SURVEILLANCE ON DISEASE INCIDENCE AND SEVERITY OF *Papaya ringspot virus* AT SELECTED LOCATIONS OF BANGLADESH

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CERTIFICATE

*This is to certify that thesis entitled, "**Surveillance on disease incidence and severity of Papaya ringspot virus disease in selected locations of Bangladesh**", submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in Plant Pathology**, embodies the result of a piece of bona-fide research work carried out by **Nishan Chakma,** Registration No. **09-03501** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

.....
Dated:
Place: Dhaka, Bangladesh

.....
Dr. Fatema Begum
Supervisor
Associate Professor
Department of Plant Pathology
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DEDICATED TO MY BELOVED PARENTS AND ELDER SISTERS

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SURVEILLANCE ON DISEASE INCIDENCE AND SEVERITY OF *Papaya ringspot virus* AT SELECTED LOCATIONS OF BANGLADESH

ABSTRACT

An experiment was conducted to survey the prevalence of disease incidence and severity of *Papaya ringspot virus* (PRSV) at eight locations of four districts in Bangladesh. Papaya is one of the popular fruit in the whole world. It suffers from several diseases and the causal organisms are fungi, bacteria, nematodes and viruses. Among them, viral diseases are found considerable yield loss and the most important one is PRSV-P. The survey was conducted from July 2016 to December 2016. Three plain districts and one hill tract area were selected for the survey. Six different symptoms were found in the selected field which was identified as PRSV based on symptomology. Symptoms were mild mosaic (MM), mosaic (MO), severe mosaic (SM), fern leaf (FL), leaf distortion (LD) and vein clearing (VC). The highest incidence (36.24%) was found in BSMARU farm in Gazipur and the lowest (12.04%) was found in Panchari, Khagrachari hill tract. The maximum severity (11.53%) was found in BSMARU campus, Gazipur and the lowest severity (2.50%) was found in Panchari, Khagrachari hill tract. The yield contributing parameters and yield of papaya found to be different significantly among the surveyed area. The lowest fruit weight (324.3 gm.) due to PRSV-P infection was recorded in BSMARU, Gazipur while the highest fruit weight (643.6 gm.) was conducted in SAU Campus, Dhaka.

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LIST OF SYMBOLS AND ABBREVIATION

% = Percentage

et al. = And others

spp = Species

no. = Number

df = Degree of freedom

& = And

Etc. = Etcetera

cm. = Centimeter

gm. = Gram

i.e. = That is

SAU = Sher-e-Bangla Agricultural University

JU = Jahangir Nagar University

BADC= Bangladesh Agriculture Development Corporation

BSMARU =Bangabandhu Sheikh Mujibur Rahman Agricultural University

FAO = Food and Agriculture Organization

BBS = Bangladesh Bureau of Statistics

PRSV-P = Papaya Ringspot Virus-Papaya Strain

MM = Mild mosaic

MO = Mosaic

SM = Severe Mosaic

LD = Leaf distortion

FL = Fern leaf

VC = Vein clearing

PH = Plant height

NL = Number of leaf

PL = Petiole length

LA= Leaf blade area

NF = Number of fruits

FW = Fruit weight

CV = Coefficient Variance

PDI = Percent Disease Index

LSD = Least Significant Difference

RCBD = Randomize block design

DMRT = Duncan’s Multiple Range Test

CHAPTER I

INTRODUCTION

Papaya (*Carica papaya* L), belongs to the family Caricaceae is an important fruit of tropical and subtropical regions in the world. It is an important popular vegetable as well as fruit crop in respect of its food value and taste. Papaya fruit is rich in vitamin A, which refers to the second highest among of fruits next to mango (Aykroyed, 1951). It contains fair amount of vitamin C, riboflavin and niacin and is a good source of moisture, protein, fat, carbohydrate, calcium, phosphorus and iron (Bosh, 1985). Papaya has also regarded as medicinal as well as industrial cash crop. Carpaine, an alkaloid present in papaya can be used as heart depressant, amoebicide and diuretic (Burdic, 1971). Papain, extracted from green papaya can be used in beverage food and pharmaceutical industries. Besides fruits and papaya leaves are used as a poultice for nervous pain, reducing elephantoid growth and for washing clothes. Barks of papaya tree are used for making rope. Roots cure piles and seeds have abortic properties (Watt, 1989).

Although papaya is successfully grown all over Bangladesh, statistic regarding the papaya production is not satisfactory. In 2009-2010, papaya has been cultivated in 2790 ha of land with a total production of 40.42 t/ha (BBS, 2009-2010) in contrast about 1,13,52,202 tons' production of papaya in 2010 was in the world (FAOSTAT Database, FAO, 2010).

Papaya production is greatly hampered due to some constraints, of which disease play an important role. Among the diseases, viral diseases took a great importance. There are several viral diseases occur to papaya, like as *Papaya ring spot virus, Papaya leaf curl virus, Papaya yellow mosaic* virus etc. viral diseases have been recognized as a major limiting factor for

commercial papaya production throughout the tropics and subtropics (Jensen 1949a and b, Cook and Zettler, 1970). The virus infects the plant at any growth stage (seedling to maturity). The infected plants produce different type of symptoms on leaves like mild to severe mosaic, vein-clearing, vein-yellowing, chlorotic spots, fern leaf, shoe-sting etc. (Rahaman, 2003). The infected plants produce small flower and distorted fruits showing stunted growth. The distorted fruits bear conspicuous ringspot symptom and taste become watery. The disease drastically deteriorates the yield, market value, and quality of the fruits (Purcifull *et al*. 1984). The yield loses due to papaya viral disease infection may reach even up to 100% very often. In Bangladesh, the virus causes 70%-100% yield reduction of papaya depending upon the stage of infection as estimated by Akanda (1991). The virus also infects various cucurbitaceous crops like cucumber, bitter gourd, sweet gourd, ash gourd, ribbed gourd etc. and causes tremendous yield losses of those crops (Akanda *et al*. 1991 a, b and c).

The papaya viral diseases may show peculiarity in their prevalence and symptom development throughout the year. Sometimes, masking of the symptom occur in the infected plants depending upon the seasons (Kiranmai *et al*. 1998)

The papaya plant may get infected by PRSV-P at any stage of the growth (seedling to maturity) and yield loss might reach even upto 100% very often (Purcifull *et al.* 1994). For causing devastating disease that severely interfere with commercial papaya production. *Papaya ringspot virus*-Papaya strain (PRSV-P) is well recognized in all papaya growing countries of tropic and sub-tropic (Gonsalves, 1998). Akhter and Akanda (2008) stated that seven symptoms namely mild mosaic (MM), mosaic (MO), severe mosaic (SM),

leaf distortion (LD), fern leaf (FL), vein clearing (VC), chlorotic leaf spot (CS) all are reacted positively in DAS-ELISA against the antisera of *Papaya ringspot virus*- papaya strain (PRSV-P).

The economically sustainable production of papaya needs efficient management of the disease caused by papaya viruses. Several attempts were made like roughing, controlling of insect vectors by spraying mineral oil and insecticides, mulching, inter-cropping with barrier crop (corn), protecting young seedlings with plastic bag, developing papaya varieties resistant to papaya viral disease for managing the disease (Yeh *et al*. 1988) but none of the measures gave satisfactory results. Considering the above facts, the present experiment was undertaken to achieve following objectives.

Objectives

1) To conduct a survey on PRSV-P disease in the field at selected location of Bangladesh.
2) To quantify the effect of PRSV-P disease on the growth and yield contributing parameters of papaya.
3) To know the reduction of growth and yield parameters of papaya due to PRSV-P infection.

CHAPTER II

REVIEW OF LITERATURE

2.1. Food and Nutritional value of papaya

Boshra and Tajul (2013) has stated that Papaya (*Carica papaya*), an herbaceous fruit crop belonging to the family Caricaceae, has garnered popularity among researchers due to its nutritional and Pharmaceutical value. They also showed that, papaya is not only known for its nutritional benefits but also considered to possess medicinal properties. It was rich in calories and in natural vitamins and minerals, like vitamin C, vitamin A, thiamine, iron and fiber. Papain found in papaya latex has natural digestive properties beneficial to the human digestive system.

Papaya (*Carica papaya* L.) belongs to the family Caricaceae grown in Australia, Hawaii, Philippines, Sri Lanka, South Africa, India, Bangladesh, Malaysia and other countries in tropical America (Anuara *et al.* 2008).

Different fruit parts contain different content. Such as, the fleshy part of the fruit contains protein, fat, fiber, carbohydrates, mineral: calcium, phosphorous, iron, vitamin C, thiamine, riboflavin, niacin and carotene, amino acids, citric and malic acids (green fruit). Juice carries N-butyric acids, n-hexanoic and n-octanoic acids, lipids, Myristic, planets, stars, linolec, linolenic and *cis*-vaccenic and oleic acid. Seed also have Fatty acids, crude protein, crude fiber, papaya oil, carpaine, benzylisothiocynate, benzylglucosinolate, glucotropacolin, bemzylthiourea, hentriacontane, β-sitostrol and enzyme myrosin (Boshra and Tajul, 2013)

Papaya plant also contains different elements. Boshra and Tajul (2013) proved that, papaya plant root contains Carposide and enzyme myrosin,

leaves conatin alkalodiscarpain, pseudocarpain and dehyrocarpaineand, choline, carposide vitamin C and E. Its bark has β-sitosterol, glucose, fructose, sucrose and xylitol. Proteolytic enzymes, papain and chemopapain, glutamine, cyclortransferase, chymopapains A, B and C, peptidase A and B and lysozymes found in the latex of the papaya.

2.2. Historical preview of viral disease of papaya

2.2.1. Papaya ringspot virus

PRSV is the most destructive disease of papaya in all over the world. Linder *et al*. (1945) has published about PRSV and brought it in literature for the first time. An investigation was done by Jensen (1949) about the virus infecting papaya in Hawaii and named the virus as *Papaya ring spot virus* (PRSV).

In 1945, for the first time it was reported about the presence of PRSV in Taiwan, where most of the plant was destroyed by virus in the commercial orchard (Wang *et al.* 1978).

In Japan, *Papaya leaf distortion mosaic virus* was first reported by Maoka *et al*. (1995) as a distinct member of genus *Potyvirus* that was later proved to be an isolate of PRSV.

Gonzalvez *et al.* (1997) established that PRSV caused three types of leaf symptom on papaya which are mosaic, yellowing and deformation.

In Andhra Pradesh of India, in commercial plantation papaya plants with severe mosaic, leaf distortion and filiform leaves were suspected to be infected with PRSV reported by Kiranmai *et al.* (1998).

PRSV is described as a typical member of *Potyvirus* and grouped PRSV into PRSV-P i.e., *Papaya ringspot virus*- papaya strain which infect papaya and cucurbtis and PRSV-W i.e., *Papaya ringspot virus*- watermelon strain which infects cucurbits but not papaya (Purcifull, 1984). He also described that

PRSV-P is radially transmitted by mechanical inoculation using sap of infected plants and host range is limited to the members of Caricaceae, Chenopodiaceae and Cucurbitaceae. PRSV caused with different symptoms but typical ringspot symptom occurs on fruits of infected plats (Yeh *et al.* 1988). The virus was proved to be a non-persistently transmitted by a number of aphid species. Among all of the species *Aphis gossypii* is the most common. It was established by Bateson *et al.* (1994) that PRSV-P could have been originated through mutation of PRSV-W.

The most renowned papaya viral disease is PRSV-P in the world. In Hawaii, Florida, the Caribbean countries, South America, Australia and the Far East it was reported that PRSV-P is the major limiting factor in papaya growing areas (Purcifull *et al*. 1984). The distribution of PRSV-P in many areas of the world including USA, Mexico, Caribbean Countries, Germany, France, Italy, India, Taiwan, Middle East and South Africa was reported by Brunt *et al*. (1990).

2.2.2. Papaya mosaic virus

Papaya mosaic virus is one of the most important virus of papaya in the world. It causes leaf mosaic and stuning in papaya. It was first described by Conover (1962, 1964) and de Bokx *et al*. (1965). It is transmitted by several species of Aphids (*Zettler et al.* 1968).

2.3. Economic importance of papaya viral disease

Papaya is a very demanding fruit in the world with various purposes like as a fruit and vegetable. It has high nutritional, medicinal value. It has a great economical contribution in the world. But the papaya viral disease causes great hamper in papaya production both in quality and quantity. It reduces the production and hamper commercial papaya production.

Jensen (1949b) stated that PRSV destroyed the papaya industry in Hawaii and shifted the papaya cultivation from Hawaii to Oahu. On the other hand, its destructive effect forced Brazilian papaya industry to move from Sao-Paulo and Rio-De-Zaneiro states to more remote northern state Espiritu-Santo and Bahia (Costa *et al*. 1969). From 1973-78 Sao-Paulo and Rio-De-Zeneiro accounted 90% of the total papaya acreage but 27% in 1984 due to PRSV prevalence over there (Costa *et al*. 1969)

Papaya ringspot virus (PRSV) destroyed most of the papaya plant in commercial orchard along the West Coast of Taiwan, reported by Wang *et al.* (1978). The total yield of papaya dropped from 41,595t in 1974 to 18,950 in 1977.

Papaya ringspot virus disease has been reported as a destructive disease causing severe economic loss of papaya production reported by Yeh *et al*. (1988) and Gonsalves (1989). The virus was designed as a major obstacle to wide scale planting of papaya.

Chan and Doon (1990) described PRSV as a potential threat to the local papaya industry in Malaysia.

Akanda (1991) reported that *Papaya ringspot virus* - papaya strain causes 70-100% yield reduction of papaya depending upon the stage of infection in Bangladesh.

Yon (1994) determined that papaya cultivation of 17170 ha of land in 1982 was clopped down to less than 6000 ha in 1991 in Indonesia due to severe prevalence of PRSV.

Gonzalez *et al*. (1997) recorded that *Papaya ringspot virus* –type P (PRSV-P) is the most harmful viral disease of papaya in Mexico. It causes losses in fruit yield and quality and showed at least three types of leaf symptoms

(mosaic, yellowing and leaf deformation). Result showed that leaf deformation may cause the highest reduction in photo assimilate synthesis and fruit growth.

PapMV infection initiated at the seedling stage on pawpaw (*Carica papaya*) results in a slightly stunted plant with leaf mottle, still able to produce fruit with no significant reduction on size or yield. Other species of the Caricaceae family are also subject to infection by PapMV which, in some, is lethal.

Mixed infections of viruses in pawpaw induce disease symptoms that can be more serious than those associated with a single infection. Severe leaf mottle and malformation, shortened petioles and rapid decline may occur. PapMV alone causes symptoms of minor concern, as do milder strains of *Papaya ringspot virus*, but a combination of the two viruses can result in lethal decline (Conover, 1964; Cook and Zettler, 1970).

2.4. Symptomatology

2.4.1. Papaya ringspot virus

The virus causes different disease in papaya produced different types of symptom on papaya as noted by different workers Jensen (1949a) described the symptoms caused by PRSV on papaya as mottling and distortion of leaves, ringspot on fruits and streaks on stem and petioles and named the virus as *Papaya ringspot virus*. Besides, stunted plant growth and reduced fruit number were reported by Jensen (1949a).

Symptoms on *Carica papaya* by PRSV are variable and depend on stage of infection, plant vigor, temperature, virus strain, plant size. Seedling show prominent vein clearing and downward cupping of the young leaves stated

by Conover (1964). After several weeks, the leaves become mottled and distorted.

Story and Halliwell (1969) recorded that distortion due to ringspot virus (which was synonym of PRSV as reported by Zettler *et al*. 1968) disease on younger plants initially is indicated by oil like streaking and spotting on the main stem. These symptoms are followed by vein-clearing and mosaic of the younger foliage. In older plants, early symptoms are streaking on stem and petioles/or green ring spotting on the fruits.

Symptomatological difference was used to distinguish between *Papaya ringspot virus* and *Papaya mosaic virus* by Cook and Zettler (1970). They noted PRSV induced mosaic along with leaf distortion and streaking on the stem and green ringspot on the fruits, where as *Papaya mosaic virus* produced small chlorotic spots resulting mosaic and rogusity of the infected leaves.

In papaya, PRSV causes mottling and distortion of leaves, ringspot on fruit and water soaked streaks on stem and petioles. The disease stunts the plant and drastically reduces the size of the fruit (Yeh *et al*. 1988).

Papaya ringspot virus causing yellow mosaic, stunting, ringspot on fruit and water soaked streaks on papaya stem in the areas of Panjab in India described by Husain and Verma (1994).

Severe mosaic, leaf distortion, oily streaks or spots on papaya fruits, whereas, blisters and shoe-string, mosaic or yellow mosaic, blister on cucurbits symptom was identified by Dahal *et al*. (1997) on papaya and cucurbits induced by PRSV.

2.4.2. Papaya mosaic virus

PapMV causes mild mosaic symptoms on papaya leaves and stunting of the plant (Wikipedia). Symptoms of infection include mild leaf mosaic and

stunting. No symptoms appear on petioles, stems or fruit. Stunting is only apparent when healthy plants are present for comparison (Conover, 1964).

Approximately 5 days after inoculation, young greenhouse-grown seedlings show vein-clearing and downward cupping of leaves. Leaf mosaic develops after 15-20 days (Purcifull and Hiebert, 1971).

2.5. Host range

Host ranges, so far, studied from the beginning of the appearance of the virus until to date as a routine work. However, the host range of papaya viral disease seems to be limited of fifteen dicotyledonous species in the families Caricaceae, Chenopodiaceae and Cucurbitaceae (Jensen 1949a, Conover 1964, Wang *et al*. 1978).

Prasad and Sarker (1989) reported two species belonging to Solanaceae family as host of PRSV, i.e., *Cyphomandra betaceae* and *Solanum nigrum*.

According to Brunt *et al.* (1990) the experimental host range of PRSV is narrow. The dicotylednous hosts are to be recorded as *Carica pepo, Chenopodium* spp.

2.6. Transmission

Papaya ringspot virus is transmitted in nature by insect vectors belonging to the Aphididae as stated by Jensen (1949a). *Myzuspersicae* and *Aphis gossypii*, were found to transmit the virus in a non-persistent manner. He also reported that PRSV-P is also mechanically transmitted as its response to mechanical inoculation was found to be highly positive.

Story and Halliwell (1969) determined that *Distortion ringspot virus* was mechanically transmitted to healthy papaya plants. The virus was transmitted from infected roots, fruits and leaves but not from the latex. Transmission of the virus from infected plants to healthy plants by the leafhopper, *Empoasca papayae* was unsuccessful.

Aphis gossypii, A. craccivora and *A. citricola* were the most common and were also efficient vectors of *Papaya ringspot virus* in papaya plants in Bihar out of 11 aphid species as investigated by Prasad and Sarker (1989).

Prasad and Singh (1990) reported that *Papaya ringspot virus* might be transmitted by *Cuscuta spp.*

Bayot *et al*. (1990) observed that only 2 of 1355 seedlings grown from seeds of papaya fruits infected with PRSV showed symptoms that closely resembled to those of PRSV. Similar symptoms were observed when sap from infected seedlings was mechanically inoculated to healthy seedlings. Presence of the virus was confirmed by aphid transmission and indexing.

The relationship between *Papaya ringspot virus* (PRSV) and *Aphis carccivora* was investigated by Taya and Singh (1997). They observed that unstarved apterae adult aphids transmitted the virus by upto 25%. Transmission increased with an increase in the number of aphids and maximum transmission was recorded with 8 nymphs and 8 apterae adult aphids per last plant.

2.7. Electron microscopy cytopathology of PRSV-P

The PRSV-P was revealed by electron microcopy that the virus particles are filamentous, usually flexuous, not enveloped and modal length of 760nm to 800nm and which of 12nm (de Bokx 1965, Story and Halliwell, 1969, Brunt *et al.* 1990).

Zettler *et al*. (1968) reported cytoplasmic inclusions similar to pinwheel, circular and bundle like structures infected the leaf tissues by ultra thin sections of PRSV revealed by electron microscopy.

Edwardson and Christy (1978) published review on the inclusion bodies induced by viruses and its importance in classification and diagnosis of plant viruses. All *Potyvirus* including PRSV produced characteristics cylindrical

inclusion body with scroll and pinwheel structures in the cytoplasm reported by them. They also stated that inclusion bodies are grouped specific and could be used for diagnosis of plant viruses at last upto group level.

Edwardson *et al*. (1984) published a paper on the inclusion body of *Potyvirus* infected plants. Cylindrical inclusion body seemed to be the unique characteristics of all *Potyvirus* including PRSV-P.

Gonsalves *et al*. (1984) and Martelli and Russo (1984) also supported the opinion of Edwardson *et al*. (1984).

Brunt *et al*. (1990) described that PRSV possesses nucleic acid 5.5%, protein 94.5%, genome containing single standard RNA, linear in one part and molecular weight of segment (s) $3.8X10^6$.

Brunt *et al*. (1990) again reported the cytopathology of *Papaya ringspot virus*. The virus particles found in all parts of the host plant, in the cytoplasm and cell vacuole. Inclusion body found in infected cells is amorphous x-bodies and pinwheels and these do not contain virus particles.

Jain *et al*. (1998) characterized PRSV isolates from India al nuclear inclusion b (Nib) gene, the complete capsid protein (CP) gene and the untranslated region (UTR) of both P and W isolates. The CP genes of the two isolates were similar, with 87% nucleotide identity and 93% amino acid identity. The partially sequenced Nib genes were also 90% identical; but isolate W contained an additional amino acid (threonine).

2.8. Management of papaya viral disease

The commercial production of papaya needs effective management of the disease caused by different viral disease like as *Papaya mosaic virus*, *Papaya ringspot virus* etc.

2.8.1. Integrated management system

Becerra (1991) stated that plantation of purple colored papay and roselle plants (*Hibiscus sabdariffa*) as barrier plants delayed the aphid infestation, which are the vector of different papaya viral disease. After harvesting the roselle barrier, the number of diseased papaya increased.

Flores *et al*. (1995) reported that isolated control measure were ineffective for *Papaya ringspot virus* (PRSV) control in Mexico. So, some actions include in an integrated crop management (ICM) strategy to attempt PRSV control include 1) seed beds covered with an insect proof polypropylene mesh, 2) high density papaya planting (2222 plants/ha) allowed roughing of diseased plant, 3) foliage and soil nutrients to improve plant vigor, 4) poisoned plant barrier (2 lines of maize and 2 lines of *Hibiscus sabdariffa*), 5) 2-plastic strips, 5 cm wide with shiny grey metallic color above each row of papaya plants and 6) biweekly spray with 1.5% mineral oil.

Andrade *et al*. (1995) reported research on integrated management of PRSV. The protection of papaya nurseries with insect proof nets, the use of vegetation screens based on maize and sorrel and the application of citroline at 1.5% are the measure that were combined in a trial. These gave an increase of 191% production and 197% in net income compared with traditional control method.

Rezende *et al*. (1995) discussed alternative methods for controlling *Papaya ringspot virus* on papaya. These included planting of crops in partially isolated areas with roughing of diseased plants, planting in screen houses, screening for plants resistant to or tolerant of the disease and cross protection with mils strains of the virus.

Ray *et al*. (1999) recommended that transplanting of papaya in October and heavy but balanced fertilizer can help reduce the severity of *Papaya ringspot virus* outbreaks. They carried out the experiment on papaya plant cv. Pusa delicious which usually suffered from PRSV under the condition of Northern Bihar in India. The best performance, with a marked reduction in viral infection was observed with transplanting in October and with a heavy manorial doses consisting of 10kg FYM, 2-kg castor cake, 1kg cake-o-meal, 200gm N, 200gm K_20 and 200gm P_2O_5 per plant per year applied in 2 split, once in June and three months later.

Hernandez *et al*. (2000) evaluated three system of management of PRSV. The system was integrated pest management (IPM), integrated management without citroline (mineral oil derived from petroleum) (IPM-SC) and conventional farmer management (CM) as control. In IPM and IPM-SC the final incidence of the virus was 85% and 88%, respectively, whilst with conventional management it was recorded as 100%. The yield of the crop was with conventional management were 17055.80 kg/ha, with IPM 3110.60 kg/ha and 33741.30 kg/ha with IPM-SC.

2.8.2. Use of cross protection

Cross protection was first found by Mckinney (1929) with *Tobacco mosaic virus* the phenomenon in which plants systematically infected with one strain of a virus are protected from the effects of infection by a second related strain of the same virus.

Yeh and Gonsalves (1984) conducted a research on evaluation of induced mutant of *Papaya ringspot virus* for control by cross protection. Nitrous acid was used to induce mutant from PRSV Hawaii isolates. Two symptomless

mutants, PRA HA 5-1 and PRV HA 6-1 were obtained from artificial induction. Seedlings of *Cucumis metuliferus*, papaya and zucchini squash infected with these isolates exhibited light vein clearing with no reaction in vigor or growth. All the plants infected with PRAHA 5-1 or PRV HA 6-1 had strong positive reaction in ELISA. These isolates behaved differently from their parental severe strain HA and caused almost no damage to papaya plants that were considered as mutants of PRV HA.

Wang *et al*. (1978) studied on the effectiveness of cross protection by nitrous acid induced mild mutant of *Papaya ringspot virus* for controlling ringspot disease. Under greenhouse condition the mutants (selected as PRV HA 5-1 and 6-1) caused mild or symptomless infection on papaya plants. HA 5-1 and HA 6-1 also proved a high degree of protection in papaya against the severe strain of PRSV. In field condition, when the protected and unprotected plants were established in solid blocks and where the disease pressure inside the orchard was minimized by roughing, protected trees showed a greater suppression of the disease.

Cross protection technique was conducted by Gonsalves (1989) for controlling plant viruses. Among the virus, *Papaya ringspot virus* was successfully controlled in Taiwan. Nitrous acid induced mutants (PRS HA 5-1 and 6-1) were designed for cross protection test. He also developed high pressure spray for mass inoculation of papaya seedling with mild mutants. The mild strains were effective enough against PRV strain from Taiwan to warant field evaluation trials. Gonsalves (1989) also suggested that cross protection alone would not give high level of disease control throughout the entire crop field due to lack of homology between mild and severe strains,

extreme challenge pressure, adverse environmental condition etc. Therefore, integrated cross protection and crop management was desired.

Sheen *et al*. (1999) worked on the mild nitrous-induced mutant (HA 6-1) of *Papaya ringspot virus* (PRSV) in the greenhouse and in the field. In the greenhouse, it caused mild or symptomless infection on test plants as well as on papaya cultivars and showed a high degree of protection against severe PRSV strains from Taiwan. Field trials of cross protection showed that the protected plants delayed the expression of severe symptom by 1-5 months compared with controls.

2.8.3. Resistance Breeding

The development of resistant papaya lines against PRSV-P was proved to be hardly possible (Conover and Litz, 1978). Moreover, many workers conducted experimentation on resistant breeding.

In a breeding program a "Florida" variety (F-1-77-5) crossed with 'Costa Rica Red' resulted a hybrid papaya, 'Tainung No. 5' with a high level of resistance against *Papaya ringspot virus* stated by Lin *et al*. (1990).

Magdalita *et al*. (1997) screened the interspecific hybrids of *Carica papaya* and *C. cauliflora* for resistance to Australian *Papaya ringspot virus* isolates. In glass house and in the field both manually inoculated all the hybrid plants and these were proved resistance to PRSV-P isolates.

Inter- and intra-specific hybridization to obtain *Papaya ringspot virus* resistant papaya plants was carried out by Wang and Hsu (1998) twenty foreign introductions were susceptible. *Carica papaya* varieties Sunrise and Tainung 2, *C. cauliflora, C. pubescens and C. stipulate* were used for interspecific breeding. Twenty promising tolerant lines were selected out of

75 lines from intra-specific crosses. Papaya line 84-19 was selected for releasing as a tolerant line.

Drew *et al*. (1999) developed procedure for hybridization of papaya (*Carica papaya*) with related *Carica* species that were resistant to PRSV (*C. cauliflora, C. quercifolia and C. pubescens*). The hybrid plants grew vigorously in the field with a high level of resistance against PRSV.

2.9. Disease incidence

Singh and Prasad (1990) reported that planting of papaya varieties (viz. Pusa Majesty, Pusa Delicious, Pusa Dwarf etc.) in October showed the superiority over June planting. Besides, a lower incidence of *Papaya ringspot virus* disease and 28% higher average fruit yield were recorded in October planting compared to June planting.

Vallejo (1999) recorded highest papaya yield of 66 kg/plant and was obtained with the application of 366 kg N/ha, showed leaf deficiency of K and the highest percentage of plant with virus compared with other treatments. The virus incidence was not significantly different among treatment with N applications.

2.10. Related research work conducted in Bangladesh

In Bangladesh research activities on papaya viral disease are scanty. Just few works were done on *Papaya ringspot virus*. Akanda (1991) worked on PRSV for the first time through systematical approach to identify the virus. He conducted symptomatological studies, electron microscopy, ultra thin sectioning, mechanical inoculation, host range study and serological test and concluded that the virus is producing mosaic, fern leaf, vein clearing, leaf distortion, shoe-string etc. symptoms on papaya plants.

Akanda *et al*. (1991a) published a paper about PRSV infecting papaya in Bangladesh. PRSV-P has effect on growth contributing character such as plant height, leaf number, petiole length and leaf blade area.

Rahaman (2003) found seven symptoms during investigation about PRSV-P. He showed there are seven symptoms are visible in the infected plants. Maximum number of symptom was observed in BADC, Gazipur.

An experiment was conducted by Akhter (2007) who showed the reduction percentage of growth and yield of the plant due to infection of PRSV-P in different location of Bangladesh.

Mowlick (2004) also conducted an experiment on development of PRSV-P mild strains using mutagenic treatment.

Akhter and Akanda (2008) showed effect of *Papaya ringspot virus*- papaya strain (PRSV-P) on growth and yield on papaya. So far, these are the research on PRSV on papaya conducted in Bangladesh.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted to survey the incidence and severity of *Papaya ringspot virus*-papaya strain (PRSV-P) on the basis of symptoms in the field. The survey was conducted at eight locations of four districts in Bangladesh.

3.1. Selection of the survey area

The survey was conducted at eight locations of four districts in Bangladesh.

3.2. Selection of the locations

There were eight locations of four districts in Bangladesh were selected for survey. In table 1 the locations are given.

Table 1. Selection the locations of the surveyed area

District	Location
Dhaka	Sher-e-Bangla Agriculture University (SAU) Campus, Dhaka
	Jahangirnagar University (JU) Campus, Savar
Gazipur	Bangladesh Agriculture Development Corporation (BADC), Kashimpur
	BSMARU Campus, Salna, Gazipur
Narayanganj	Narayanganj Sadar Upazilla
	Sonargaon Upazilla
Khagrachari	Khagracchari Sadar Upazilla
	Panchari Upazilla

3.3. Survey period

Eight surveys were conducted during the period from 25 May 2016 to 14 December 2016. The first, second, third, fourth, fifth, sixth, seventh and eight surveys were done on 25 May 2016, 5 July 2016, 12 July 2016, 25 July 2016, 19 August 2016, 22 August 2016, 25 October 2016 and 14 December 2016, respectively.

3.4. Observation of the symptoms

Symptom of the disease PRSV-P was identified by visual observation described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008)

3.5. Data collection during survey

During the survey, twelve plants were selected randomly in a location. Each four plant was considered as one replication. From every location three replications was collected. In every district, two locations were selected to collect the data. Following the procedure, twenty-four replications were selected from eight locations in four districts. The data was collected on following parameters:

1. Plant height per plant (cm)
2. Number of leaves per plant (no.)
3. Petiole length of a leaf (cm)
4. Leaf area of a leaf (cm^2)
5. Number of fruits per plant (no.)
6. Weight of fruits per plant (gm)

3.6. Determination of the disease incidence and severity

Every plant in the field was counted to determine the disease incidence and severity. Then it was expressed in percentage.

The disease incidence in plant was calculated following formula (Agrios, 2005)

$$Disease\ incidence\ (\%) = \frac{No.\ of\ diseased\ plant\ (or\ parts)}{No.of\ total\ plant\ (or\ parts)} \times 100$$

The disease severity was expressed in PDI. The PDI was computed by using standard formula (Paper *et al.*, 1996) is giving below:

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

3.7. Quantitative assessment on the effect of PRSV-P on growth and yield contributing parameters of papaya

The effect of PRSV-P infection on growth and yield contributing characters of papaya plants were assessed by collecting the data on six selected parameters below:

i. Plant height (cm.)
ii. Number of leaf
iii. Petiole length (cm.)
iv. Leaf area (cm^2)
v. Fruit number
vi. Fruit weight (gm.)

Percent reduction of growth and yield parameters was calculated using formula:

$$\%\ Reduction = \frac{A - B}{A} \times 100$$

Where,

A= Any parameter of healthy plant

B= That parameter of infected plant

3.8. Statistical analysis

Randomized block design (RCBD) was used to analyze the different parameters through computer software MSTAT-C (Anonymous, 1989). To calculate the level of significant difference and to separate the means within the parameters Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD) test were performed.

CHAPTER IV

RESULTS

4.1. Survey and identification of PRSV-P in papaya at eight locations of four districts

For observing the prevalence of disease incidence and severity of PRSV-P virus in papaya, eight locations of four districts in Bangladesh were selected and surveyed. The PRSV-P disease was identified by symptomology of infected papaya plant and compared with standard literature. The survey results on the symptom of PRSV-P infecting papaya plant are summarized in Table 2-9 and presented in Plate 1-8. It was found that there were six distinct symptoms prevalent at eight locations of four districts in Bangladesh.

The seven different symptoms so far categorized and named as mild mosaic (MM), mosaic (MO), severe mosaic (SM), fern leaf (FL), vein clearing (VC), leaf distortion (LD) and ringspot on fruit. The development of streaking on the young stem and leaf petioles as well as green ring spot developing on leaf was common and consistent with all the six types of symptom. Yellowing of the leaf and distortion of fruits were observed in association with other symptoms.

4.2. Survey and identification of PRSV-P on the basis of field symptom in Dhaka district

In Dhaka, four symptoms were found viz. mosaic (MO) leaf distortion (LD), fern leaf (FL) and mild mosaic (MM). The descriptions of these disease symptoms are given in Table (2, 3) and Plate (1, 2).

4.2.1. Survey of PRSV-P disease in SAU campus

Two typical symptoms were found in SAU campus during survey period. These were mosaic (MO) and leaf distortion (LD) on papaya plant. The types of symptoms are shown in Table and Plate (Table 2 and Plate 1).

Table 2: Survey of PRSV-P disease in SAU campus based on symptom

Symptoms		Virus identification	Remarks
Type	Descriptions	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Mosaic (MO)	Systemic mosaic developed on the leaves scattered, covering the whole leaf blades. Newly developing leaves of the infected plant showed distinct mosaic symptoms. In the advance stage the leaves developed yellowing but mosaic persist. Notable effect on growth of the infected plant is common.		
Leaf distortion (LD)	Leaf distortion appeared in the young growing leaves initially as irregular serration, blistering of the leaves and severe mottling resulting shoe string, curling of	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964),

	the leaf blade and cupping in the same cases. Texture of the leaf formed irregularly and some patches become leathery. Petioles and stems are severely affected with reduced growth.		Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).

(a) (b)

Plate 1: Symptoms of Papaya plant in SAU campus (a) MM (b) LD

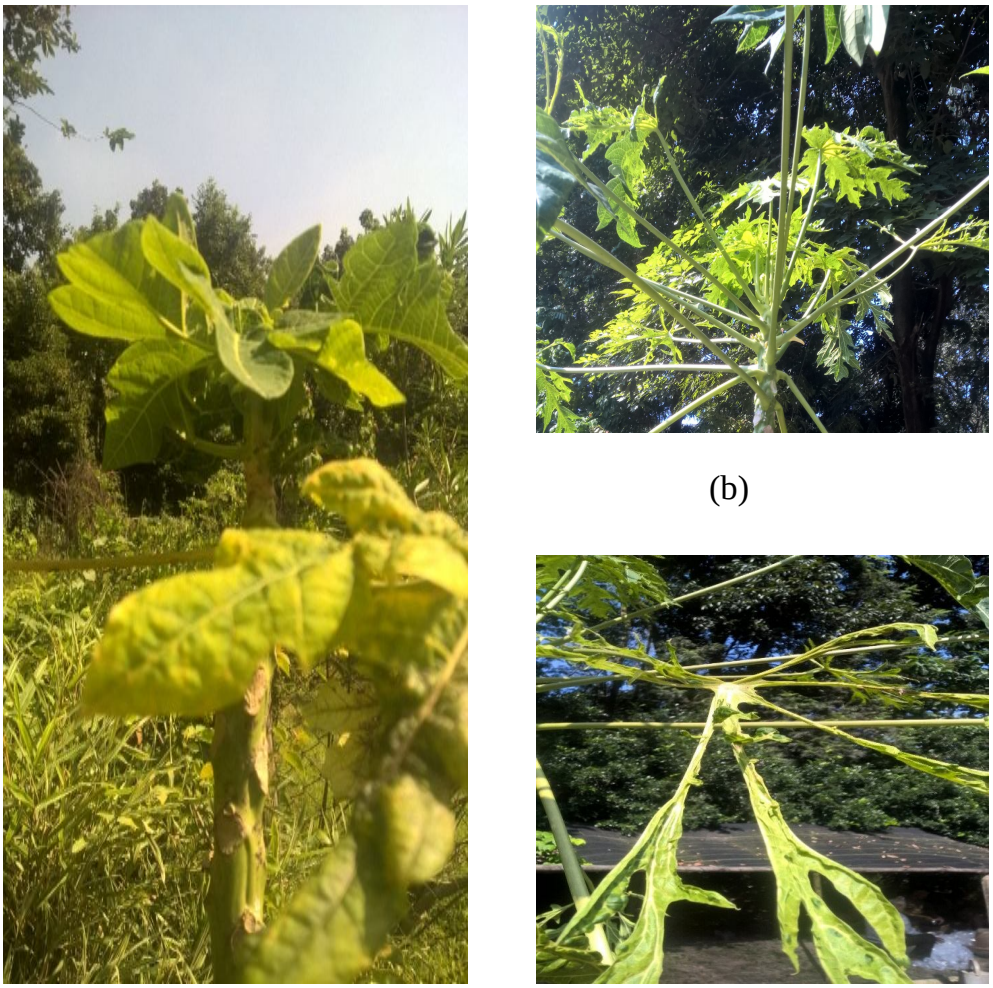
4.2.2. Survey of PRSV-P disease in Jahangirnagar University (JU) campus

In JU campus three distinct symptoms were found in papaya plants namely LD, MM, and FL by observing symptomology. The symptoms of this area are shown in Table 3 and Plate 2.

Table 3: Survey of PRSV-P disease in JU campus based on symptom

Symptoms		Virus identification	Remarks
Type	Descriptions	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Leaf distortion (LD)	Leaf distortion appeared in the young growing leaves initially as irregular serration, blistering of the leaves and severe mottling resulting shoe string, curling of the leaf blade and cupping in the same cases. The plants are badly affected showing growth cessation resulting remarkable stunted plant bearing poor number of flowers and fruits. Petioles and stems are severely affected with reduced growth.		
Mild mosaic (MM)	Initial symptom appeared in the younger leaves as diffuse mosaic and faint yellowing. Mostly	PRSV-P	Symptom that showed by PRSV-P described by

	confined around the whole of the leaf blade. Symptom extended to the whole leaf very rare, under dry and infertile soil condition or 2-3 years aged plant. Progress of symptoms on petioles and stem of the infected plant is very seldom. Green ringspot not seen in the fruits. Plant looks almost healthy without close observation. Stunting and growth reduction are not remarkable.		Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Fern leaf (FL)	Leaf distortion which turns the leaf blade of the infected plants into fern appearance. Mottling and malformation of leaves occur. Fern like growth of the leaf blade is persistent in the leaves. In some plant the symptom frequently accompanies with vein clearing and mild yellowing. Stunting of the plant is quite prominent, reduce number of flower. Fruit distortion in young fruits occurred.	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).



(a) (b) (c)

Plate 2: Symptoms of Papaya plant in JU campus (a) LD (b) MM (c) FL

4.3. Survey and identification of PRSV-P on the basis of field symptom in Gazipur district

Five symptoms viz. MM, MO, SM, LD, FL. were observed in Gazipur district. The descriptions and photographs of symptoms are presented in Table 4 and Table 5 and Plate 3 and 4, respectively.

4.3.1. Survey of PRSV-P disease in BADC complex

Different categories of PRSV-P symptoms were observed in BADC complex. These were VC, FL, MO, SM. The symptoms of four different types are described in Table 4 and Plate 3.

Table 4: Survey of PRSV-P disease in BADC complex based on symptom

Symptoms		Virus identification	Remarks
Type	Descriptions	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Vein clearing (VC)	Symptoms initiate with the clearing of the main veins of the whole leaf, which gradually extends all other veins and interveins. Deformations of the leaves are occasional. The infected plants can be identified by distinct symptoms. Symptom is not prominent on petioles and stems.		
Fern leaf (FL)	Leaf distortion which turns the leaf blade of the infected plants into fern appearance. Mottling and malformation of leaves occur. Fern like growth of the leaf blade is persistent in the	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999),

	leaves. In some plant the symptom frequently accompany with vein clearing and mild yellowing. Stunting of the plant is quite prominent, reduce number of flower.		Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Mosaic (MO)	Systemic mosaic developed on the leaves scattered by covering the whole leaf blades. Newly developing leaves of the infected plant showed distinct mosaic symptoms. In the advanced stage, the leaves developed yellowing but mosaic persists. All the symptoms are not so severe but very much prominent in observation.	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Severe Mosaic (SM)	Mosaic developed with large yellow patches all over the leaf as initial symptoms. The vein clearing restricted near the whorl of the leaf and very rarely progress towards main veins. With the progressed of the	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and

	disease, severe mottling occurred resulting irregular deformation of the leaf. The symptom persisted in the infected plant showing reduced growth. The young fruits developed water soaked spots, which turned into depressed conspicuous ring spots. The malformed fruits of the infected plant loose the growth, vigor and steam girth. The canopy size reduced showing tapering towards apex. Plants were less bearing with reduced fruit size.		Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Leaf distortion (LD)	Leaf distortion appeared in the young growing leaves initially as irregular serration, blistering of the leaves and severe mottling resulting shoe string, curling of the leaf blade and cupping in the same cases. The plants are badly affected showing growth cessation resulting remarkable stunted plant bearing poor number of flowers and fruits.	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).

	Petioles and stems are severely affected with reduced growth.		

(a)

(b)

(c)

(d)

Plate 3: PRSV symptom in papaya in BADC Complex (a) VC (b) FL (c) MO (d) LD

4.3.2. Survey of PRSV-P disease in BSMARU campus

Two distinct symptoms of PRSV-P disease were observed in BSMARU campus. These were mild mosaic (MM) and leaf distortion (LD). The symptoms of two different types are described in Table 5 Plate 4.

Table 5: Survey of PRSV-P disease in BSMARU campus based on symptom

Symptoms		Virus identification	Remarks
Type	Descriptions	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Mild mosaic (MM)	Initial symptom appeared in the younger leaves as diffuse mosaic and faint yellowing. Mostly confined around the whorl of the leaf blade. Symptom extended to the whole leaf very rare, under dry and infertile soil condition or 2-3 years aged plant. Progress of symptoms on petioles and stem of the infected plant is rare. Green ringspot not seen in the fruits. Plant looks almost healthy without close observation. Stunting and growth reduction are not remarkable.		
Leaf distortion (LD)	Leaf distortion appeared in the young growing leaves initially as irregular serration, blistering of the leaves and	PRSV-P	Symptom that showed by PRSV-P described by Jensen

	severe mottling resulting shoe string, curling of the leaf blade and cupping in the same cases. Texture of the leaf formed irregularly and some patches become leathery. Petioles and stems are severely affected with reduced growth. The plants are badly affected showing growth cessation resulting remarkable stunted plant bearing poor number of flowers and fruits.		(1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003) Akhter and Akanda (2008).

(a)

(b)

Plate 4: PRSV symptom in papaya at BSMARU complex (a) MM (b) LD

4.4. Survey and identification of PRSV-P on the basis of field symptom in Narayanganj district

Four different types of symptoms viz. MO, SM, LD, VC, and FL were observed in Narayanganj district.

4.4.1. Survey of PRSV-P disease in Narayanganj Sadar

In Table 6 and Plate 5, the symptoms that found in Narayanganj sadar Upazilla are described. Mild mosaic (MM), vein clearing (VC) and fern leaf (FL) were found in this location.

Table 6: Survey of PRSV-P disease in Narayanganj Sadar based on symptom

Symptoms		Virus identification	Remarks
Type	Descriptions	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Mild mosaic (MM)	Initial symptom appeared in the younger leaves as diffuse mosaic and faint yellowing. Mostly confined around the whorl of the leaf blade. Symptom extended to the whole leaf very rare, under dry and infertile soil condition or 2-3 aged plant. Progress of symptoms on petioles and stem of the infected plant is very seldom. Green ringspot not seen in the fruits. Plant looks almost healthy without close observation. Stunting and growth		

	reduction are not remarkable.		
Vein clearing (VC)	Symptoms initiate with the clearing of the main veins of the whole leaf, which gradually extends all other veins and interveins. Deformations of the leaves are occasional. The infected plants can be identified by distinct symptom. Symptom is not prominent on petiole s and stems.	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Fern leaf (FL)	Leaf distortion which turns the leaf blade of the infected plants into fern appearance. Mottling and malformation of leaves occur. Fern like growth of the leaf blade is persistent in the leaves. In some plant the symptom frequently accompany with vein clearing and mild yellowing. Stunting of the plant is quite prominent, reduce number of flower. Fruit distortion in young fruits occurred. Stunting of the plant is quite prominent.	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).

(a)

(b)

(c)

Plate 5: The PRSV symptom in papaya plant in Narayanganj Sadar Upazilla (a) MM (b) VC (c) FL

4.4.2. Survey of PRSV-P disease in Sonargaon

Two types viz. Leaf distortion (LD), and fern leaf (FL) symptoms were found in Sonargaon upazilla under Narayangonj district. The results of symptomology of PRSV-P are shown in Table 7 and Plate 6.

Table 7: Survey of PRSV-P disease in Sonargaon based on symptom

Symptoms		Virus identification	Remarks
Type	Descriptions	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Fern leaf (FL)	Leaf distortion which turns the leaf blade of the infected plants into fern appearance. Mottling and malformation of leaves occur. Fern like growth of the leaf blade is persistent in the leaves. In some plant the symptom frequently accompanies with vein clearing and mild yellowing. Stunting of the plant is quite prominent, reduce number of flower. Fruit distortion in young fruits occurred. Stunting of the plant is quite prominent.		
Leaf distortion	Leaf distortion appeared in the young growing leaves initially as irregular	PRSV-P	Symptom that showed by PRSV-P

(LD)	serration, blistering of the leaves and severe mottling resulting shoe string, curling of the leaf blade and cupping in the same cases. The plants are badly affected showing growth cessation resulting remarkable stunted plant bearing poor number of flowers and fruits. Petioles and stems are severely affected with reduced growth.		described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Severe mosaic (SM)	Mosaic developed with large yellow patches all over the leaf as initial symptoms. The vein clearing restricted near the whorl of the leaf and very rarely progress towards main veins. With the progressed of the disease, severe mottling occurred resulting irregular deformation of the leaf. The symptom persisted in the infected plant showing reduced growth. The young fruits developed water soaked spots, which turned into depressed conspicuous ring spots. The malformed fruits of the infected plant loose the growth, vigor and steam girth. The canopy size reduced	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).

	showing tapering towards apex. Plants were less bearing with reduced fruit size.		

(a)

(b)

(c)

Plate 6: PRSV symptom in papaya plant in Sonargaon Upazilla (a) FL (b) LD (c) SM

4.5. Survey and identification of PRSV-P on the basis of field symptom in Khagrachari hill tract

In Khagrachari hill tract area four symptoms were observed and they were ringspot on fruit, MM, LD, FL and MO. Table (8, 9) and Plate (7, 8) described the symptoms which were observed in Khagrachari Sadar and Panchari, respectively.

4.5.1. Survey of PRSV-P disease in Khagrachari Sadar

In Table 8 and Plate 7 the description of symptoms are shown for Khagrachari Sadar. Fern leaf (FL), leaf distortion (LD), mild mosaic (MM), mosaic (MO) and ringspot on fruit was found in this location.

Table 8: Survey of PRSV-P disease in Khagrachari Sadar based on symptom

Symptoms		Virus identification	Remarks
Type	Descriptions	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and
Mosaic (MO)	Systemic mosaic developed on the leaves scattered by covering the whole leaf blades. Newly developing leaves of the infected plant showed distinct mosaic symptoms. In the advanced stage the leaves developed yellowing symptom but mosaic persist. All the symptoms are not so severe but very much prominent in		

	observation. Notable effect on growth of the infected plant is common.		Akanda (2008).
Leaf distortion (LD)	Leaf distortion appeared in the young growing leaves initially as irregular serration, blistering of the leaves and severe mottling resulting shoe string, curling of the leaf blade and cupping in the same cases. Texture of the leaf formed irregularly and some patches become leathery. Petioles and stems are severely affected with reduced growth. The plants are badly affected showing growth cessation resulting remarkable stunted plant bearing poor number of flowers and fruits.	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Mild mosaic (MM)	Initial symptom appeared in the younger leaves as diffuse mosaic and faint yellowing. Mostly confined around the whorl of the leaf blade. Symptom extended to the whole leaf very rare, under dry and infertile soil condition or 2-3 aged plant. Progress of symptoms on petioles and stem of the infected plant is rare. Green ringspot not seen in the fruits.	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and

	Plant looks almost healthy without close observation. Stunting and growth reduction are not remarkable.		Akanda (2008).
Fern leaf (FL)	Leaf distortion which turns the leaf blade of the infected plants into fern appearance. Mottling and malformation of leaves occur. Fern like growth of the leaf blade is persistent in the leaves. In some plant the symptom frequently accompanies with vein clearing and mild yellowing. Fruit distortion in young fruits occurred. Stunting of the plant is quite prominent, reduce number of flower.	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Ringspot on fruit	Green and dark green circular ring on the fruit is the typical symptom of this disease. Several green circular rings is found on the fruit.	PRSV-P	Conover (1964), Jensen (1949a).

Plate 7: PRSV symptom in papaya in Khagrachari Sadar (a) MO (b) LD (c) MM (d) Ringspot on fruit.

4.5.2. Survey of PRSV-P disease in Panchari

Fern leaf (FL), mild mosaic (MM) and mosaic (MO) symptoms were found in Panchari upazilla. These described in Table 9 and Plate 8.

Table 9: Survey of PRSV-P disease in Panchari based on following symptoms

Symptoms		Virus identification	Remarks
Type	Descriptions	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Fern leaf (FL)	Leaf distortion which turns the leaf blade of the infected plants into fern appearance. Mottling and malformation of leaves occur. Fern like growth of the leaf blade is persistent in the leaves. In some plant the symptom frequently accompany with vein clearing and mild yellowing. Fruit distortion in young fruits occurred. Stunting of the plant is quite prominent, reduce number of flower.		
Mild mosaic (MM)	Initial symptom appeared in the younger leaves as diffuse mosaic and faint yellowing. Mostly confined around the whorl of the leaf blade. Symptom extended to the whole leaf very rare,	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves

	under dry and infertile soil condition or 2-3 aged plant. Progress of symptoms on petioles and stem of the infected plant is very seldom. Green ringspot not seen in the fruits. Plant looks almost healthy without close observation. Stunting and growth reduction are not remarkable.		*et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).
Mosaic (MO)	Systemic mosaic developed on the leaves scattered by covering the whole leaf blades. Newly developing leaves of the infected plant showed distinct mosaic symptoms. In the advanced stage the leaves developed yellowing but mosaic persist. All the symptoms are not so severe but very much prominent in observation. Notable effect on growth of the infected plant is common.	PRSV-P	Symptom that showed by PRSV-P described by Jensen (1949a), Conover (1964), Gonsalves *et al.* (1999), Akanda (1991) and Rezende *et al.* (1995), Rahaman (2003), Akhter and Akanda (2008).

(a)

(b)

(c)

Plate 8: PRSV symptom in papaya in Panchari Upazilla (a) LD (b) MM (c) MO

4.6. Incidence and severity at eight locations of four districts in Bangladesh

Significant difference were found in disease incidence at eight locations in Bangladesh. The disease incidence and severity of PRSV-P in eight locations of four districts are shown in table 10. The incidence ranged from 12.04% to 36.24% and the severity ranged from 2.50% to 11.53%.

4.6.1. Disease incidence

The highest incidence (36.24%) was found in BSMARU, Gazipur and the lowest incidence (12.04%) was found in Panchari, Khagrachari hill tract.

4.6.2. Disease severity

Significant differences were found in disease severity among eight locations in Bangladesh. Highest disease severity (11.53%) was in BSMARU campus followed by SAU (9.03%), where the lowest disease severity (2.50%) was found in Panchari upazilla preceded by Narayanganj Sadar (3.44%).

Table 10. The disease incidence and severity of PRSV-P in papaya at eight locations of four districts in Bangladesh

Locations		Disease Incidence (%)	Disease Severity (%)
Dhaka	SAU, Dhaka	29.84 b*	9.03 b
	JU, Savar	30.05 b	7.49 bc
Gazipur	BADC, Kashimpur	17.40 c	3.05 e
	BSMARU, Salna	36.24 a	11.53 a
Narayanganj	Narayanganj Sadar	17.60 c	3.44 e
	Sonargaon, Upazilla	30.17 b	6.25 cd
Khagrachari hill tract	Khagrachari Sadar	17.72 c	4.16 de
	Panchari, Upazilla	12.04 d	2.50 e
Coefficient of Variation (CV%)		13.64	17.88
LSD value (0.05)		5.216	2.166

*Values within the same column with a common letter(s) do not differ significantly (P=0.05).

4.7. Comparison of incidence and severity of PRSV-P at eight locations of four districts in Bangladesh

The comparison of incidence and severity of different locations is shown in figure 1. From the figure, the highest incidence and severity was found in BSMARU and the lowest result in Panchari upazilla.

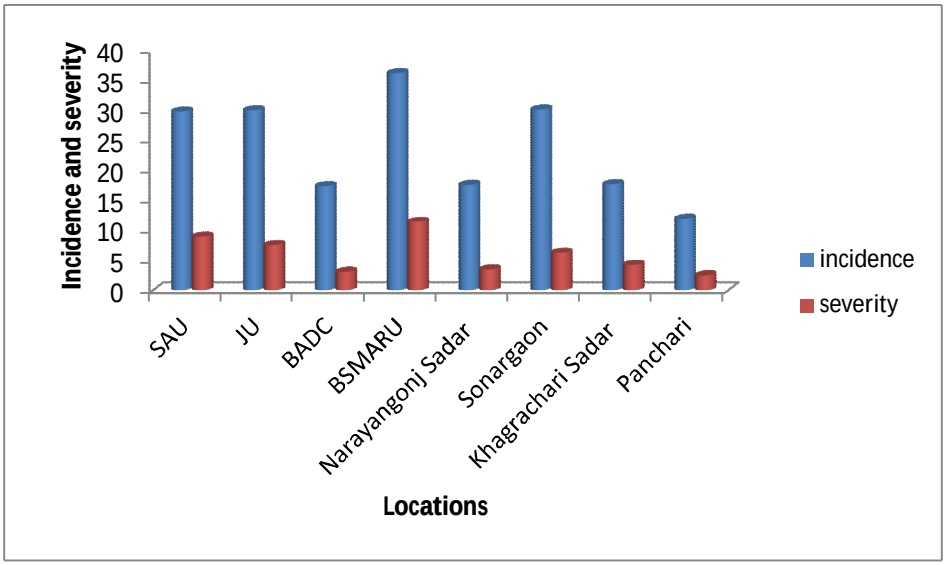


Fig 1: Comparison of incidence and severity of PRSV-P at eight locations of four districts in Bangladesh

4.8. Effect of PRSV-P on growth and growth parameters in papaya

The effect of growth and growth parameters due to PRSV-P infection in papaya are shown in Table 11.

4.8.1. Plant height

The value of plant height differs significantly are shown in Table 1. The highest height (178.6 cm) in SAU campus followed by Panchari upazilla (159.6 cm) while the lowest (127.5 cm) found in Sonargaon upazilla preceded by (146.0 cm) at BADC complex.

4.8.2. No. of leaf

Maximum number of leaf (20.67) was found in Sonargaon upazilla followed by SAU campus (19.08) while minimum was found in BSMARU (15.67).

4.8.3. Petiole length

Among eight locations, the highest petiole length (53.66 cm) was found in Khagrachari sadar followed by SAU campus (47.95 cm). On the other hand, lowest petiole length was found in BSMARU campus (30.90 cm) that was significantly similar to Narayanganj Sadar.

4.8.4. Leaf area

The highest leaf area was found in Panchari upazilla (1330 cm^2) where the lowest leaf area was found in BSMARU (535.1 cm^2).

Table 11. Effect of PRSV-P on growth parameters at eight locations of four districts in Bangladesh

Location		Plant Growth parameters			
		Plant height (cm)	No. of leaf (no.)	Petiole length (cm)	Leaf area (cm^2)
Dhaka	SAU	178.6 a*	19.08 b	47.95 b	1167 c
	JU	146.9 de	16.08 de	35.39 cd	959.9 d
Gazipur	BADC	146.0 e	16.67 cde	31.86 de	857.4 g
	BSMARU	150.5 cde	15.67 e	30.90 e	535.1 h
Narayanganj	Narayanganj Sadar	153.1 bcd	17.60 c	30.95 e	887.4 f
	Sonargaon Upazilla	127.5 f	20.67 a	36.62 c	954.9 e
Khagrachari hill tract	Khagrachari Sadar	155.8 bc	18.58 b	53.66 a	1186 b
	Panchari upazilla	159.6 b	17.08 cd	34.93 cd	1330 a
Coefficient of Variation (CV %)		7.52	9.82	16.93	21.19
LSD value (0.05)		6.314	0.9750	3.543	3.234

*Values within the same column with a common letter(s) do not differ significantly (P=0.05).

4.9. Growth and growth parameters of papaya plant due to PRSV-P infection

Effect on growth and growth parameters due to PRSV-P infection are shown in fig- 2,3,4,5 based on different locations.

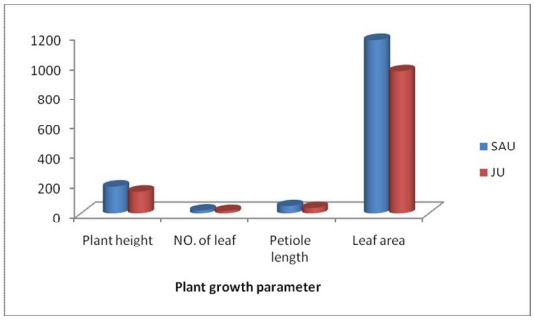


Fig 2: Effect of growth and growth parameters due to PRSV-P disease in two locations of Dhaka district

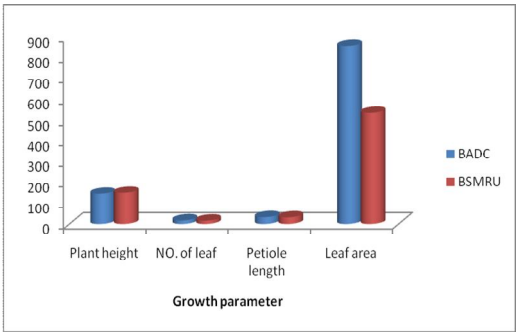


Fig 3: Effect of growth and growth parameters due to PRSV-P disease in two locations of Gazipur district

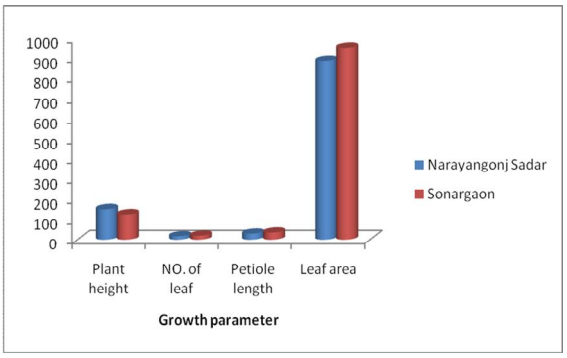


Fig 4: Effect of growth and growth parameters due to PRSV-P disease in two locations of Narayanganj district

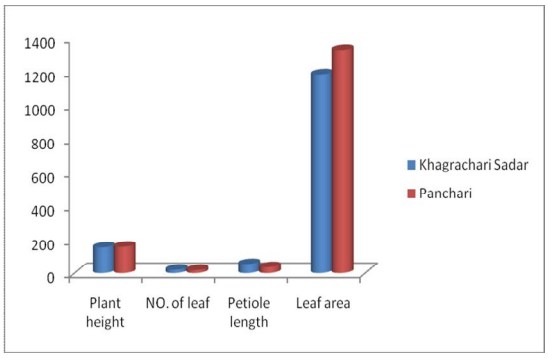


Fig 5: Effect of growth and growth parameters due to PRSV-P disease in two locations of Khagrachari hill tract.

4.10. Effect of PRSV-P on yield parameters at different locations of four districts in Bangladesh

Yield and yield parameters at eight locations of four districts in Bangladesh were varied significantly due to PRSV-P infection in papaya. The results are shown in Table 12. In case of fruit number, the range of fruit number was 6.167-4.583. The maximum number (6.167) was found in BSMARU and the lowest number (4.583) was found in BADC and Panchari which were statistically similar with Sonargaon (4.917). The fruit weight came to the highest (643.6gm) was found in SAU campus and the lowest (324.3gm) was found in BSMARU. The range of fruit weight was 643.6-324.3.

Table 12: Effect of yield contributing character at eight locations of four districts in Bangladesh due to PRSV-P infection

Location		Fruit number per plant	Fruit weight per fruit
Dhaka	SAU, Dhaka	5.500 b*	643.6 a
	JU, Savar	5.000 bc	527.7 cd
Gazipur	BADC, Kashimpur	4.583 c	567.8 bc
	BSMARU, Salna	6.167 a	324.3 e
Narayanganj	Narayanganj Sadar	5.517 b	515.5 cd
	Sonargaon Upazilla	4.917 c	491.2 d
Khagrachari hill tract	Khagrachari Sadar	5.083 bc	504.6 d
	Panchari Upazilla	4.583 c	584.5 b
Coefficient of Variation (CV %)		8.12	18.11
LSD value (0.05)		0.4859	51.56

*Values within the same column with a common letter(s) do not differ significantly (P=0.05).

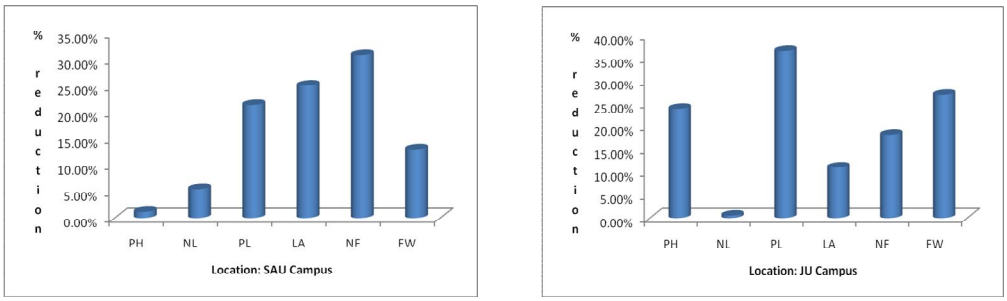


Fig 5: Percent reduction of growth and yield parameters in Dhaka district

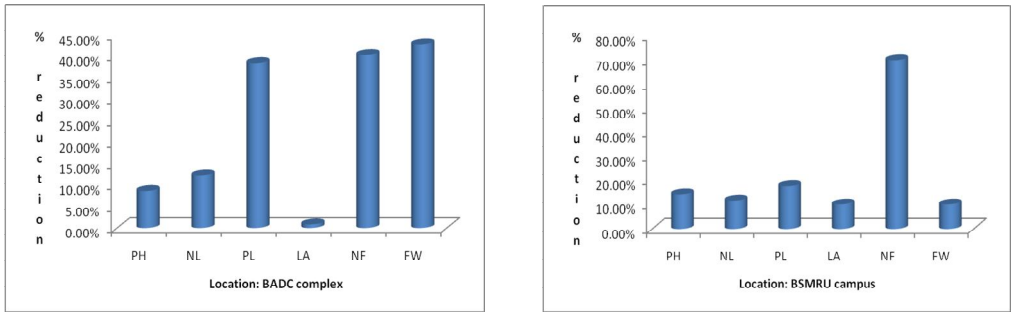


Fig 6: Percent reduction of growth and yield parameters in Gazipur district

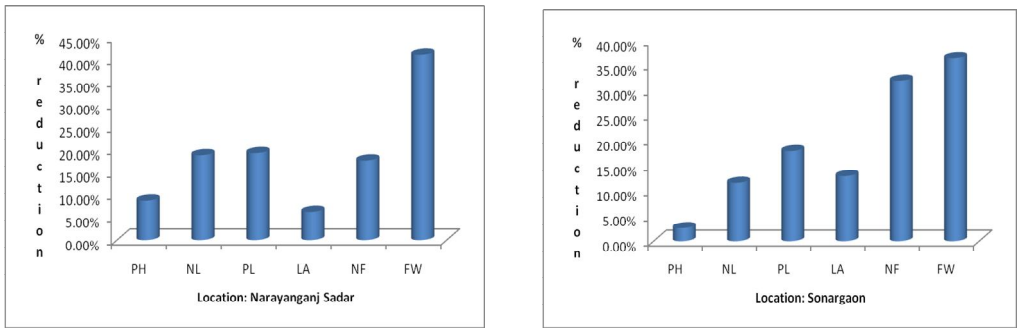


Fig 7: Percent reduction of growth and yield parameters in Narayanganj district

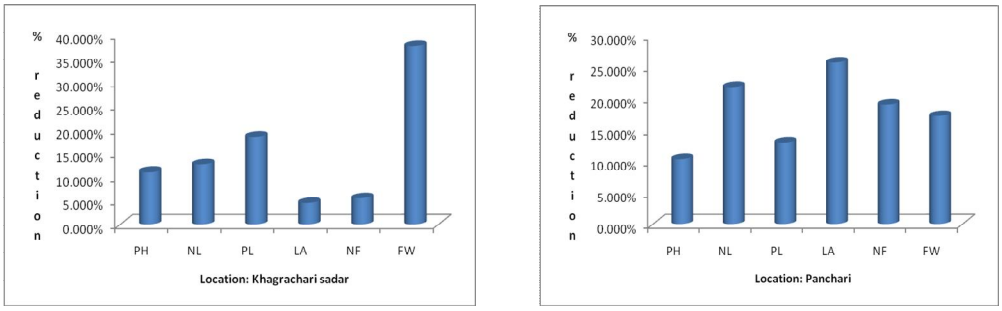


Fig 8: Percent reduction of growth and yield parameters in Khagrachari hill tract

4.11. Percent reduction in growth and yield parameters of papaya due to PRSV-P disease infection at different location of four districts

The percent reduction of growth and yield parameters are shown in Table 13. The Minimum plant height reduced (1.17%) in SAU and the maximum (23.85%) in JU. In case of leaf number, the lowest reduction (5.5%) was found in SAU and the highest reduction (21.9%) was found in Panchari. In Panchari the lowest reduction (13.04%) of petiole length was found while the highest reduction (38.46%) was found in BADC. Fruit number reduced the highest (70.33%) in BSMARU and the lowest (5.46%) in Khagrachari. But in case of fruit weight, maximum reduction (42.93%) was found in BADC while the lowest reduction (12.90%) was in SAU.

Table 13: Percent reduction of growth and yield contributing characters of papaya due to PRSV-P infection

Locations		%RPH	%RNL*	%RPL	%RLA	%RNF	%RFW
Dhaka	SAU	1.17	5.5	21.52	25.19	31.03	12.90
	JU	23.85	5.63	36.71	11.02	18.29	26.86
Gazipur	BADC	8.59	12.35	38.46	0.88	40.5	42.93
	BSMARU	14.13	11.54	17.50	9.87	70.33	9.87
Narayanganj	Narayanganj Sadar	8.67	18.82	19.29	6.23	17.64	41.34
	Sonargaon	2.56	11.62	17.94	12.97	32	36.61
Khagrachari hill tract	Khagrachari Sadar	10.97	12.59	18.44	4.43	5.46	37.62
	Panchari	10.37	21.9	13.04	25.69	19.05	17.18

*****RPH= Reduction of plant height, RNL= Reduction of number of leaf, RPL= Reduction of petiole length, RLA= Reduction of leaf area, RNF= Reduction of number of fruit, RFW= Reduction of fruit weight.

CHAPTER V

DISCUSSIONS

Papaya (*Carica papaya* L.) belongs to the family Caricaceae grown in Australia, Hawaii, Philippines, Sri Lanka, South Africa, India, Bangladesh, Malaysia and other countries in tropical America (Anuara *et al*. 2008). In 2009-2010, papaya has been cultivated in 2790 ha of land with a total production of 40.42 tons/ha (BBS, 2009-2010) in contrast to about 11352202 tons' production of papaya in 2010 in the world (FAOSTAT Database, FAO, 2010). The papaya fruit is very rich in vitamin A, which refers to the second highest among of fruits and comes next to mango (Aykroyed, 1951).

There were several diseases causing damage is papaya cultivation. Some are fungal diseases some bacterial and viral also. The diseases were very harmful for commercial cultivation. Among the viral diseases PRSV-P causes severe damage in growth and yield. Linder (1945) has published report about PRSV and brought it in literature for the first time. An investigation was done by Jensen (1949) about the virus infecting papaya in Hawaii and named the virus as *Papaya ring spot virus* (PRSV).

In the present survey, eight locations of four districts in Bangladesh were selected to observe the disease incidence and severity of PRSV-P. Among eight locations six different types of symptoms viz. mild mosaic (MM), mosaic (MO), severe mosaic (SM), leaf distortion (SM), fern leaf (FL) and vein clearing (VC) were found which may caused by PRSV-P based on symptomology. In the present survey highest (36.24%) incidence was found in BSMARU, in Gazipur whereas, the lowest value found in Panchari

(12.04%), Khagrachari. The maximum severity was recorded in BSMARU (11.53%) while minimum severity was recorded in Panchari (2.50%). Similar survey was done by Akhter (2007). He showed the reduction percentage of growth and yield of the plant due to infection of PRSV-P in different locations in Bangladesh. Seedling showed prominent vein clearing and downward cupping of the young leaves stated by Conover (1964). Gonzalvez *et al.* (1997) established that PRSV caused three types of leaf symptom on papaya which are mosaic, yellowing and deformation. Rahaman (2003) also found similar symptom during investigation about PRSV-P in Kashimpur farm, BSMRAU farm and nineteen other districts. He showed there are seven symptoms visible in the infected plants. Among them, maximum number of symptom was observed in BADC, Gazipur.

Meixico Castro *et al*. (2015) had done similar type of survey to study incidence and severity in the state of Guerrero and found similar results.

Viral disease is recognized as a major limiting factor for economic papaya production throughout the tropics and subtropics (Jensen 1949a and b, Cook and Zettler, 1970). During the survey, it was recorded that PRSV-P has a great influence in growth and yield parameters. PRSV-P also affected on growth contributing character such as plant height, leaf number, petiole length and leaf area too. Similar experiment was conducted by Akhter, (2007). He had noted down the reduction of seedling height due to inoculation of seven symptoms of four locations. Similar experiment was done by Akhter and Akanda (2008). They showed remarkable effect of *Papaya ringspot virus*- papaya strain (PRSV-P) on growth and yield on papaya.

The yield loss due to papaya viral disease infection may reach even up to 100% very often. In this survey, BSMRAU was recorded for maximum fruit number/plant (6.167). On the other hand, the minimum fruit number 4.583 was calculated in BADC and Panchari respectively. On the other hand, maximum fruit weight per fruit was found at SAU (643.6 gm) and minimum was found at BSMARU (324.3gm). There was similarity found by Akanda (1991) and showed that the virus causes 70%-100% yield reduction of papaya depending upon the stage of infection as estimated in Bangladesh.

During the survey, minimum plant height reduction was at SAU (1.17%) and the maximum at JU (23.85%). In case of leaf number, the lowest number was found at SAU (5.5%) and highest number found at Panchari (21.9%). In Panchari (13.04%) the lowest value of petiole length has found and the highest was at BADC (38.46%). Leaf number reduction was the highest (21.9%) at Panchari and the lowest value (5.5%) was found in SAU campus. Rahman (2003) also showed similar reduction of different characters of papaya due to PRSV-P infection. He described different parameters like plant height, flower per plant, fruit per plant fruit weight and fruit yield. In every parameter there was remarkable reduction caused by PRSV-P infection.

CHAPTER VI

SUMMARY & CONCLUSIONS

Papaya (*Carica papaya* L.) is belong to the family of Caricaceae is a very important fruit all over the world. Though its demand is increasing day by day the production of papaya is not satisfactory. Lack of proper knowledge of papaya production and management of its disease is the main reason of this situation. Papaya is vulnerable to the disease especially viral disease but very scanty information regarding its distribution, incidence, severity and management are available. Therefore, the present experiment was designed to study the surveillance and identification of PRSV-P disease based on symptomology and to observe the disease incidence and severity of PRSV-P at eight locations of four districts in Bangladesh.

There were six symptoms were found such as mild mosaic, mosaic, severe mosaic, leaf distortion, fern leaf and vein clearing during the survey. Among them maximum number of symptoms were found at BADC complex.

In survey period, among eight locations highest incidence was found at BSMARU (36.24%) and lowest at Panchari (12.04%). On other hand, in case of severity, maximum severity was found at BSMARU (11.53%) and minimum was recorded at Panchari (2.50%), BADC (3.057%) and Narayanganj Sadar (3.443%).

In selected locations, the growth contributing characters also recorded. Plant height, leaf number, petiole length and leaf blade area were recorded. Maximum plant height was found at SAU campus (178.6cm) and minimum at Sonargaon (127.5cm). In case of leaf number, the highest was at Sonargaon (20.67) and lowest (15.67) at BSMRAU campus. Petiole length was highest (53.66cm) at Khagrachari Sadar and the lowest (30.90cm) was at BSMARU.

The PRSV-P is economically very important disease and can cause yield loss from 70% to 100%. The highest number of fruit was harvested from BSMRAU campus (6.167) as yield contributing characters and the lowest number was found at JU and Panchari (4.583). But in case of fruit weight maximum was found at SAU (643.6gm) and minimum was recorded at BSMARU (324.3gm).

Maximum plant height (23.85%) reduction was found at JU while minimum (1.17%) was at SAU. In case of leaf number, the highest reduction (21.9) was found in Panchari and lowest (5.5%) was recorded in SAU campus. Petiole length reduction was highest (38.46) at BADC and at low (13.04%) at Panchari had. Leaf area reduction was highest (25.69%) at Panchari while BADC had the lowest (0.88) position. Fruit number reduction was the lowest (5.46%) at Khagrachari and the highest value (70.33%) was at BSMRU. Reduction of fruit weight was low (9.87%) at BSMARU campus and BADC got the highest value (42.93%).

From the findings of the study it may be concluded that the symptoms, disease incidence and severity and its effects on growth, yield and yield contributing characters varied significantly in surveyed areas by the infection of PRSV-P in papayas the lowest incidence and severity was found at Panchari (Hill tract area). The farmer may be suggested to cultivate papaya in that region. However, further study need to be continued to have information prissily.

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APPENDICES

Appendices 1. Weather report of Bangladesh during the survey was conducted.

High & Low Weather Summary for May 2016

	Temperature	Humidity	Pressure
High	52 °C (3 May, 12:00)	97% (12 May, 21:00)	1012 mbar (12 May, 21:00)
Low	22 °C (3 May, 06:00)	19% (3 May, 12:00)	995 mbar (21 May, 15:00)
Average	29 °C	74%	1005 mbar

* Reported 1 May 06:00 — 29 May 09:00, Dhaka. Weather by CustomWeather, © 2017

High & Low Weather Summary for July 2016

	Temperature	Humidity	Pressure
High	34 °C (31 Jul, 15:00)	100% (3 Jul, 06:00)	1010 mbar (3 Jul, 06:00)
Low	20 °C (13 Jul, 12:00)	55% (31 Jul, 15:00)	996 mbar (7 Jul, 18:00)
Average	29 °C	81%	1002 mbar

* Reported 1 Jul 09:00 — 31 Jul 21:00, Dhaka. Weather by CustomWeather, © 2017

High & Low Weather Summary for August 2016

	Temperature	Humidity	Pressure
High	35 °C (25 Aug, 15:00)	97% (10 Aug, 06:00)	1010 mbar (10 Aug, 06:00)
Low	25 °C (22 Aug, 06:00)	53% (30 Aug, 15:00)	993 mbar (16 Aug, 18:00)
Average	30 °C	77%	1001 mbar

* Reported 1 Aug 06:00 — 31 Aug 18:00, Dhaka. Weather by CustomWeather, © 2017

High & Low Weather Summary for October 2016

	Temperature	Humidity	Pressure
High	36 °C (20 Oct, 15:00)	100% (8 Oct, 06:00)	1014 mbar (8 Oct, 06:00)
Low	24 °C (25 Oct, 06:00)	29% (20 Oct, 15:00)	1002 mbar (4 Oct, 18:00)
Average	29 °C	73%	1007 mbar

* Reported 1 Oct 00:00 — 31 Oct 21:00, Dhaka. Weather by CustomWeather, © 2017

High & Low Weather Summary for December 2016

	Temperature	Humidity	Pressure
High	30 °C (21 Dec, 09:00)	97% (30 Dec, 06:00)	1017 mbar (30 Dec, 06:00)
Low	16 °C (8 Dec, 06:00)	34% (8 Dec, 12:00)	1009 mbar (11 Dec, 18:00)
Average	23 °C	68%	1013 mbar

* Reported 1 Dec 00:00 — 31 Dec 18:00, Dhaka. Weather by CustomWeather, © 2017