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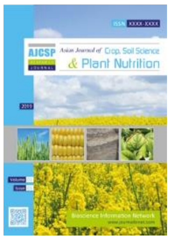

Effect of sowing time on disease incidence and severity of *Cucumber mosaic virus* (CMV) on pumpkin

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ABSTRACT

A field experiment was conducted at the experimental farm of Sher-e-Bangla Agricultural University, Dhaka, during the period from November 2016 to March 2017 to evaluate the effect of sowing time on disease incidence and severity of Cucumber mosaic virus (CMV) in selected four pumpkin genotypes under the Modhupur Tract (AEZ-28). The genotypes used in the experiment were collected from four districts of Bangladesh and these districts are Narayanganj (L_1), Narshingdi (L_2), Dhaka (L_3), and Gazipur (L_4). The experiment was set up as an RCBD with three replications in the main plot. Between the two sowing times, the first sowing (25th October) reported the lowest disease incidence (%) and severity (%) than the second sowing (5th November). In both sowing times, the genotype from Narshingdi (L_2), had a greater percentage of disease incidence (%) and severity (%) than the genotype from Dhaka (L_3). Growth parameters and yield and yield contributing characters showed significant variation in both sowings. The higher yield (kg/ha) was found in the first sowing than in the second sowing. The relationship analysis revealed a negative correlation between yield, disease incidence (%), and severity (%). Considering all measurement variables, managing the sowing time may be suggested to have a higher yield and prevent disease.

Key Words: *Evaluation, Viral disease, Growth parameters, Negative correlation and Yield contributing parameters.*

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I. Introduction

Pumpkin (*Cucurbita moschata*) belongs to the family Cucurbitaceae. It is locally known as 'Misti kumra' or 'Misti lau' or 'Misty kadu'. It is considered to have originated from Central and North America (Whitaker and Davis, 1962) and distributed widely such as Southeast Asia, Tropical Africa, Central America (Peru and Mexico), the Caribbean and most parts of the tropics. It is widely cultivated in many countries, like India, China, Malaysia, Taiwan, and Bangladesh. The young leaves, male flowers and mature or immature fruit of pumpkin are used as vegetable and cattle feed (Shanmugavelu, 1989). Moreover, it has the highest storability among all vegetables; well-matured fruits can be stored for 2-4 months under ambient conditions (Yawalkar, 1985).

Viral diseases can cause 60-70% economic losses in crops (Nagendran et al. 2017). The prominent and destructive widely reported virus of vegetables is *Cucumber mosaic virus* (CMV), which belongs to the genus Cucumovirus and the family Bromoviridae (Lovisolo 1981; Roossinck 2002; Jacquemond, 2012; Ayo-John et al., 2014). It causes huge yield losses in cucurbit crops (Akbar et al., 2015). In the southern part of Bangladesh, the incidence of *CMV* was 24.0% (Saifullah et al., 2003). It can be transmitted by at least 60 species of aphids in a nonpersistent manner (Kennedy et al., 1962; Krenz et al., 2015; Nalam et al., 2019; Palukaitis and García-Arenal, 2019). CMV has a very wide host range of over 1,200 plant species and exhibits various symptoms with varying degrees of severity (Hull, 2013; Palukaitis and García-Arenal, 2019). This viral infection usually causes systematic mosaic symptoms of light and dark green patterns in leaves, which can result in plant stunting and fruit deformity (Zitikaite et al., 2011).

Genetic resistance is the most effective management strategy for this virus, as it has a wide host range of insect vectors and cannot be stopped by pesticides. (Lin CY et al., 2012). However, due to the incompatibility of the many cucurbit species, breeding for host resistance is challenging (Zitter and Murphy, 2009). Therefore, evaluating other cultural methods to control viral diseases such as the sowing time could help develop alternative strategies. In tropical regions, it is well recognized that the planting date is crucial (Hull, 2013). Changing the sowing time reduced virus infection in okra, mung beans and tomatoes (Muqit et al., 1999). Similar measures also successfully avoided tungro virus disease in rice (Manwan, 1987) and viruses in groundnuts in African countries (Hull, 2009). Considering the above circumstances, the research experiment was conducted to evaluate the effect of sowing time on CMV disease incidence and severity of pumpkin under field conditions and to detect the effect of sowing time against CMV disease among collected farmers saved pumpkin seed.

II. Materials and Methods

Experimental location and research period

The field experiment was carried out at Sher-e-Bangla Agricultural University (SAU) central farm under the Department of Plant Pathology, Dhaka-1207 from November'2016 to March'2017.

Experimental soil characteristics and climate condition

The location of the experimental site was at 23◦46′ N latitude and 90◦24′ longitude with the elevation of 9 meters above the sea level belonging to the Modhupur tract under the Agro Ecological Zone (AEZ) 28. The soil texture was silty loam, non-calcareous, dark grey soil of Tejgaon soil series with a pH 6.7. There was very low or no rainfall during December and January. The average highest temperature during the period of investigation was 29.88 °C and the lowest temperature was 13.64 °C.

Seed collection

Seed samples were collected from farmers saved seed from four districts of Dhaka division. These locations are namely – Narayanganj (Rupganj), Narshingdi (Belabo), Dhaka (Savar) and Gazipur (Kashimpur). Collected seeds are named as – (I) L_1 = Narayanganj (Rupganj), (II) L_2 = Narshingdi (Belabo), (III) L_3 = Dhaka (Savar) and (IV) L_4 = Gazipur (Kashimpur).

Seedling raising

Seeds of the collected genotypes were sown in polybag (15 x 10 cm). Each polybag received 2 kg of soil mixed with decomposed cow dung. Three replications were in per treatment and 2 seeds were sown in each polybag. Intensive care was taken to produce healthy seedlings. The first sowing was done on 25th October 2016 and second sowing was done on 5th November 2016.

Transplanting of pumpkin seedling

At the age of twenty days, the seedlings were transplanted in the main field. Procedures of land cultivation preparation of bed and pits, application of manures and fertilizers, transplanting of seedlings, intercultural operations were done by following the standards. The experiment was an RCBD with three replications in the main plot. Pits of 45 cm x 45 cm x 40 cm size was dug maintaining row to row and pit to pit spacing of 2.0 m and 2.0 m, respectively. Recommended doses of fertilizers @ of 175kg Urea,175 kg TSP, 150kg MP, 100 kg Gypsum and 10 kg Borax, and 16000 kg cow dung per hectare were applied (Bhuyan, 2010). At the time of the final land preparation, the whole amount of

TSP, Gypsum, and Boron was applied, along with half of the cow dung. The first transplanting was done on 15th November 2016 and the second on 25th November 2016.

Intercultural Operation

The seedlings were always kept under careful observation. Necessary intercultural operations such as thinning and gap filling, irrigation, weeding, mulching, and drainage were done through the cropping season for proper growth and development of the experimental plants.

Identification of viruses

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

Harvesting

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

Parameters assessed

Data were collected based on growth and yield contributing characters of infected plant or plant parts. They are as follows-

- Number of infected leaf/plants
- Vine length/plant (cm)
- Leaf length (cm)
- Leaf breadth (cm)
- Number of fruits /plants
- Fruit weight (kg)
- Length of the fruits (cm)
- Breadth of the fruits (cm)
- Thickness of fleshy part in fruit (cm)
- Thickness of placenta (cm)

Disease incidence and disease severity

Data on disease incidence and severity of virus disease in experimental field were recorded through frequent visits after appearance of symptoms. Disease incidence was calculated by using a standard formula (Agrios, 2005):

$$\text{Disease Incidence (\%)} = \frac{X1}{X} \times 100$$

X1 = Number of infected plants (or parts)
X = Total number of plants (or parts) observed

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

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

The disease severity of pumpkin was indexed on a 0-5 indexing scale, where 0 = no visible disease symptoms, 1 = mild mosaic on 10% of leaf area, 2 = mosaic patches and/or necrotic spots on 50% of leaf area, 3= leaves near apical meristem deformed slightly, yellow and reduced in size; 4= severe distortion of apical meristem with mosaic, and 5= extensive mosaic and serious deformation of leaves (Xu et al., 2004).

Statistical analysis

The collected data were subjected to analyses of variance (ANOVA) and the means were separated with the least significant difference (LSD) method at 5% level of significance. The statistical package MSTAT-C was used for this purpose.

III. Results and Discussion

Effect of sowing time on *CMV* disease incidence (%) of pumpkin at both sowing time

Disease incidences of different treatments are presented in (Table 01). There were significant differences found between the treatments. In early sowing, in 50, 65, 80, 95, and 110 days after transplanting (DAT), highest disease incidence (%) was found in L_2 (Narshingdi) and those were 18.97, 27.33, 55.68, 69.05, and 85.64, respectively. On the contrary, in 50, 65, 80, 95, and 110 DAT, lowest disease incidence (%) was found in L_3 (Dhaka) and those were 7.25, 15.03, 24.07, 33.74, and 47.60, respectively. Similarly, in late sowing, in 50, 65, 80, 95, and 110 DAT, highest disease incidence (%) was found in L_2 (Narshingdi) and those were 36.54, 45.39, 66.16, 78.47, and 90.08, respectively. On the other hand, in late sowing in 50, 65, 80, 95, and 110 DAT, lowest disease incidence (%) was found in L_3 (Dhaka) and those were 19.97, 21.68, 36.21, 40.63, and 49.85, respectively.

Table 01. CMV disease incidence (%) of pumpkin at both sowing time

Sowing time	Collected seeds	Disease incidence (%)				
		50 DAT	65 DAT	80 DAT	95 DAT	110 DAT
First sowing	L_1**	12.35 b*	19.30 b	35.75 bc	46.66 bc	66.09 bc
	L_2	18.97 a	27.33 a	55.68 a	69.05 a	85.64 a
	L_3	7.257 c	15.03 b	24.07 c	33.74 c	47.60 c
	L_4	13.91 b	22.57 ab	42.91 b	56.09 ab	71.07 ab
	CV (%)	27.74%	37.75%	30.06%	28.76%	28.18%
	LSD value	3.637	7.942	11.89	14.76	19.03
Second sowing	L_1	24.97 bc	32.97 b	46.73 b	62.67 b	70.86 b
	L_2	36.54 a	45.39 a	66.16 a	78.47 a	90.08 a
	L_3	19.97 c	21.68 c	36.21 c	40.63 c	49.85 c
	L_4	30.33 ab	38.87 ab	57.87 a	70.58 ab	81.02 ab
	CV (%)	23.64%	23.86%	18.56%	15.64%	20.49%
	LSD value	6.60	8.28	9.60	9.86	14.93

*Significant at 5% level of probability; ** L_1 = Narayanganj, L_2 = Narshingdi, L_3 = Dhaka and L_4 = Gazipur

Effect of sowing time on *CMV* disease severity (%) of pumpkin at both sowing time

Disease severity of different treatments is presented in (Table 02). There were significant differences found between the treatments. In early sowing, in 50, 65, 80, 95, and 110 DAT, the highest disease severity (%) was found in L_2 (Narshingdi) and those were 31.67, 53.33, 68.33, 73.33, and 88.33, respectively. But, in 50, 65, 80, 95, and 110 DAT, the lowest disease severity (%) was found in L_3 (Dhaka) and those were 6.67, 30.00, 38.33, 41.67, and 53.33, respectively.

In late sowing, in 50, 65, 80, 95, and 110 DAT, highest disease severity (%) was found in L_2 (Narshingdi) and those were 35.00, 56.67, 68.33, 81.67, and 93.33, respectively. On the contrary, in 50, 65, 80, 95, and 110 DAT, lowest disease severity (%) was found in L_3 (Dhaka) and those were 11.67, 28.33, 40.00, 48.33, and 56.67, respectively.

Table 02. CMV disease severity (%) of pumpkin at both sowing time

Sowing time	Collected seeds	Disease severity (%)				
		50 DAT	65 DAT	80 DAT	95 DAT	110 DAT
First sowing	L_1**	15.00 c*	38.33 bc	53.33 b	60.00 b	71.67 b
	L_2	31.67 a	53.33 a	68.33 a	73.33 a	88.33 a
	L_3	6.667 d	30.00 c	38.33 c	41.67 c	53.33 c
	L_4	25.00 b	43.33 ab	60.00 ab	66.67 ab	78.33 ab
	CV (%)	17.02	25.95	21.48	18.91	14.41
	LSD value	3.330	10.69	11.80	11.41	10.50
Second sowing	L_1	16.67 b	43.33 b	56.67 b	68.33 b	78.33 b
	L_2	35.00 a	56.67 a	68.33 a	81.67 a	93.33 a
	L_3	11.67 c	28.33 c	40.00 c	48.33 c	56.67 c
	L_4	33.33 a	51.67 ab	61.67 ab	73.33 ab	85.00 ab
	CV (%)	14.22	18.79	16.04	13.61	11.89
	LSD value	3.43	8.45	9.08	9.23	9.31

*Significant at 5% level of probability; ** L_1 = Narayanganj, L_2 = Narshingdi, L_3 = Dhaka and L_4 = Gazipur

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Here, periodic increment of viral disease incidence and severity was found at two sowing dates. Similar findings were also reported by Begum *et* al. (2016), Mitchell et *al.* (2005), Harvell et al. (2002). They worked on different pumpkin virus's incidences at field conditions. This disease's progression suggests that as plants became older, their sensitivity to virus infection increased. But this observation is opposite to the finding of Fargette et al. (1993), they found the susceptibility of the cassava plants to *African cassava mosaic virus* (ACMV) infection reduced with age.

Effect of both sowing time on *CMV* diseases on growth parameters of pumpkin

Leaf area (cm^2) and vine length (cm)

Leaf area of different treatments are presented in (Figure 01). In case of late sowing, the highest leaf area (cm^2) and vine length (cm) were found in L_3 (Dhaka) those are 108.4 and 198.6, respectively. In contrast, the lowest leaf area (cm^2) and vine length (cm) were in L_2 (Narshingdi) those are 76.66 and 126.9, respectively. Vine length of different treatments are presented in (Table 03). In early sowing, the highest leaf area (cm^2) and vine length (cm) were found in L_3 (Dhaka), 117.7 and 199.6, respectively, whereas the lowest leaf area (cm^2) and vine length (cm) were in L_2 (Narshingdi) those are 79.44 and 164.3, respectively. Similar works were also done by Begum et al. 2016 and Begum et al. 2015. They revealed that virus infection decreased different growth parameters similar to this finding.

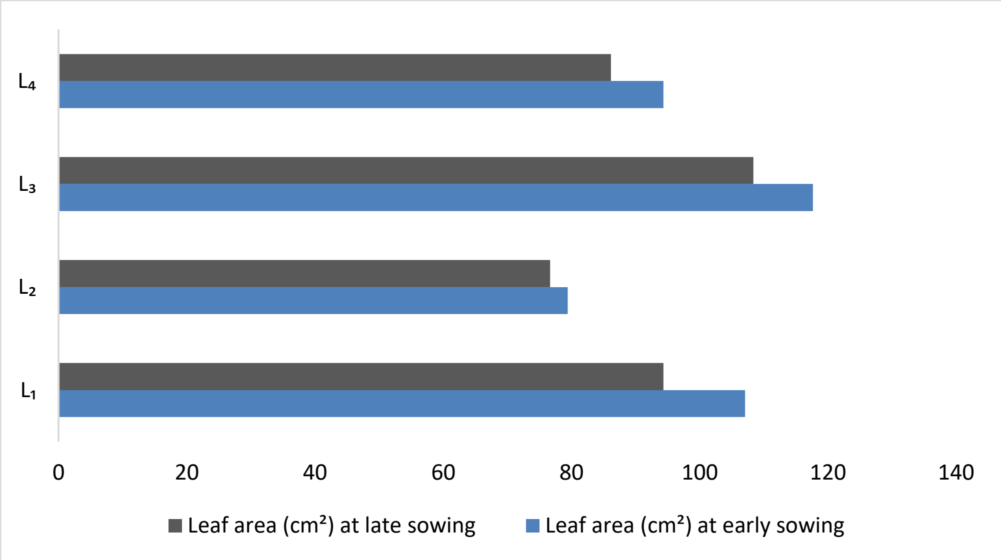

Figure 01. Leaf area of different pumpkin genotypes

Table 03. Effect of both sowing time on CMV diseases pumpkin on leaf area (cm2) and vine length (cm)

Collected seeds	Different growth parameters	
	Vine length (cm) at first sowing	Vine length (cm) at second sowing
L_1 **	188.9 b*	190.4 a
L_2	164.3 c	126.9 b
L_3	199.6 a	198.6 a
L_4	184.8 b	188.6 a
CV (%)	20.11%	2.09%
LSD value	53.91	7.68

*Significant at 5% level of probability; ** L_1 = Narayanganj, L_2 = Narshingdi, L_3 = Dhaka and L_4 = Gazipur

Effect of both sowing date on pumpkin at different yield and yield contributing parameters

Different yield and yield contributing parameters such as number of fruits, fruit weight (kg), yield (kg), fruit diameter (cm), and flesh thickness (cm) are presented in (Table 04). In early sowing, the highest number of fruits, fruit weight (kg), yield (kg), fruit diameter (cm), and flesh thickness (cm) were found in L_3 (Dhaka) and those were 7.00, 1.87, 13.07, 14.03, and 4.00, respectively. On the contrary, the lowest number of fruits, fruit weight (kg), yield (kg), fruit diameter (cm), and flesh thickness (cm) were found in L_2 (Narshingdi) and those were 3.23, 1.40, 4.45, 12.99, and 3.27, respectively. Similarly, in late sowing, the highest number of fruits, fruit weight (kg), yield (kg), fruit diameter (cm), and flesh thickness (cm) were found in L_3 (Dhaka) and those were 6.00, 1.79, 10.67, 13.80, and 3.73, respectively. On the other hand, the lowest number of fruits, fruit weight (kg), yield (kg), fruit diameter (cm), and flesh thickness (cm) were found in L_2 (Narshingdi) and those were 3.67, 1.32, 5.26, 12.80, and 3.03, respectively. Similar works were also done by Begum et al. 2016 and Begum et al. 2015. They revealed that virus infection decreased different growth parameters similar to these findings.

Table 04. Effect of both sowing date on pumpkin at different yield and yield contributing parameters

Sowing time	Collected seeds	Different yield and yield contributing parameters				
		No. of fruit/ plot	Fruit weight /plot(kg)	Yield (kg/ha)	Fruit diameter (cm)	Flesh thickness (cm)
First sowing	L_1**	5.33 a*	1.77 ab	7.74 b	13.72 ab	3.75 ab
	L_2	3.23 b	1.40 b	4.45 c	12.99 b	3.27 c
	L_3	7.00 a	1.87 a	13.07 a	14.03 a	4.00 a
	L_4	3.33 b	1.47 ab	5.59 c	13.11 b	3.35 bc
	CV (%)	18.23%	13.55%	11.80%	3.07 %	6.01 %
	LSD value	1.73	0.44	1.84	0.826	0.43
Second sowing	L_1	4.67 ab	1.69 ab	6.12 b	13.53 ab	3.50 ab
	L_2	3.67 b	1.32 b	5.26 c	12.80 b	3.03 b
	L_3	6.00 a	1.79 a	10.67 a	13.80 a	3.73 a
	L_4	4.33 b	1.37 ab	6.08 bc	12.87 b	3.13 b
	CV (%)	14.73%	14.19%	6.07 %	2.95 %	7.56%
	LSD value	1.37	0.44	0.85	0.78	0.50

*Significant at 5% level of probability; ** L_1 = Narayanganj, L_2 = Narshingdi, L_3 = Dhaka and L_4 = Gazipur

Relationship between the CMV disease incidence (%) at 110 DAT and yield (kg/ha) at both sowing time

Relationship between the CMV disease incidence (%) at 110 DAT and yield (kg/ha) at first sowing the field are shown in (Figure 02). A negative correlation exists between the disease incidence (%) and yield (kg/ha). It means that with the increase in disease incidence (%), yield (kg/ha) can decrease. The contribution of the regression (R^2 = 0.927) indicated that 92.7 % yield (kg/ha) in pumpkins would be affected by disease incidence (%). Similarly, at second sowing relationship between the CMV disease incidence (%) at 110 DAT and yield (kg/ha) are shown in (Figure 03). A negative correlation also exists between the disease incidence (%) and yield (kg/ha). The contribution of the regression (R^2 = 0.8892) indicated that 88.92 % yield (kg/ha) in pumpkins would be affected by disease incidence (%).

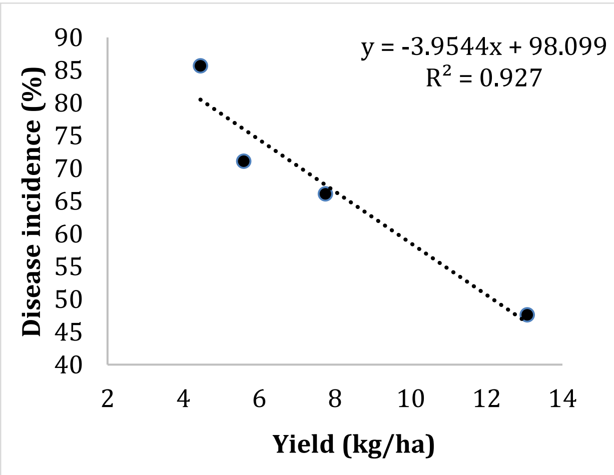

Figure 02. Relationship between disease incidence (%) and yield (kg/ha) at first sowing.

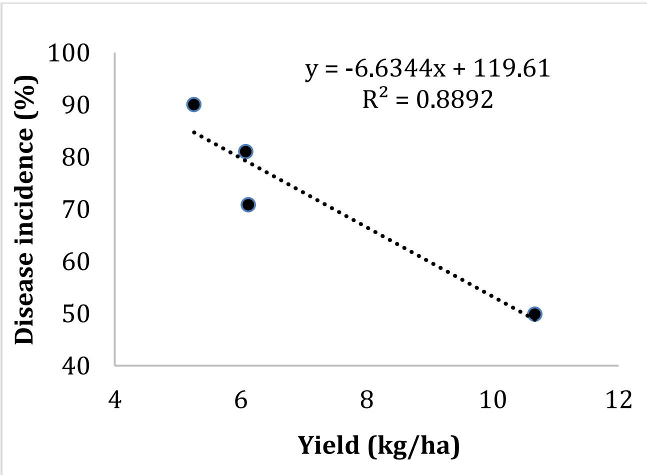

Figure 03. Relationship between disease incidence (%) and yield (kg/ha) at second sowing.

V. Conclusion

The experiment was conducted to determine how disease incidence and severity of Cucumber Mosaic Virus (CMV) were affected by sowing time. The collected seeds were sown in two sowing times by maintaining 10-day intervals. From the study, it was found that the second sowing (5th November) resulted in the highest disease incidence (%) and severity (%) than the first sowing (25th October). The genotype from Narshingdi (L_2), reported a greater percentage of disease incidence (%) and severity (%) than the genotype from Dhaka (L_3) at both sowing times. But regarding growth parameters, yield and yield contributing characters, first sowing was recorded as the best for having good plant growth (vine length and leaf area) and yield. In both sowing time, Dhaka (L_3) resulted in more plant growth (vine length and leaf area) and yield (kg/ha) than the genotype from Narshingdi (L_2). From the study, it can be concluded that last week of October is suitable for pumpkin seed sowing to have the best yield and avoid disease.

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