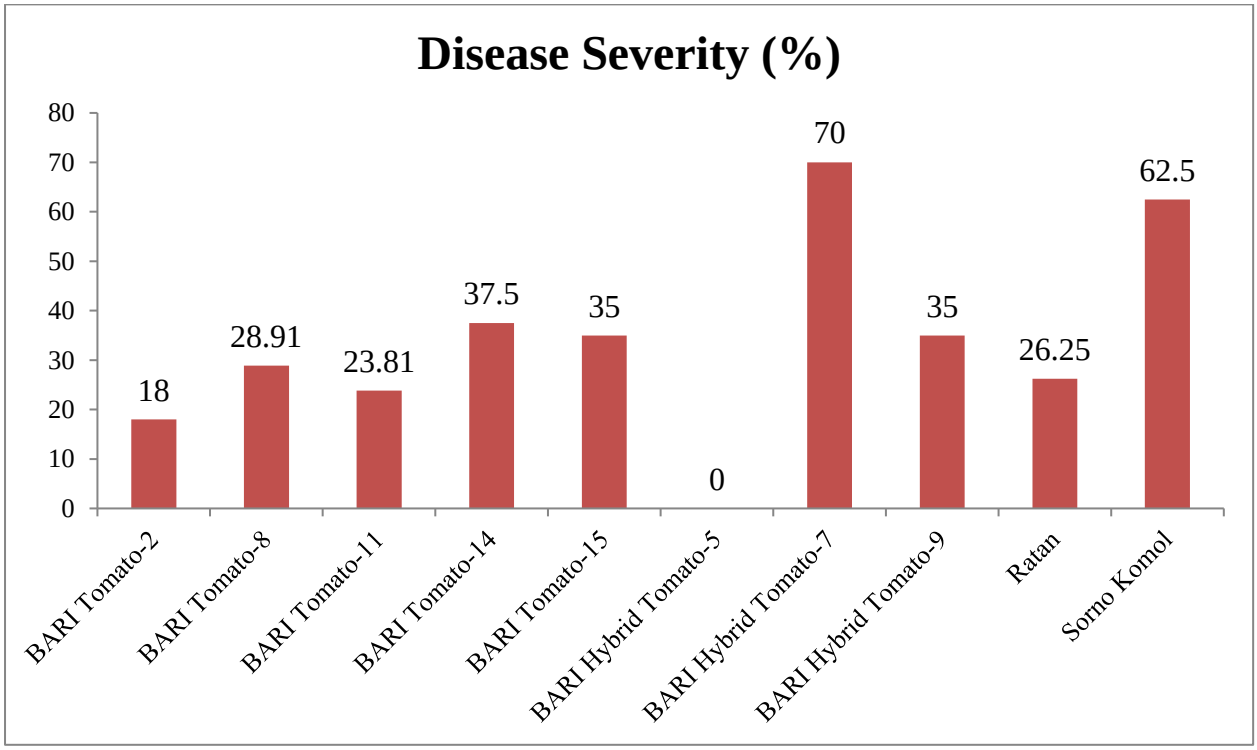


Figure 5: Disease Severity (%) of different tomato varieties against *Tomato Yellow Leaf Curl Virus* (*TYLCV*)

4.3. Infestation of whitefly per Leaf:

The maximum number of whitefly per leaf was obtained in BARI Hybrid Tomato-5 (27.00) followed by BARI Tomato-14 (23.67), Ratan (19.33), BARI Tomato-15 (18.67) and BARI Hybrid Tomato-9 (17.67). The minimum number of whitefly per leaf was found in BARI Tomato-2 (10.67) preceded by Sorno Komol (13.33), BARI Hybrid Tomato-7 (13.67), BARI Tomato-11 (16.33) and BARI Tomato-8 (16.67). Among the varieties BARI Hybrid Tomato-5 (27.00)

and BARI Tomato-2 (10.67) was statistically different. The variety BARI Tomato-14 (23.67), Ratan (19.33), BARI Tomato-15 (18.67), BARI Hybrid Tomato-9 (17.67), BARI Tomato-8 (16.67), BARI Tomato-11 (16.33), BARI Hybrid Tomato-7 (13.67) and Sorno Komol (13.33) were statistically identical. Results of white fly association are presented in figure 6.

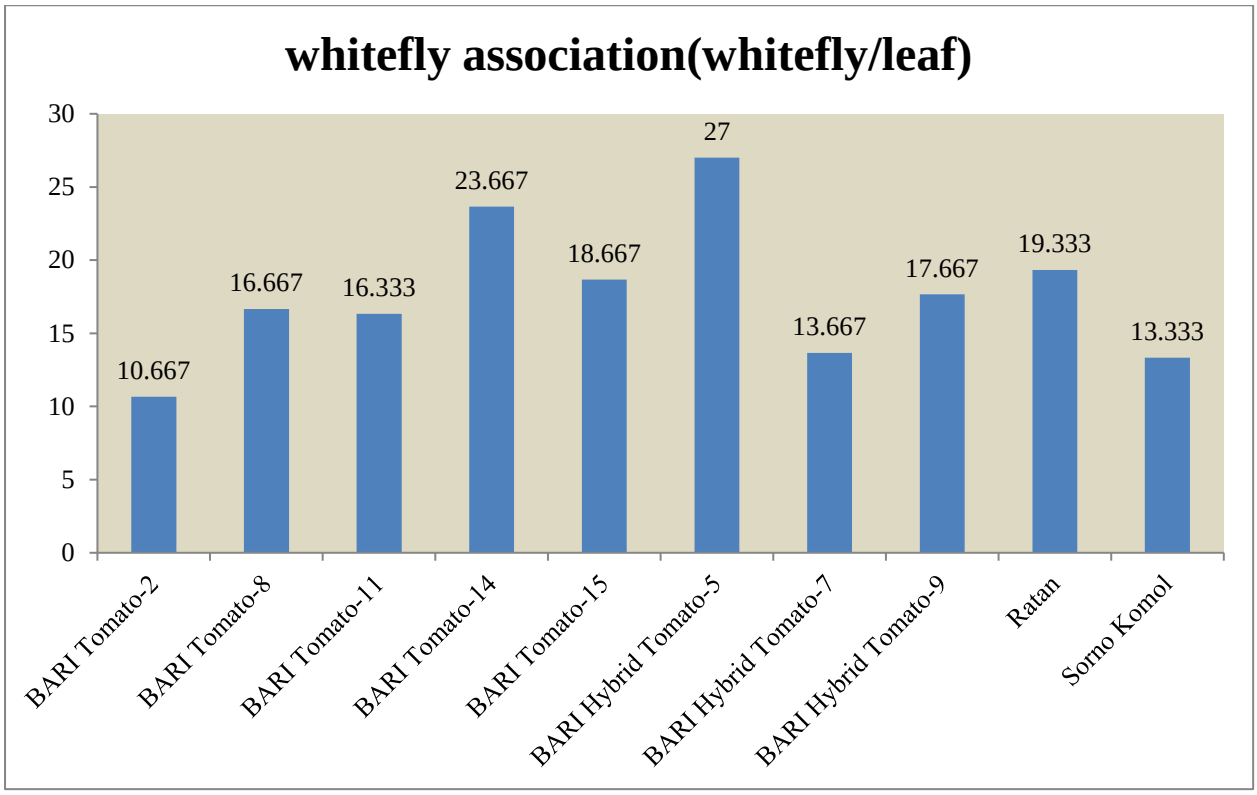


Figure 6: Graphical representation of whitefly per leaf of selected tomato varieties against *Tomato yellow leaf curl virus (TYLCV).*

4.4. Virus Detection through DAS-ELISA test

The serological test, Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) was done to detect the *TYLCV*. For DAS-ELISA test leaf samples were collected from the all selected tomato varieties including BARI Hybrid Tomato-5. In the micro titer plate 12th column was used as a negative control and 11th column was used as a positive control and

the first rows of 1-10th columns were used for test samples. Yellow color indicate that all the selected varieties used in this experiment showed positive reaction with virus antigen of *TYLCV,* (Figure-7 and Table-5). Although the variety BARI Hybrid Tomato-5 was not shown any kind of typical symptoms when investigated on the basis of biological properties symptomology and transmission test.

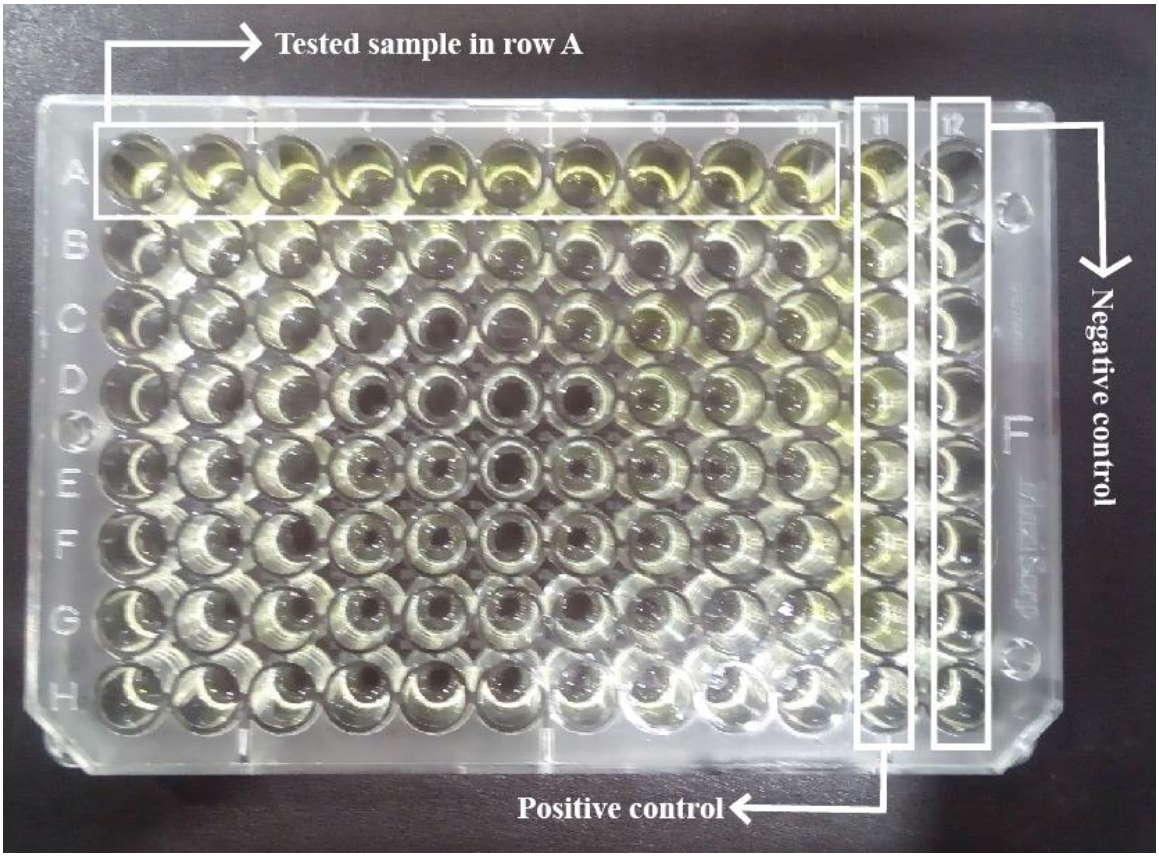


Figure 7: DAS-ELISA test to identify viruses of the selected tomato varieties (development of yellow colour in micro titer wells indicating presence of virus)

Table 5. Virus identification of the selected tomato varieties through DAS-ELISA test

Sl. No.	Variety	Tested Viruses	Results
1.	BARI Tomato-2	*TYLCV*	+
2.	BARI Tomato-8	*TYLCV*	+
3.	BARI Tomato-11	*TYLCV*	+
4.	BARI Tomato-14	*TYLCV*	+
5.	BARI Tomato-15	*TYLCV*	+
6.	BARI Hybrid Tomato-5	*TYLCV*	+
7.	BARI Hybrid Tomato-7	*TYLCV*	+
8.	BARI Hybrid Tomato-9	*TYLCV*	+
9.	Ratan	*TYLCV*	+
10.	Sorno Komol	*TYLCV*	+

**TYLCV= Tomato Yellow Leaf Curl Virus*

4.5. The morphological features of yield and yield contributing character in tomato against *Tomato yellow leaf curl virus (TYLCV)*

4.5.1. Number of leaves and branches per plant of selected tomato varieties

There were significant differences were found in case of number of leaf per plant. The results are shown in table 5.The maximum number of leaves per plant was obtained in the variety BARI Tomato-8 (31.67) followed by variety BARI Tomato-2 (24.67), BARI Tomato-11(20.67), Ratan (20), and BARI

Tomato-14 (18). The minimum number of leaves was obtained in variety Sorno Komol (11.67), preceded by BARI Hybrid Tomato-9 (14.33), BARI Hybrid Tomato-7 (14.33), BARI Hybrid Tomato-5 (14.67) and BARI Tomato-15 (14.67). Among the varieties BARI Tomato-8 (31.67) was significantly different from Sorno Komol (11.67), BARI Hybrid Tomato-9 (14.33) and BARI Hybrid Tomato-7 (14.33). There was no significant difference among the varieties BARI Tomato-2 (24.67), BARI Tomato-11 (20.67), Ratan (20), BARI Tomato-14 (18), BARI Hybrid Tomato-5 (14.67) and BARI Tomato-15 (14.67).

The maximum number of branches per plant was recorded in the variety BARI Tomato-11 (9.33) followed by BARI Tomato-8 (8.33), BARI Tomato-2 (6.33), BARI Hybrid Tomato-7 (5.33) and BARI Hybrid Tomato-5 (5.00). The minimum number of branches per plant was found in BARI Tomato-15 (3.33) preceded by Sorno Komol (3.67), BARI Hybrid Tomato-9 (4.00), BARI Tomato-14 (4.00) and Ratan (4.67). Among the varieties BARI Hybrid Tomato-7 (5.33), BARI Hybrid Tomato-5 (3.33), Ratan (4.67), BARI Tomato-14 (4.00), BARI Hybrid Tomato-9 (4.00) and Sorno Komol (3.67) were statistically identical. BARI Tomato-8 (8.33) and BARI Tomato-2 (6.33) were also statistically identical but different than others. BARI Tomato-11 (9.33) and BARI Tomato-15 (3.33) were significantly different.

Table 6: Number of leaves and branches per plant of selected tomato varieties against *Tomato yellow leaf curl virus (TYLCV)*

Variety	No of leaves/plant	No of branches/plant
BARI Tomato-2	24.67 ab	6.33 bc
BARI Tomato-8	31.67 a	8.33 ab
BARI Tomato-11	20.67 bc	9.33 a
BARI Tomato-14	18.00 bc	4.00 cd
BARI Tomato-15	14.67 bc	3.33 d
BARI Hybrid Tomato-5	14.67 bc	5.00 cd
BARI Hybrid Tomato-7	14.33 c	5.33 cd
BARI Hybrid Tomato-9	14.33 c	4.00 cd
Ratan	20.00 bc	4.67 cd
Sorno Komol	11.67 c	3.67 cd
CV (%)	10.26	2.71
LSD Value (0.05)	4.92	1.29

4.5.2. Number of flowers and fruits per plant of selected tomato varieties

The maximum number of flowers per plant was recorded in the variety BARI Tomato-11 (90.67) followed by BARI Tomato-2 (44.67), BARI Hybrid Tomato-5 (30.00), Ratan (29.67), BARI Tomato-8 (28.33). The minimum number of flowers per plant was found in the variety Sorno Komol (10.67) preceded by BARI Hybrid Tomato-9 (13.33), BARI Tomato-15 (16.00), BARI Tomato-14 (16.33) and BARI Hybrid Tomato-7 (16.33). Among the varieties BARI Tomato-11 (90.67) and Sorno Komol (10.67) were significantly different. There was no significant difference among the varieties BARI Tomato-2 (44.67), BARI Hybrid Tomato-5 (30.00), Ratan (29.67), BARI Tomato-8 (28.33), BARI Hybrid Tomato-7 (16.33), BARI Tomato-14 (16.33), BARI Tomato-15 (16.00), BARI Hybrid Tomato-9 (13.33).

The highest number of fruits per plant was recorded in the variety BARI Tomato-11 (81.33) followed by BARI Hybrid Tomato-5 (12.33), BARI Tomato-14 (12.00), BARI Tomato-8(11.33) and BARI Tomato-2 (7.00). On the other hand lowest number of fruits per plant was recorded in Ratan (5.33) preceded by Sorno Komol (5.67), BARI Hybrid Tomato-9 (6.00), BARI Hybrid Tomato-7 (6.00) and BARI Tomato-15 (6.33). Among the varieties BARI Tomato-11 (81.33) was significantly different than other varieties. BARI Hybrid Tomato-5 (12.33), BARI Tomato-14 (12.00), BARI Tomato-8 (11.33) and BARI Tomato-2 (7.00), BARI Tomato-15 (6.33), BARI Hybrid Tomato-7 (6.00), BARI Hybrid Tomato-9 (6.00) and Sorno Komol (5.67) were stastically similar. Significant varieties of flowers/plant and fruits/plant were found in selected tomato varieties during experimental period. The results of variation flowers/plant and fruits/plant are shown in table 7.

Table 7: Number of flowers and fruits per plant of selected tomato varieties against *Tomato yellow leaf curl virus (TYLCV)*

Variety	No of flowers/Plant	No of fruits/Plant
BARI Tomato-2	44.667 b	7.0000 b
BARI Tomato-8	28.333 bc	11.333 b
BARI Tomato-11	90.667 a	81.333 a
BARI Tomato-14	16.333 c	12.000 b
BARI Tomato-15	16.000 c	6.3333 b
BARI Hybrid Tomato-5	30.000 bc	12.333 b
BARI Hybrid Tomato-7	16.333 c	6.0000 b
BARI Hybrid Tomato-9	13.333 c	6.0000 b
Ratan	29.667 bc	5.3333 b
Sorno Komol	10.667 c	5.6667 b
CV (%)	20.913	11.116
LSD Value (0.05)	10.026	5.3292

4.5.3. Fruits diameter (cm), Individual fruit weight (g) and yield (kg) of different tomato varieties

The maximum number of fruits diameter was measred in the variety BARI Tomato-8 (6.27 cm) followed by BARI Hybrid Tomato-5 (5.12 cm), BARI Tomato-2 (6.27 cm), BARI Hybrid Tomato-9 (4.92 cm) and BARI Tomato-14 (4.72 cm). The minimum number of fruits diameter was obtained in BARI Tomato-15 (1.17 cm) preceded by Sorno Komol (1.27 cm), Ratan (2.57 cm), BARI Tomato-11 (2.63 cm) and BARI Hybrid Tomato-7 (3.52 cm). Among the

varieties BARI Tomato-8 (6.27 cm) and BARI Tomato-15 (1.17 cm) were significantly different. The varieties BARI Hybrid Tomato-5 (5.12 cm), BARI Tomato-2 (6.27 cm), BARI Hybrid Tomato-9 (4.92 cm) and BARI Tomato-14 (4.72 cm) were statistically identical but different than Ratan (2.57 cm), BARI Tomato-11 (2.63 cm) and BARI Hybrid Tomato-7 (3.52 cm). The varieties BARI Hybrid Tomato-7 (3.52 cm)**,** BARI Tomato-11 (2.63 cm), Ratan (2.57 cm), and Sorno Komol (1.27 cm) were also statistically same.

The maximum number of individual fruits weight was measured in the variety BARI Tomato-8 (69.80 g) followed by BARI Tomato-2 (64.95 g), BARI Hybrid Tomato-5 (53.08 g), BARI Tomato-14 (46.96 g) and BARI Hybrid Tomato-9 (41.65 g). The minimum number of individual fruits weight was recorded in the variety BARI Tomato-15 (6.14 g) preceded by Sorno Komol (8.78 g), BARI Tomato-11 (10.34 g), Ratan (17.52 g) and BARI Hybrid Tomato-7 (19.29 g). Among the varieties BARI Tomato-8 (69.80 g) and BARI Tomato-2 (64.95 g) were statistically similar. These varieties were statistically different than BARI Hybrid Tomato-7 (19.29 g), Ratan (17.52 g), BARI Tomato-11 (10.34 g), Sorno Komol (8.78 g) and BARI Tomato-15 (6.14 g). The varieties BARI Hybrid Tomato-7 (19.29 g), Ratan (17.52 g), BARI Tomato-11 (10.34 g), Sorno Komol (8.78 g) and BARI Tomato-15 (6.14 g) were statistically identical. The varieties BARI Hybrid Tomato-5 (53.08 g), BARI Tomato-14 (46.96 g) and BARI Hybrid Tomato-9 (41.65 g) were also statistically similar.

The highest yield per plant was obtained in the variety BARI Tomato-11 (0.794 kg) followed by BARI Tomato-8 (0.744 kg), BARI Hybrid Tomato-5 (0.644 kg), BARI Tomato-14 (0.535 kg) and BARI Tomato-2 (0.451 kg). The lowest yield was recorded in BARI Tomato-15 (0.112 kg) preceded by BARI Hybrid Tomato-7 (0.119 kg), Sorno Komol (0.145 kg), Ratan (0.150 kg) and BARI Hybrid Tomato-9 (0.352 kg). The variety BARI Tomato-11 (0.794 kg) was significantly different than BARI Tomato-15 (0.112 kg) and BARI Hybrid Tomato-7 (0.119 kg). There was no significant difference in varieties BARI

Tomato-8 (0.744 kg), BARI Hybrid Tomato-5 (0.644 kg), BARI Tomato-14 (0.535 kg) and BARI Tomato-2 (0.451 kg), BARI Hybrid Tomato-9 (0.352 kg), Ratan (0.150 kg) and Sorno Komol (0.145 kg). The results are presented in table no 7.

Table 8: Effect of yield parameters of different selected tomato varieties due to *TYLCV* infection

Variety	Fruits Diameter (cm)	Individual fruit weight (g)	Yield (kg)
BARI Tomato-2	5.10 ab	64.94 a	0.4514 bcd
BARI Tomato-8	6.27 a	69.80 a	0.7439 ab
BARI Tomato-11	2.64 cdef	10.33 c	0.7939 a
BARI Tomato-14	4.72 abcd	46.69 b	0.5351 abc
BARI Tomato-15	1.17 f	6.14 c	0.1124 e
BARI Hybrid Tomato-5	5.12 ab	53.07 ab	0.6443 abc
BARI Hybrid Tomato-7	3.52 bcde	19.29 c	0.1187 e
BARI Hybrid Tomato-9	4.92 abc	41.65 b	0.3515 cde
Ratan	2.57 def	17.52 c	0.1503 de
Sorno Komol	1.27 ef	8.78 c	0.1453 de
CV (%)	2.29	17.82	0.3104
LSD Value (0.05)	1.09	8.54	0.1488

4.5.4. Effect of shoot length (cm) and root length (cm) of different tomato varieties due to *TYLCV* infection

The highest shoot length was measured in the variety BARI Tomato-11 (126.33 cm) followed by BARI Tomato-14 (96.00 cm), BARI Tomato-8 (93.33 cm), BARI Hybrid Tomato-5 (86.67 cm) and BARI Hybrid Tomato-9 (82.33 cm).

The lowest shoot length was observed in the variety Sorno Komol (40.33 cm) preceded by BARI Tomato-15 (52.33 cm), BARI Tomato-2 (53.67 cm), Ratan (61.33 cm) and BARI Hybrid Tomato-7 (72.67 cm). Among the varieties the value of variety BARI Tomato-11 (126.33 cm) was significantly different from all other varieties. There was no significant difference among the varieties BARI Tomato-14 (96.00 cm), BARI Tomato-8 (93.33 cm), BARI Hybrid Tomato-5 (86.67 cm) and BARI Hybrid Tomato-9 (82.33 cm). The variety BARI Hybrid Tomato-7 (72.67 cm) and Ratan (61.33 cm) were statistically same but different than BARI Tomato-2 (53.67 cm), BARI Tomato-15 (52.33 cm) and Sorno Komol (40.33 cm). The varieties BARI Tomato-2 (53.67 cm), BARI Tomato-15 (52.33 cm) and Sorno Komol (40.33 cm) were statistically same.

The highest root length was measured in the variety BARI Tomato-11 (50.67 cm) followed by BARI Tomato-8 (48.67 cm), BARI Hybrid Tomato-5(47.33 cm), BARI Tomato-14 (40.00 cm) and BARI Tomato-2 (28.67 cm). The lowest root length was observed in the variety Sorno Komol (21.33 cm) preceded by BARI Hybrid Tomato-9 (21.33 cm), BARI Hybrid Tomato-7 (21.33 cm), Ratan (21.33 cm) and BARI Tomato-15 (24.67 cm). The variety BARI Tomato-11 (50.67 cm) and BARI Tomato-8 (48.67 cm) were statistically similar but significantly different than BARI Hybrid Tomato-5(47.33 cm), BARI Tomato-14 (40.00 cm) and BARI Tomato-2 (28.67 cm), BARI Tomato-15 (24.67 cm), Ratan (21.33 cm), BARI Hybrid Tomato-7 (21.33 cm), BARI Hybrid Tomato-9 (21.33 cm) and Sorno Komol (21.33 cm). The variety BARI Hybrid Tomato-5 (47.33 cm), BARI Tomato-14 (40.00 cm) and BARI Tomato-2 (28.67 cm), BARI Tomato-15 (24.67 cm), Ratan (21.33 cm), BARI Hybrid Tomato-7 (21.33 cm), BARI Hybrid Tomato-9 (21.33 cm) and Sorno Komol (21.33 cm) were statistically identical. The results are presented in table no 9.

Table 9: Effect of shoot length (cm) and Root length (cm) per plant of different selected varieties against *Tomato yellow leaf curl virus (TYLCV)* infection

Variety	Shoot Length (cm)	Root Length (cm)
BARI Tomato-2	53.667 ef	28.667 c
BARI Tomato-8	93.333 b	48.667 a
BARI Tomato-11	126.33 a	50.667 a
BARI Tomato-14	96.000 b	40.000 b
BARI Tomato-15	52.333 ef	24.667 c
BARI Hybrid Tomato-5	86.667 bc	47.333 ab
BARI Hybrid Tomato-7	72.667 cd	21.333 c
BARI Hybrid Tomato-9	82.333 bc	21.333 c
Ratan	61.333 de	21.667 c
Sorno Komol	40.333 f	21.333 c
CV (%)	18.720	7.7864
LSD Value(0.05)	8.9740	3.7327

4.6. Relationship between Disease Severity (%) and Yield (g)

Regression study was done to establish the relationship between the disease severity (%) and yield (g) of infected tomato plants. From the study it was revealed that significant relation was observed between the two parameters disease severity (%) and yield (g) of different tomato varieties. It was evident from the Figure-8 that the equation $y = -8.108x + 677.9$ gave a good fit to the data and the co-efficient of determination ($R^2 = 0.379$) showed that, fitted regression line had a significant regression co-efficient. From these relations it can be concluded that the yield of tomato was negatively related with the disease severity of the selected tomato varieties.

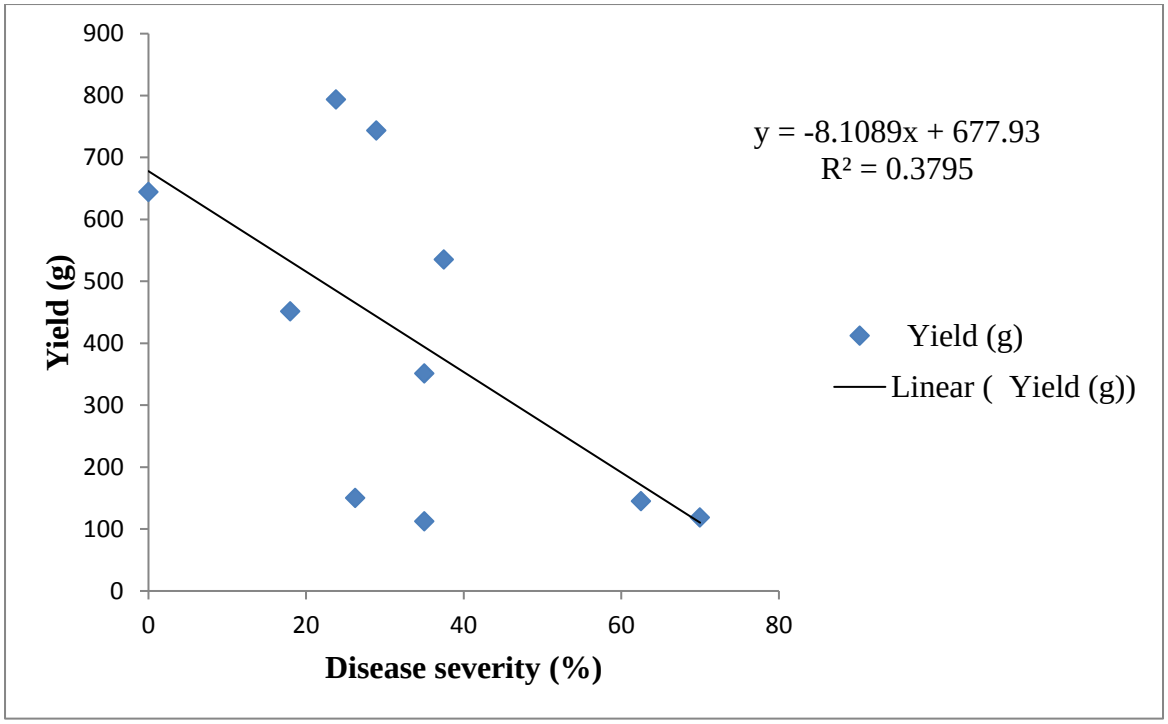


Figure 8: Relationship between Disease severity and Yield

4.7. Relationship between whitefly association and Disease incidence

The relationship between the incidence of whitefly and occurrence of *TYLCV* diseases can easily be understood from the figure 9. Whitefly was the vector of *TYLCV*. As *TYLCV* was transmitted by whitefly it was sure that where whitefly would occur there disease development rate should be high. In all the varieties of the selected tomato viz. BARI Tomato-2, BARI Tomato-8, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-5, BARI Hybrid Tomato-7, BARI Hybrid Tomato-9, Ratan and Sorno Komol whitefly incidence was seen and except BARI Hybrid Tomato-5 every variety showed more or less disease. In BARI Hybrid Tomato-5 though whitefly was present but disease development was zero. In the variety BARI Hybrid Tomato-5 whitefly per leaf was 27. This was the highest number of whitefly per leaf among the selected varieties but disease development was zero. The result is more clearly visible in the figure 9.

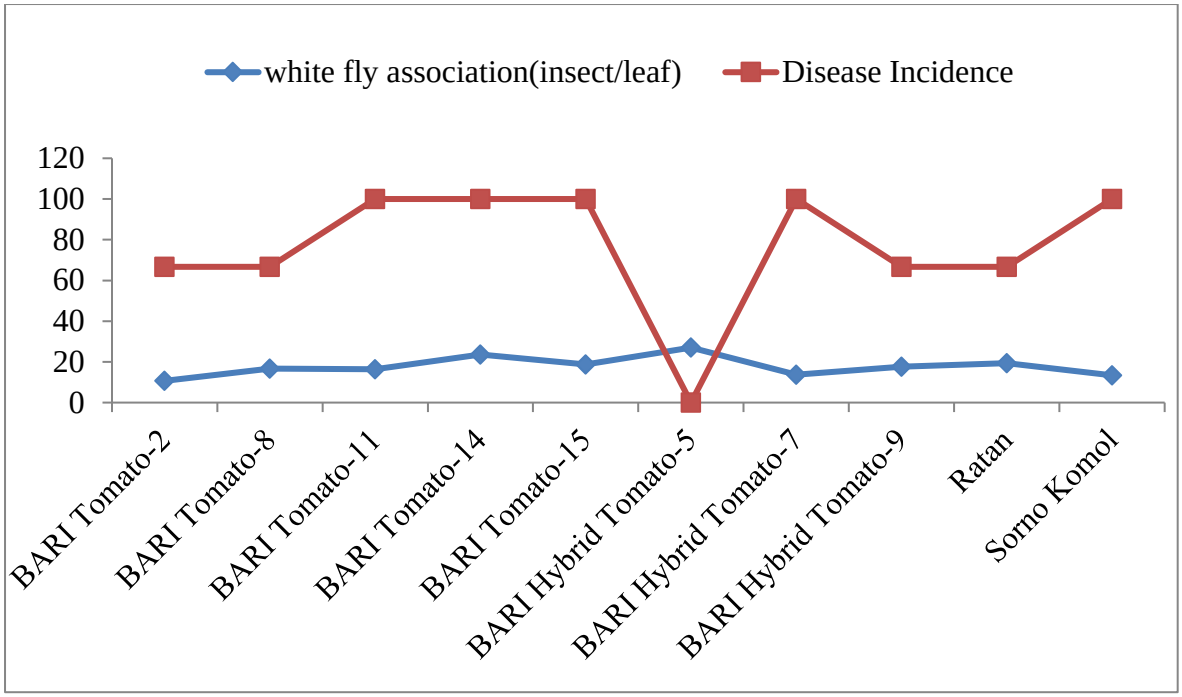


Figure 9: Relationship between incidence of whitefly and occurrence of *TYLCV* diseases.

4.8. Relationship between Disease Incidence (%) and Yield (g) of tomato varieties

Corelation study was done to establish the relationship between the Disease Incidence (%) and yield (g) of infected tomato plants. From the study it was revealed that significant relation was observed between disease incidence and yield. It was evident from the Figure-10 that the equation y = -2.952x + 631 gave a good fit to the data and the co-efficient of determination (R^2 = 0.121) showed that, fitted regression line had a significant regression co-efficient. From this relation it can be concluded that the yield of tomato was negatively related with the Disease incidence of the selected tomato varieties.

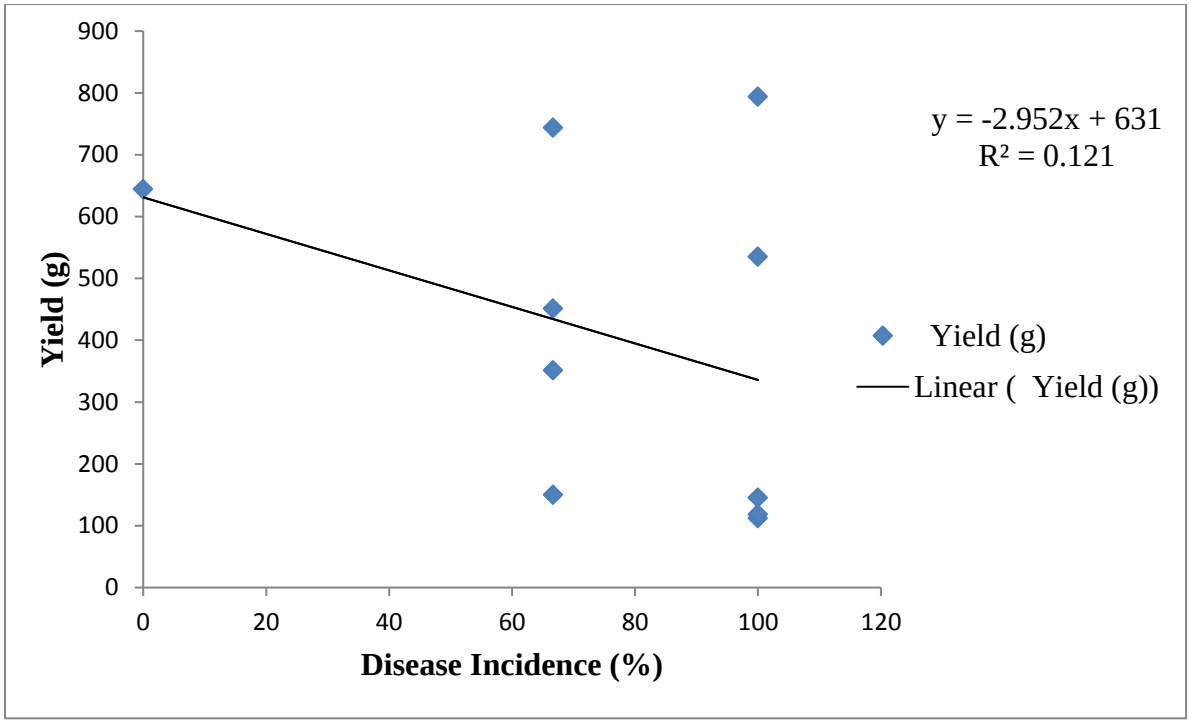


Figure 10: Relationship between Disease Incidence and Yield of selected tomato varieties

4.9. Relation between Disease Severity (%) and Number of fruits per plant:

Corelation study was done to establish the relationship between the Disease Severity (%) and No. of fruits/plant of the selected tomato plants. From the study it was revealed that significant relation was observed between the two parameters. It was evident from the Figure-11 that the equation y = -0.263x + 24.21gave a good fit to the data and the co-efficient of determination (R^2 = 0.052) showed that, fitted regression line had a significant regression co-efficient. From this relation it can be concluded that the No. of fruits/plant of the selected tomato varieties were negatively related with the Disease severity.

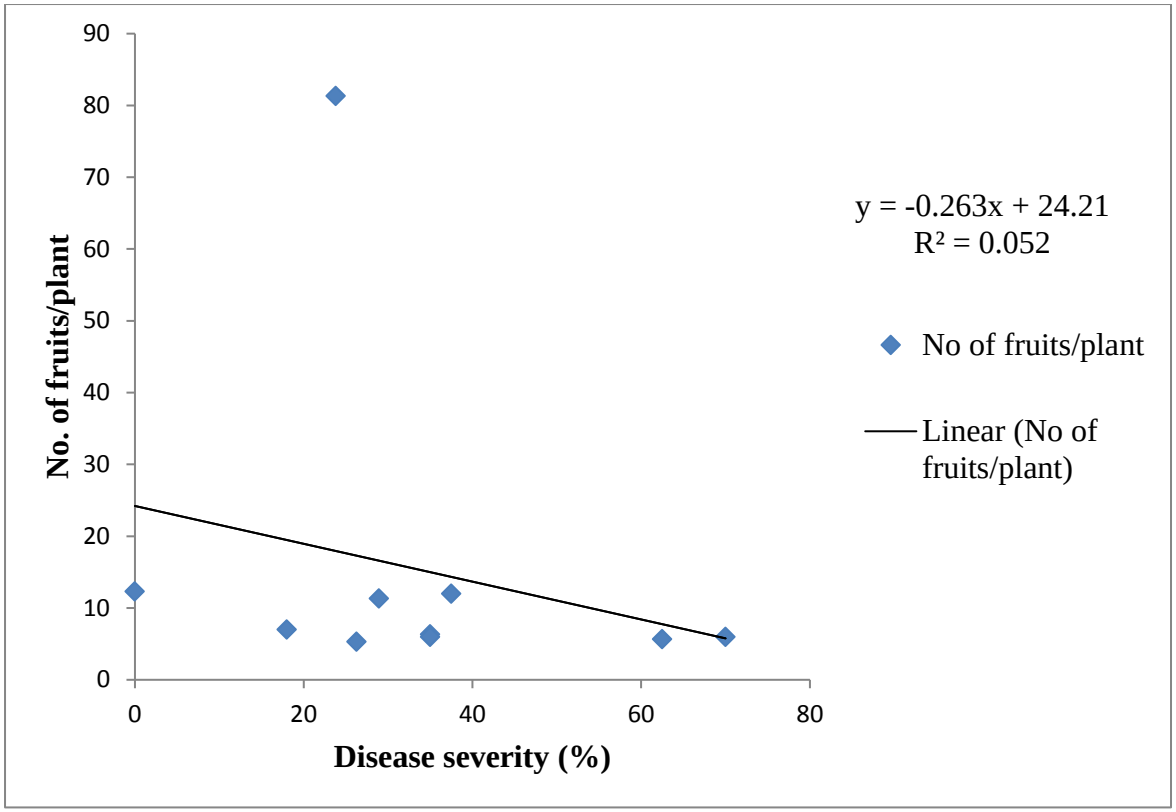


Figure 11: Relationship between Disease Severity and No of fruits/plant of selected tomato varieties

4.10. Relationship between Disease Incidence (%) and Shoot Length (cm)

Corelation study was done to establish the relationship between the Disease Incidence (%) and Shoot Length (cm) of selected tomato varieties. From the study it was revealed that significant relation was observed between the two parameters. It was evident from the Figure-12 that the equation y = -0.056x + 80.80 gave a good fit to the data and the co-efficient of determination (R^2 = 0.004) showed that, fitted regression line had a significant regression co-efficient. From this relation it can be concluded that the Shoot length of selected tomato varieties were negatively related with the Disease incidence.

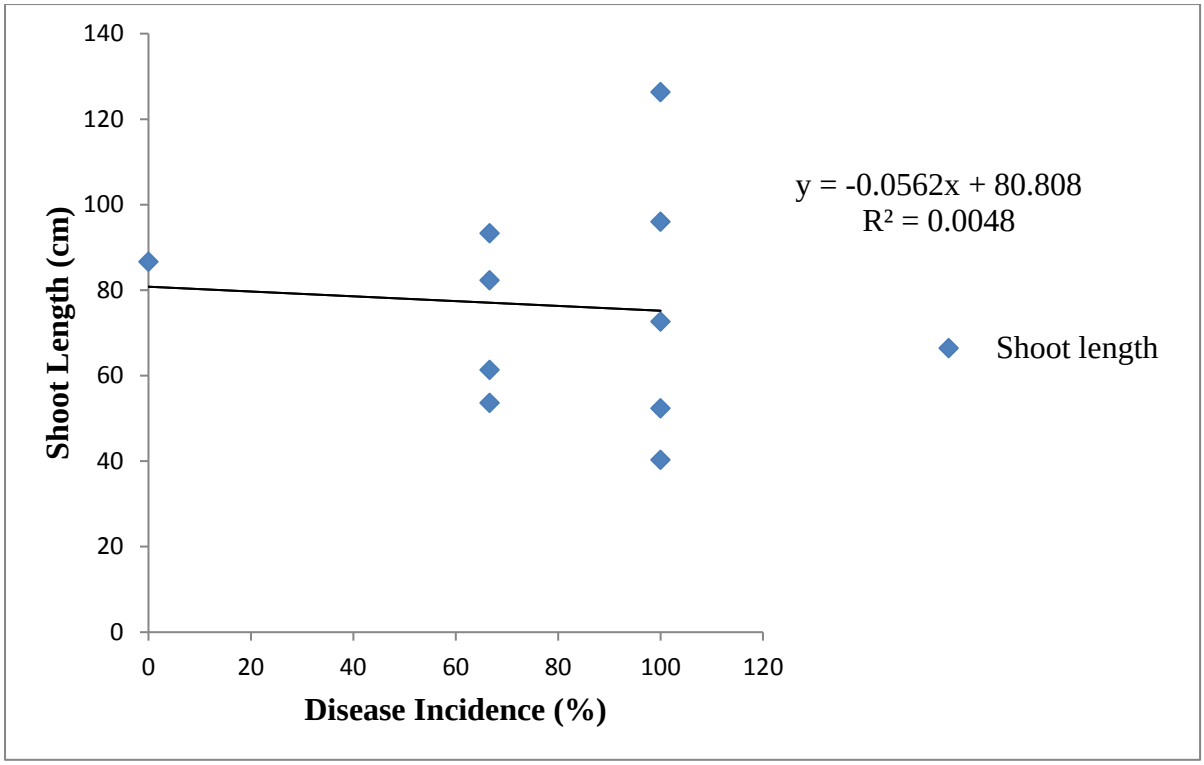


Figure 12: Relationship between Disease Incidence and Shoot Length of selected tomato varieties

4.11. Relationship between Disease Severity (%) and Shoot Length (cm)

Corelation study was done to establish the relationship between the Disease Severity (%) and Shoot Length (cm) of selected tomato varieties. From the study it was revealed that significant relation was observed between the two parameters. It was evident from the Figure-13 that the equation y = -0.423x + 90.75 gave a good fit to the data and the co-efficient of determination (R^2 = 0.112) showed that, fitted regression line had a significant regression co-efficient. From this relation it can be concluded that the Shoot length of selected tomato varieties were negatively related with the Disease severity.

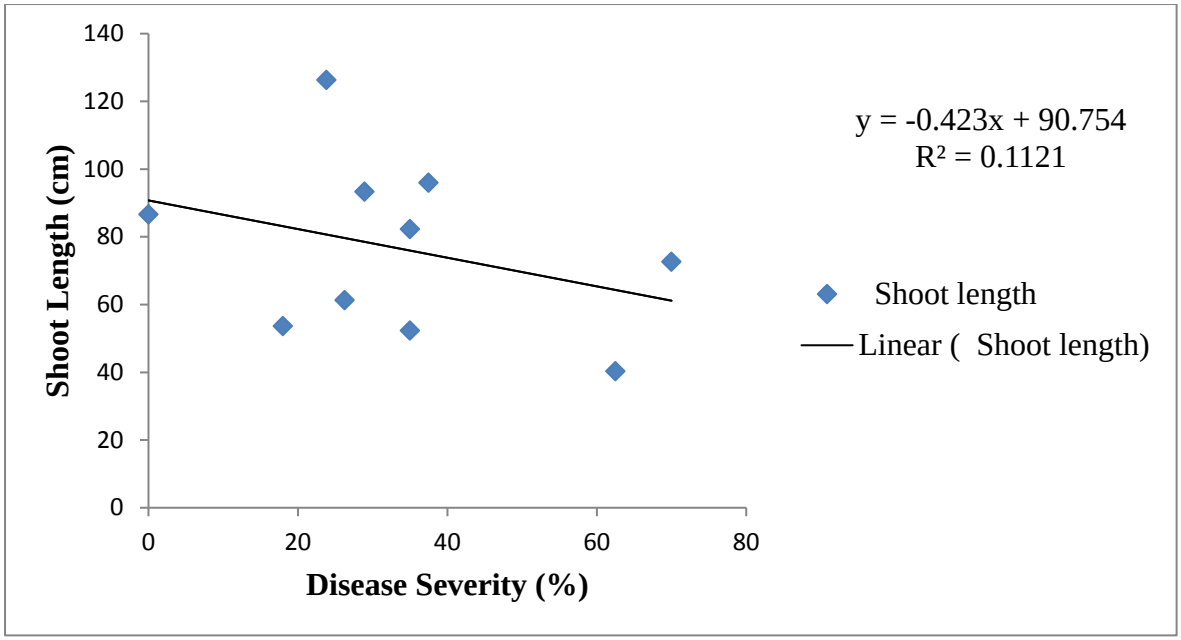


Figure 13: Relationship between Disease Severity and Shoot Length of selected tomato varieties

4.12. Discussion

Tomato (*Lycopersicon esculentum* Mill.) is an important and widely grown vegetable crop. It is a good source of antioxidant, vitamin A, C, E and minerals. It also reduces the risk of cancer. Its juice is a good blood purifier. The crop suffers from many fungal, viral, bacterial and nematode diseases which causes reduction in the yield and quality of tomato fruit. Among the viral diseases, *Tomato Yellow Leaf Curl Virus* (*TYLCV*) is the most important one which limits the tomato production to a great extent. The disease caused a serious loss in tomato production. Therefore, the present experiment was carried out to evaluate the incidence and severity level of *Tomato Yellow Leaf Curl Virus (TYLCV)* against the selected tomato varieties and to screen the resistance/tolerance of selected tomato varietiess against *TYLCV* through serogical test. The result generated during the course of investigation is discussed here:

4.12.1. Disease Incidence

All selected varieties were showed susceptibility to almost 100% *TYLCV* except BARI Hybrid Tomato-5. Among the ten varieties the highest disease incidence was found in BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-7 and Sorno Komol. These varieties were highly susceptible. On the other hand there was no disease incidence occurred in the variety BARI Hybrid Tomato-5. According to disease rating scale BARI Hybrid Tomato-5 is an immune variety against *Tomato yellow leaf curl virus (TYLCV).* Our observation is partially similar to the previous study that was conducted by (Reddy *et al*., 2011; Zeshan *et al*., 2016).

4.12.2. Disease Severity

Among the ten selected varieties the highest disease severity was observed in BARI Hybrid Tomato-7 and the lowest disease severity was recorded in BARI Hybrid Tomato-5. The disease severity of other varieties were BARI Tomato-2 (18%), BARI Tomato-8 (28.91%), BARI Tomato-11(23.81%), BARI Tomato-14 (37.5%), BARI Tomato-15 (35%), BARI Hybrid Tomato-9 (35%), Ratan (26.25%), Sorno Komol (62.5%).

From the Disease severity and Disease Incidence analysis it is observed that the variety BARI Hybrid Tomato-5 is an immune variety against *Tomato yellow leaf curl virus (TYLCV)* on the basis of biological properties. Our results also match with the previous study conducted by (Yadav and Awasthi, 2009).

4.12.3. Whitefly association

The minimum number of whitefly per leaf in was recorded in BARI Tomato-2 preceded by Sorno Komol, BARI Hybrid Tomato-7, BARI Tomato-11 and BARI Tomato-8. Whereas the maximum number of whitefly per leaf was recorded in BARI Hybrid Tomato-5 followed by BARI Tomato-14, Ratan, BARI Tomato-15 and BARI Hybrid Tomato-9.

Early infection of *TYLCV* causes drastic reduction of all the growth contributing character of all the tomato varieties. The extent of damage in different growth contributing characters was largely dependent upon the stage of infection of *TYLCV*, condition of growing seedlings and tomato varieties. Almost same phenomenon with the *TYLCV* infection was noted by Gupta, (2000).

4.12.4. DAS-ELISA test

From the DAS-ELISA test it is clearly shown that all the selected varieties viz BARI Tomato-2, BARI Tomato-8, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-5, BARI Hybrid Tomato-7, BARI Hybrid

Tomato-9, Ratan and Sorno Komol were infected by *Tomato yellow leaf curl virus (TYLCV).*

From both lab and field findings it can be concluded that although the variety BARI Hybrid Tomato-5 was showed immune in our eye observation but actually virus was present in the samples of this variety. So it is evident that no accession provided complete resistance to *Tomato Yellow Leaf Curl Virus* (*TYLCV*). Results from this study are similar to those found by (Osei *et al*., 2012).

4.12.5. Morphological features

The infected tomato plant showed different morphological responses against different morphological features. The yield of individual variety depends on the number of leaves, branch, flowers and fruits per plant. The lowest number of leaves per plant was recorded in Sorno Komol preceded by BARI Hybrid Tomato-9, BARI Hybrid Tomato-7, BARI Hybrid Tomato-5 and BARI Tomato-15. The highest number of leaves per plant was obtained in the variety BARI Tomato-8 followed by variety BARI Tomato-2, BARI Tomato-11, Ratan and BARI Tomato-14.

The minimum number of branches per plant was counted in the variety BARI Tomato-15 preceded by Sorno Komol, BARI Hybrid Tomato-9, BARI Tomato-14 and Ratan. The maximum number of branches per plant was recorded in the variety BARI Tomato-11 followed by BARI Tomato-8, BARI Tomato-2, BARI Hybrid Tomato-7 and BARI Hybrid Tomato-5. The maximum number of flowers per plant was recorded in the variety BARI Tomato-11 followed by BARI Tomato-2, BARI Hybrid Tomato-5, Ratan, BARI Tomato-8. The minimum number of flowers per plant was found in the variety Sorno Komol preceded by BARI Hybrid Tomato-9, BARI Tomato-15, BARI Tomato-14 and BARI Hybrid Tomato-7. The minimum number of fruits per plant was obtained in Ratan preceded by Sorno Komol, BARI Hybrid Tomato-9, BARI Hybrid Tomato-7 and BARI Tomato-15. Whereas The

maximum number of fruits per plant was obtained in the variety BARI Tomato-11 followed by BARI Hybrid Tomato-5, BARI Tomato-14, BARI Tomato-8 and BARI Tomato-2.

The highest yield per plant was recorded in the variety BARI Tomato-11 followed by BARI Tomato-8, BARI Hybrid Tomato-5, BARI Tomato-14 and BARI Tomato-2 . The lowest yield was obtained in BARI Tomato-15 preceded by BARI Hybrid Tomato-7, Sorno Komol, Ratan and BARI Hybrid Tomato-9. The lowest shoot length was observed in the variety Sorno Komol preceded by BARI Tomato-15, BARI Tomato-2, Ratan and BARI Hybrid Tomato-7. Whereas the highest shoot length was observed in the variety BARI Tomato-11 followed by BARI Tomato-14, BARI Tomato-8, BARI Hybrid Tomato-5 and BARI Hybrid Tomato-9.

In the present study the yield contributing parameters seemed to be affected to varying extent depending on the viral infection, Number of whitefly per leaf, growing condition and tomato variety. Similar observations were recorded by Ajlan *et al*., (2007); Olaniyi *et al*., (2010) and Gafni, (2003).

4.12.6. Relation between Disease Incidence and Yield

The highly susceptible varieties were BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-7 and Sorno Komol. The susceptible varieties were BARI Tomato-2, BARI Tomato-8, BARI Hybrid Tomato-9, Ratan. BARI Hybrid Tomato-5 is the Immune variety. The highest yield per plant was recorded in the variety BARI Tomato-11 (0.794 kg) followed by BARI Tomato-8 (0.744 kg), BARI Hybrid Tomato-5 (0.644 kg), BARI Tomato-14 (0.535 kg) and BARI Tomato-2 (0.451 kg). The lowest yield was recorded in BARI Tomato-15 (0.112 kg) preceded by BARI Hybrid Tomato-7 (0.119 kg), Sorno Komol (0.145 kg), Ratan (0.150 kg) and BARI Hybrid Tomato-9 (0.352 kg). There is a strong and negative correlation between Disease incidence and yield which shows that the equation $y = -2.952x + 631$ gave a good fit to the data, and the co-efficient of determination. From the

regression analysis it may be concluded that the yield is negatively correlated with disease incidence of plant. Similar result was recorded by Alam *et al*, (2016).

4.12.7. Relation between disease Severity (%) and yield

The highest disease severity was observed in BARI Hybrid Tomato-7 (70%) and no disease was found in BARI Hybrid Tomato-5 (0%). The disease severity of other varieties were BARI Tomato-2 (18%), BARI Tomato-8 (28.91%), BARI Tomato-11(23.81%), BARI Tomato-14 (37.5%), BARI Tomato-15 (35%), BARI Hybrid Tomato-9 (35%), Ratan (26.25%), Sorno Komol (62.5%). The highest yield per plant was recorded in the variety BARI Tomato-11 (0.794 kg) followed by BARI Tomato-8 (0.744 kg), BARI Hybrid Tomato-5 (0.644 kg), BARI Tomato-14 (0.535 kg) and BARI Tomato-2 (0.451 kg). The lowest yield was recorded in BARI Tomato-15 (0.112 kg) preceded by BARI Hybrid Tomato-7 (0.119 kg), Sorno Komol (0.145 kg), Ratan (0.150 kg) and BARI Hybrid Tomato-9 (0.352 kg). There is a strong and negative correlation between Disease severity and yield which shows that the equation y = -8.108x + 677.9 gave a good fit to the data, and the co-efficient of determination. From the regression analysis it may be concluded that the yield is negatively correlated with disease severity of plant. Similar result was recorded by Alam *et al,* (2016).

4.12.8. Relationship between whitefly association and disease development:

In all the selected varieties where whitefly was present disease was also present except the variety BARI Hybrid Tomato-5. Though it contains the highest number of whitefly per plant but there was no disease development in this variety. From this it can be concluded that BARI Hybrid Tomato-5 is an immune variety against *Tomato yellow leaf curl virus (TYLCV).*Similar findings were found by (Demichelis *et al*., 2000)

4.12.9. Relationship between disease incidence and shoot length:

The highly susceptible varieties were BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-7 and Sorno Komol. The susceptible varieties were BARI Tomato-2, BARI Tomato-8, BARI Hybrid Tomato-9, Ratan. BARI Hybrid Tomato-5 is the Immune variety. The highest shoot length was observed in the variety BARI Tomato-11 (126.33 cm) followed by BARI Tomato-14 (96.00 cm), BARI Tomato-8 (93.33 cm), BARI Hybrid Tomato-5 (86.67 cm) and BARI Hybrid Tomato-9 (82.33 cm). The lowest shoot length was observed in the variety Sorno Komol (40.33 cm) preceded by BARI Tomato-15 (52.33 cm), BARI Tomato-2 (53.67 cm), Ratan (61.33 cm) and BARI Hybrid Tomato-7 (72.67 cm). There is a strong and negative correlation between Disease incidence and Shoot length which shows that the equation $y = -0.056x + 80.80$ gave a good fit to the data, and the co-efficient of determination. From the regression analysis it may be concluded that the Shoot length is negatively correlated with disease incidence.

4.12.10. Relationship between disease severity and shoot length:

The highest disease severity was found in BARI Hybrid Tomato-7 (70%) and lowest disease severity was found in BARI Hybrid Tomato-5 (0%). The highest shoot length was observed in the variety BARI Tomato-11 (126.33 cm) followed by BARI Tomato-14 (96.00 cm), BARI Tomato-8 (93.33 cm), BARI Hybrid Tomato-5 (86.67 cm) and BARI Hybrid Tomato-9 (82.33 cm). The lowest shoot length was observed in the variety Sorno Komol (40.33 cm) preceded by BARI Tomato-15 (52.33 cm), BARI Tomato-2 (53.67 cm), Ratan (61.33 cm) and BARI Hybrid Tomato-7 (72.67 cm). There is a strong and negative correlation between Disease severity and Shoot length which shows that the equation $y = -0.423x + 90.75$ gave a good fit to the data, and the co-efficient of determination. From the regression analysis it may be concluded that the Shoot length is negatively correlated with disease severity.

From the findings of this study, it is revealed that out of ten varieties BARI Hybrid Tomato-5 showed better performance compared to other varieties against *Tomato yellow leaf curl virus (TYLCV).* Though whitefly per leaf was highest in BARI Hybrid Tomato-5 but both disease incidence and severity were low in this variety. It was also found that the variety BARI Hybrid Tomato-5 also provide better yield than the other varieties. It was reported that the variety BARI Hybrid Tomato-5 shows better performance on plant height, no of fruits, fruits diameter, individual fruit weight, root length and yield over the other varieties against *Tomato yellow leaf curl virus (TYLCV).*

CHAPTER 5

SUMMARY AND CONCLUSIONS

Tomato (*Lycopersicon esculentum* Mill.) is a herbaceous fruiting plant belonging to the family Solanaceae. It originated in Latin America and has become one of the most popular and widely cultivated vegetable crops of the world with ability to survive in diverse environmental conditions. It is grown for its edible fruit, which can be consumed, either raw or cooked or in the form of various processed products like juice, ketchup, sauce, pickle, pastes and powder. It is universally treated as "protective food" and provides almost all types of vitamins and minerals in quite fair amount. Tomato-based products are used as a preventive strategy against cancer and cardiovascular diseases. Tomato crop is attacked by large number of pathogens that infect various plant parts of the crop and greatly affect the production. Among the different viral diseases tomato yellow leaf curl disease caused by *Tomato Yellow Leaf Curl Virus* (*TYLCV*) is a major limiting factor in tomato cultivation. The virus caused severe infection and yield losses up to 90%. But the main constraint for low productivity in tomato crop is due to the attack of tomato yellow leaf curl disease. So, considering the importance of the crop a systematic study was conducted with the objectives to identify the virus, determine disease incidence and severity level and to screen the resistance of different tomato varieties.

Ten tomato varieties viz. BARI Tomato-2, BARI Tomato-8, BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-5, BARI Hybrid Tomato-7, BARI Hybrid Tomato-9, Ratan, Sorno Komol were selected for evaluation in the experiment.

In respect to disease incidence, the selected tomato varieties differed significantly among themselves. The highest disease incidence (100%) was found in BARI Tomato-11, BARI Tomato-14, BARI Tomato-15, BARI Hybrid Tomato-17 and Sorno Komol. On the other hand lowest disease incidence i.e 0% disease was found in BARI Hybrid Tomato-5. The moderate disease

incidence was found in BARI Tomato-2 (66.67%), BARI Tomato-8 (66.67%), BARI Hybrid Tomato-9 (66.67%) and Ratan (66.67%).

The highest disease severity (70% and 62.5%) was found in BARI Hybrid Tomato-7 and Sorno Komol, respectively. There was no visible symptoms was found in BARI Hybrid Tomato-5. Other tomato varieties were showed lower to medium viz. BARI Tomato-2, BARI Tomato-11, Ratan and BARI Tomato-8 were showed lower disease severity (18%, 23.81%,26.25%, 28.91% respectively), while BARI Tomato-15, BARI Hybrid Tomato-9, BARI Tomato-14 showed the moderate disease severity (35%, 35%, 37.5% respectively). In case of whitefly association, the maximum number of whitefly per leaf was recorded in BARI Hybrid Tomato-5 (27.00) and the minimum number of whitefly per leaf was recorded in BARI Tomato-2 (10.67). In case of ELISA test all the selected varieties showed positive reaction against *TYLCV*.

In case of number of leaves, the maximum number of leaves per plant was recorded in the variety BARI Tomato-8 (31.67) and the minimum in variety Sorno Komol (11.67). In case of number of branches, the maximum number of branches per plant was recorded in the variety BARI Tomato-11 (9.33) and minimum in BARI Tomato-15 (3.33). In case of number of flowers and fruits, the maximum number of flowers per plant was recorded in the variety BARI Tomato-11 (90.67) and the minimum number of flowers per plant was found in the variety Sorno Komol (10.67). The maximum number of fruits per plant was recorded in the variety BARI Tomato-11 (81.33) and the minimum number of fruits per plant was recorded in Ratan (5.33). In case of shoot length, the highest shoot length was measured in the variety BARI Tomato-11 (126.33 cm) and the lowest shoot length was observed in the variety Sorno Komol (40.33 cm). In case of yield, the highest yield per plant was obtained in the variety BARI Tomato-11 (0.794 kg) and the lowest yield was recorded in BARI Tomato-15 (0.112 kg).

From the relations of the parameters, it could be concluded that yield was negatively related with disease incidence (%) and disease severity (%); No. of fruits were also negatively related with disease severity (%) and Shoot length was also negatively related with disease incidence (%) and disease severity (%).

Considering the performance of tomato varieties it may be concluded that BARI Hybrid Tomato-5 could be graded as an immune variety against *Tomato Yellow Leaf Curl Virus (TYLCV)*. The others selected varieties were found susceptible against (*TYLCV*). In ELISA test *TYLCV* was found positive in all the selected varieties. From these different result of biological and serological test it could suggested that more emphasize should be given to find out the resistance against *TYLCV* and research should be continued with BARI Hybrid Tomato-5.

CHAPTER 6

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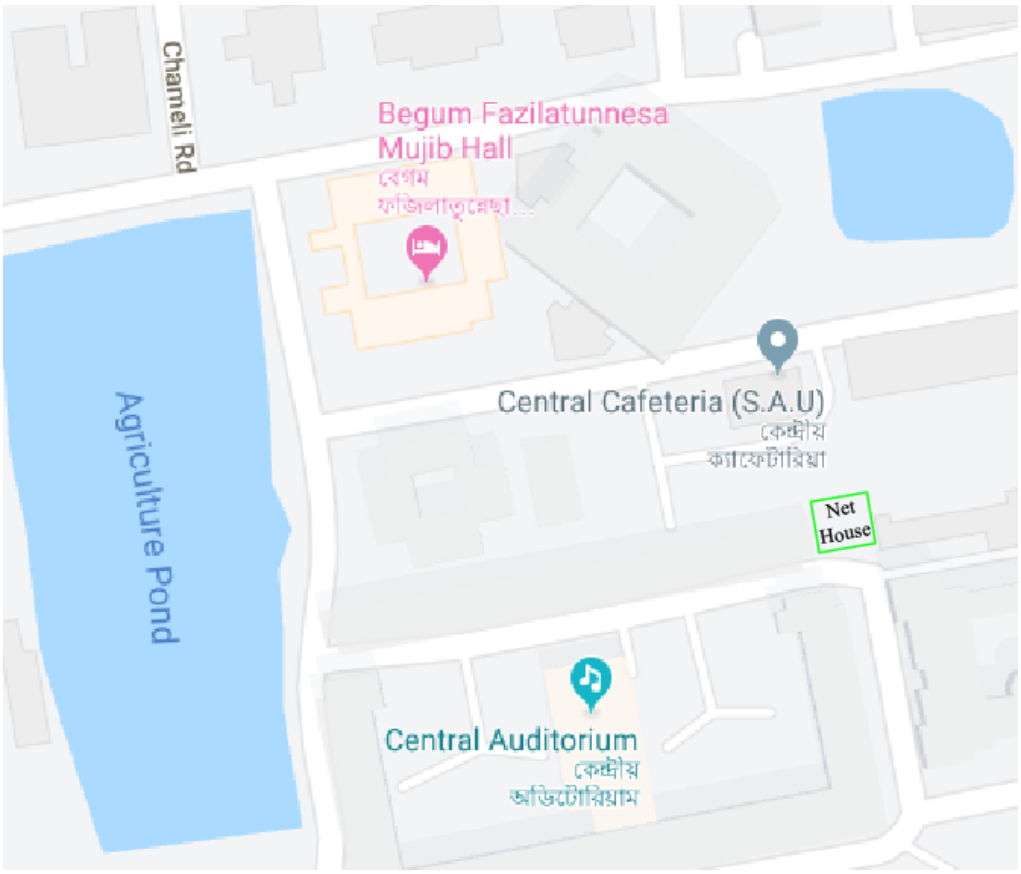
CHAPTER 7

APPENDICES

Appendix-I. Map showing the experimental site under study



Appendix-II. Physiochemical properties of soil, used in the experimental pots

Characteristics	Value
Sand (%)	25.67
Silt (%)	53.86
Clay (%)	20.48
Texture	Silty loam
pH	5.7-7.1
Organic carbon (%)	0.30
Organic matter (%)	0.55
Total N (%)	0.028
Phosphorus(µg/g soil)	23.59
Exchangeable K (milliequivalents/100 g soil)	0.61
Sulphur (µg/g soil)	28.45
Boron (µg/g soil)	0.06
Zinc (µg/g soil)	2.32

Source: Soil Resources Development Institute (SRDI), Dhaka-1207.

Appendix-III. Monthly average relative humidity, maximum and minimum temperature, rainfall and sunshine hour of the experimental period (October 2017- March 2018

Month	Average RH (%)	Average Temperature (°C)		Total Rainfall (mm)	Average Sunshine hours
		Min.	Max.		
October	79	25	32	175	6
November	65	21	30	35	8
December	74	15	29	15	9
January	68	13	24	7	9
February	57	18	30	25	8
March	57	20	33	65	7

Source: Bangladesh Meteorological Department (Climate & weather division), Agargaon, Dhaka-1207.

Appendix-IV. Disease severity calculation (BARI Tomato-2):

$$\% \text{ disease severity} = \frac{\text{Sum of total disease rating}}{\text{Total No of observation} \times \text{ Maximum grade in the scale}} \times 100$$

Sum of total disease rating:

Disease grade	Frequency (diseased leaf)	Disease rating
0	0	0
1	1	1
2	3	6
3	1	3
4	2	8
		Total=18

Sum of total disease rating= 18

Total no of observation= 25

Maximum grade in the scale= 4

$$\% \text{ disease severity} = \frac{18}{25 \times 4} \times 100$$

$$=18$$

Appendix-V. Comparison between Immune (BARI Hybrid Tomato-5) and highly diseased (BARI Hybrid Tomato-7) variety

BARI Hybrid Tomato-5

BARI Hybrid Tomato-7