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Effects of Irrigation Methods and Water Regimes on Occurrences of *Cucumo*- And *Poty*- Viruses in Watermelon

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Abstract

The response of watermelon to Cucumber mosaic virus (CMV) and Watermelon mosaic virus (WMV) under different irrigation methods and water regimes (WRs) was investigated. Watermelon varieties; Kaolak and Sugar baby were irrigated using either sprinkler or basin method of application at 50%, 75% and 100% WRs during the dry season. Results showed that at 3 and 5 weeks after planting (WAP) total irrigation at 100% water regime (WR_{100}) produced the tallest plants with basin irrigation. Mean values of 15.68 cm and 15.85 cm were obtained from Kaolak and Sugar baby respectively with basin irrigation at WR_{100}. However, irrigation at WR_{50} produced shorter plants with fewer leaves. Complete yield loss was recorded on the field due to severe virus infection. Mixed virus infections were evident in some of the treatments but basin irrigation at WR_{75} and WR_{100} had no mix infection of CMV and WMV. Also, varieties were not susceptible to CMV infection at WR_{75} and WR_{100} when basin irrigation was used. However, the most severe CMV infection occurred in Sugar baby at WR_{50} using sprinkler irrigation with virus titre of 1.285. The two varieties were susceptible to WMV irrespective of the irrigation method or WR. The highest WMV titre was 2.88 obtained from Sugar baby at WR_{50}. Total irrigation produced plants with good agronomic parameters compared with deficit irrigation. However, complete yield loss was recorded as a result of either single or double virus infections. Therefore, virus prevention and breeding for virus resistance are best approaches for the control of plant viruses.

Key words: Basin, irrigation, sprinkler, watermelon, water regime, virus

1. Introduction

Watermelon is one of the major cucurbit crops which thrive in different parts of the world especially in tropical and subtropical regions. Cultivation of watermelon requires adequate supply of water although; the crop is not regarded as high water demand crop (FAOSTAT, 2001). In Nigeria, this crop command higher prices than the local non-exotic crops especially in dry season. The challenge of pest and disease infestation could however reduce the projected revenue from cultivation even under irrigation which is an extra input into production costs. Its cultivation is hampered by numerous viral diseases that often cause economic losses in most production areas (Gaba *et al*., 2004; Papayiannis *et al*., 2005). The cucurbit crops are susceptible to at least 35 viruses (Papayiannis *et al*., 2005), among which Cucumber mosaic virus (CMV), Cucumber green mottle mosaic virus (CGMMV), and Watermelon mosaic virus (WMV) are the most damaging viruses in watermelon.

The occurrence of more than one virus species in a single plant is not uncommon in cultivated and native plant species. A mixed virus infection may lead to greater disease severity than individual viral components and this is sometimes referred to as a synergistic disease (Murphy and Bowen, 2006). Mixed infections can also modify viral traits such as host range (Hacker and Fowler, 2000; García-Cano *et al*., 2006), transmission rate (Wintermantel *et al*., 2008), cellular tropism (Wege and Siegmund, 2007), or titer. Most studies have focused on synergistic diseases caused by two ssDNA virus or ssRNA; particularly by a Potyvirus and other ssRNA virus. In most instances, the titer of the non-potyvirus increases while that of the potyvirus is not altered (Wang *et al*., 2002; Murphy and Bowen, 2006; Taiwo *et al*., 2007). This enhancement has been explained by potyvirus HC-Pro-mediated RNA silencing suppression (Valli *et al*., 2006). Nevertheless these interactions not always produce synergistic diseases (Wang *et al*., 2004; Untiveros *et al*., 2007), and depending on the particular combination of virus species, accumulation of the counterpart can also decrease (Kokkinos and Clark, 2006). Infections of cucurbit species by either potyviruses such as Zuchinni yellow mosaic virus (ZYMV) or Watermelon mosaic virus (WMV) or by CMV are very common and cause considerable damage worldwide in severe epidemics in cucurbit fields, either in single or double infections (Wang *et al*., 2002).

Cucumber mosaic virus has a worldwide distribution and a very wide host range. It has been known that the virus has the widest host range of any known plant virus (191 hosts in 40 families) (Gallitelli, 2000). It can be transmitted from plant to plant both mechanically by sap and by aphids in a stylet-borne manner (Palukaitis and Garcia-Arenal, 2003). Viruses in the genus potyvirus [e.g., Papaya ringspot virus (PRSV), Watermelon mosaic virus (WMV) and Zucchini yellow mosaic virus (ZYMV)] are transmitted by aphids in a nonpersistent manner and are considered a constant threat to cucurbit crops (Guner and Whener, 2008). With growing interest in cultivation of the crop outside the northern part of Nigeria which range between derived to sahel savannah ecology, there is need to understand the behavior of viruses associated with the growing of the crop.

The extent of manifestation of potyvirus (watermelon mosaic virus) and cucumovirus (cucumber mosaic virus) or their synergistic interactions under total irrigation, supplemental or deficit irrigation have not being well investigated. This work was to investigate the impact of irrigation type and water regimes on the growth of watermelon as well as on the titres of Watermelon mosaic virus and Cucumber mosaic virus in Ibadan which is in the Humid, Sub-Humid agro ecology.

2. Materials and Methods

2.1 Study Location and Scope

This study was carried out in one of the experimental fields of the Institute of Agricultural Research and Training, Moor Plantation Ibadan, Nigeria. The field ($7^0$38'E, $3^0$84'N and 174.3 m above sea level) is in the Humid, Sub-humid Agro Ecological Zone of Nigeria. The experiment was conducted during the dry season (between December and March, 2013) when evapotranspiration exceeds precipitation (Oke et al, 2011).

Two varieties of watermelon; Kaolak and Sugar baby were obtained from a Farmer's Support Seed shop at, Ibadan, Oyo State, Nigeria. The watermelon was raised during the dry season when crop water requirement was supplied through irrigation. Two irrigation methods and three levels of irrigation were employed.

2.2 Soil and Water Characterization

The soil samples were taken for soil test while core samples were also taken at 0-15 cm and 15 – 30 cm for soil physical characteristics examination including determination of available water of the field.

2.3 Experimental Design

The seeds were planted on 24th December, 2013 during dry season. Two irrigation treatments (Sprinkler and Basin) were the main plots while the three (3) irrigation water regimes were sub plots. The water regimes were based on Consumptive Use (C_u) computed from Reference Evapotranspiration (ET_o) and Crop Coefficient (K_c) for Watermelon. The CROPWAT 8.0 was used in computing the ET_o while the Cu was computed from Equation 1.

$$C_u = K_c * ET_o \quad (1)$$

Relative to C_u, the water regimes are 50% (WR_{50}), 75% (WR_{75}) and 100% (WR_{100}). The Net Irrigation (NIR) water applied within a developmental stage was obtained for each of the water regime using equation 2.

$$\text{NIR} = \text{WR x no of days within a stage} \quad (2)$$

Two watermelon varieties V1 (Kaolak) and V2 (Sugar baby) were investigated. The field was arranged in Split Plot with 3 replications. With each plot size of 3m x 3m, the planting spacing was 1m x 0.5m while a space of 2 m was set between plots. A space of 4m was imposed between irrigation types. Basic agronomic management practices were carried out.

2.4 Agronomic and Irrigation Data

The basin irrigation was accomplished by creating bunds (25cm) around the basin plots and the irrigated water was released into the plot using graduated bucket. Sprinkler treatment was done using 10 litres size watering can with which computed volume of water was applied. To evaluate the differences and impacts of WRs on the performance of the crop varieties, plant height, number of leaves were taken at three Weeks after Planting (WAP) and 5 WAP on tagged plants per plot. Numbers of flowers and fruits were also counted from the tagged plants. Volume of water used at the various regimes were noted

2.5 Determination of the Effect of Irrigation Methods and Water Regimes on Virus Titres

Leaf samples were plucked from the same position from the tagged plants that received the different treatments and they were kept in envelopes and transported to the laboratory in an ice box. The determination of the presence of single and mixed infections of Cucumber mosaic virus (CMV), a cucumovirus and Watermelon mosaic virus (WMV), a potyvirus was carried out by using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instruction (Agdia Inc. Elk. IN, USA) and as described by Taiwo *et al*. (2007). The absorbance of the optical density (OD) at 405nm was measured in an ELISA plate reader (ELx800). Absorbance values were considered positive when the OD ratio of infected samples/negative contro (healthy plant)l is greater than 2.

2.6 Statistical Analysis

Data were subjected to analysis of variance with levels of significance defined by the Least Square Difference (LSD). Means from the interactions of the treatments (irrigation, irrigation regimes and varieties) were separated using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$. The GENSTAT and Statistical Package for Social Scientists (SPSS 16.0) were used to analyse the data obtained.

3. Results

3.1 Water use and crop coefficient of Watermelon

Crop developmental stages and corresponding crop coefficient (K_c) are shown in Figure 1. Development stages of watermelon are based on Richard *et al*. (1998). Following the evapotranspiration characteristics of the production location, the variation in water need is reflected in the crop coefficient (K_c). The K_c shows the fraction of ET_o that must be met. The crop coefficient of 1.0 during the flowering and fruit set and 0.8 during the fruit and maturity show how much water was used during these critical stages. Thus, the water applied during production is presented in Table 1. The NIR which is the total irrigation water applied based on the ET_c were 228.4mm, 319.8mm and 456.9mm for WR_{50}, WR_{75} and WR_{100} respectively. Virus infections were noticed from 7 - 8 WAP during fruiting. The fruits aborted and rotted leading to total yield loss.

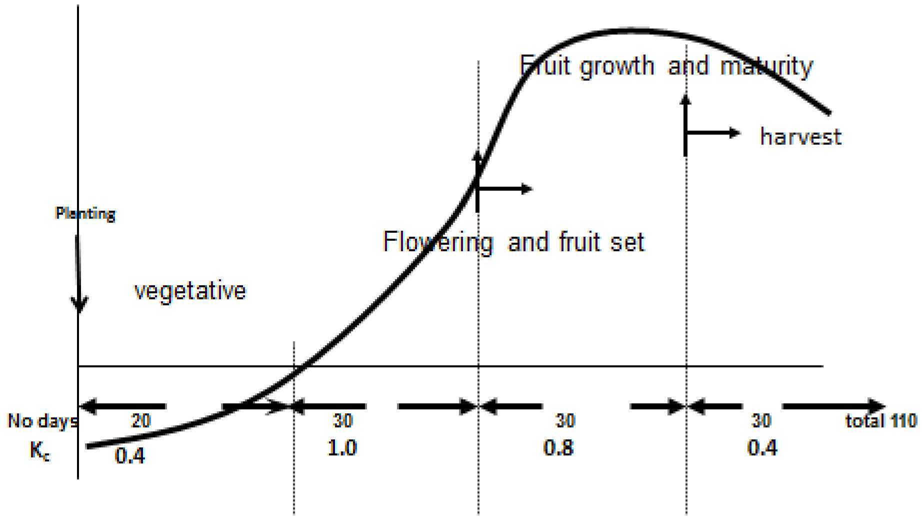

Figure 1. The Watermelon Crop Development Stages

Table 1. Water application for water melon growth stages

Crop Stages	*Days*	*K_c*	*Et_o*	*Et_c*	*Water Regimes WR (mm)*			*Net Irrigation NIR (mm)*		
Stages					*WR_{50}*	*WR_{75}*	*WR_{100}*	*WR_{50}*	*WR_{75}*	*WR_{100}*
Initial	*20*	*0.4*	*5.3*	*2.1*	*1.1*	*1.5*	*2.1*	*21.2*	*29.7*	*42.4*
Development	*30*	*1.0*	*7.1*	*7.1*	*3.6*	*5.0*	*7.1*	*106.5*	*149.1*	*213.0*
Mid	*30*	*0.8*	*5.7*	*4.3*	*2.1*	*3.0*	*4.3*	*64.1*	*89.8*	*128.3*
Late	*30*	*0.4*	*6.1*	*2.4*	*1.2*	*1.7*	*2.4*	*36.6*	*51.2*	*73.2*
TOTAL	*110*							*228.4*	*319.8*	*456.9*

K_c = Crop Coefficient; Et_o = Reference Evapotranspiration; Et_c = Consumptive Use or Crop Water Requirement

3.2 Effect of Irrigation Type, Irrigation Regimes and Variety On Growth of Watermelon

Table 2 presents the mean values of growth data, CMV and WMV at 3WAP and 5WAP. It was observed that variety does not impact any significant difference in the plant height at 3WAP while the plant height of 15.2 cm recorded from Kaolak was significantly difference from what was observed at 5WAP (13.8cm) posted by Baby Sugar variety. No significant difference was recorded in the number of leaves at 3WAP and 5WAP irrespective of variety. The incidence of CMV was not also significant in the two varieties even though the opposite was observed in WMV.

Furthermore, the impact of irrigation type did not produce any significance difference in the number of leaves at 3WAP or 5WAP whereas at 3WAP, plant height from Basin irrigation was observed to be significantly higher than plant height from 5WAP. This implies that the impact of method may not be critical on the growth of watermelon beyond the initial few weeks of crop life. Although, the level of CMV and WMV were significantly difference across the irrigation method employed. Sprinkler irrigation poses significantly higher level of virus incidence with CMV and WMV being 1.0 and 1.3 respectively. From Table 2 also, it was observed that irrigation water regime did not significantly affect growth parameters at P <0.05. However, significant differences were noticed at higher level of probability. Although, the plant heights and number of leaves at 3WAP and 5WAP were better at WR_{75} and best at $WR_{100,}$ the reverse was the case with the virus incidence which was worst in least level of irrigation (WR_{50}). At higher irrigation regime (WR_{100} or WR_{75}), when more water was supplied, virus incidences were significantly lower (p<0.001).

Table 2. Analysis of Variance of the Varieties, Irrigation Methods and Irrigation Regimes

Treatment	Plant Height 3WAP (cm)	Plant Height 5WAP (cm)	No of leaves 3WAP	No of leaves 5WAP	CMV	WMV
Varieties						
Kaolak	12.4	15.2	11.8	39.7	0.82	0.81
Baby Sugar	12.2	13.8	11.4	38.3	0.86	1.44
LSD	1.28ns	1.15*	0.78ns	4.92ns	0.09ns	0.21***
Irrigation						
Sprinkler	11.6	14.5	11.4	37.7	1.0	1.30
Basin	13.0	14.5	11.8	40.4	0.68	0.95
LSD	1.28*	1.15ns	0.78ns	4.92ns	0.09***	0.21**
Irrigation Regime						
50%	10.9	13.5	11.1	29.1	1.07	1.59
75%	11.9	14.0	11.5	38.6	0.74	1.08
100%	14.1	16.0	12.1	49.4	0.72	0.71
LSD	1.57***	1.40**	0.96ns	6.03**	0.12***	0.25***

** LSD value is significant at p<0.05; ** LSD value is significant at p=0.01; ***LSD value is significant at p<0.001; ns means not significant*

3.3 Impact of irrigation type and water regimes on growth and virus incidences on watermelon

It was observed that at 3 WAP, treatment with total irrigation (without deficit) (WR_{100})

applied in basin produced the tallest plant with average values of 15.68 cm and 15.85 cm for Kaolak and Sugar baby respectively. This was followed by sprinkler irrigation of Sugar baby at WR_{100} having an average value of 14.5 cm. The shortest plants were produced at WR_{50} using basin irrigation with average values of 9.67 cm and 9.92 cm for Kaolak and Sugar baby respectively (Table 3). At 5 WAP, it was observed that the highest significant ($P \leq 0.05$) plant height values of 15.67 cm and 16.67 cm were obtained from Sugar baby and Kaolak irrigated with WR_{100} in basin irrigation. These values were not significantly different from 15.42 cm obtained from Sugar baby irrigated at WR_{75} using basin (Table 3).

The number of leaves at 3 WAP revealed that Sugar baby and Kaolak that received WR_{100} using basin produced the highest mean number of leaves with values of 12.75 and 12.83 respectively while the least number of leaves (9.92) was obtained from Kaolak when irrigated at WR_{50} using basin (Table 2). Mean number of leaves at 5 WAP ranged from 23.75 to 55.75. Irrigation at WR_{50} led to the formation of very few leaves by the two varieties while irrigation with basin at WR_{100} led to the formation of more leaves by Sugar baby (Table 2). However, no significant difference was observed in the number of leaves at 5WAP in Basin or Sprinkler irrigation at WR_{75}.

Table 3. Interaction of different irrigation regimes, irrigation type and varieties on plant height and number of leaves

Water regime (WR)	Irrigation type	Variety	Plant height (cm) at 3 WAP	Plant height (cm) at 5 WAP	No. of leaf at 3 WAP	No. of leaf at 5 WAP
	basin	1	9.67c	13.99ab	9.92b	24.83e
		2	9.92c	13.08ab	11.50ab	23.75e
50%	sprinkler	1	10.67bc	12.38b	11.17ab	34.75cde
		2	13.50abc	14.41ab	11.67ab	33.25de
	basin	1	12.00abc	14.25ab	11.92ab	39.67bcd
		2	13.96abc	15.42a	11.58ab	47.58abc
75%	sprinkler	1	10.67bc	12.73b	11.33ab	33.42de
		2	12.33abc	12.31b	11.25ab	33.58de
	basin	1	15.68a	16.67a	12.83a	46.75abcd
		2	15.85a	15.67a	12.75a	55.75a
100%	sprinkler	1	13.50abc	14.33ab	11.17ab	46.50abcd
		2	14.50ab	14.38ab	11.67ab	48.50ab

Mean values followed by the same letter are not significantly different at 5% level of probability according to Duncan's multiple range test. 1= Kaolak, 2= Sugar baby.

Although, insecticide (Cypermethrin) was applied at 2WAP and 5WAP, the manifestation of infestations in the field was quite high shortly after flowering (which was noticed from 8WAP). The yield could not be recorded because the plants experienced flower abortions and as a result very few plant stands were able to produce fruits. There was complete yield loss as the fruits produced were malformed and they could not develop to form normal fruits with the expected shapes and sizes of normal watermelon. Eventually all the fruits rotted away on the field. This problem, in relation to water regimes was observed to be worst in WR_{50} followed by WR_{75} and WR_{100}. Nevertheless, the yield loss was total irrespective of the type of irrigation.

3.4 Effect of different water regimes and irrigation types on virus titres

The result on the effect of different water regimes and type of irrigation of the two varieties of watermelon showed that the plants had varying degree of susceptibility to CMV. Cucumber mosaic virus was present in all the treatments except at WR_{75} and WR_{100} using basin irrigation. However, Watermelon mosaic virus was present in all the treatments. Result on the reaction of watermelon varieties to mixed virus infections showed that there was no mixed infection of CMV and WMV when basin type of irrigation was used at WR_{75} and WR_{100} (Table 4).

Kaolak was not infected with CMV at WR_{75} and WR_{100} when basin method of application was used. Meanwhile, they were susceptible when sprinkler method was used and the titre values were 0.877 and 1.002 respectively. Kaolak was susceptible to WMV infection at all the WR levels. Although, the plants showed positive response to WMV at WR_{100} but the susceptibility was moderate, especially those that were irrigated with basin which had titres of 0.606 (Table 5).

The highest significant titre of CMV ($P \leq 0.05$) was observed in Sugar baby irrigated using sprinkler method at WR_{50} with a mean of 1.285, followed by Sugar baby irrigated with basin at WR_{50} with an average value of 1.105. Cucumber mosaic virus was absent at WR_{75} and WR_{100} using basin method of application and the titres were 0.419 and 0.455 respectively. With sprinkler irrigation at WR_{50}, a significantly high WMV infection was recorded in Sugar baby with an average titre of 2.8795. Also, the effect of WMV on Sugar baby at WR_{50} using basin irrigation was pronounced with a mean titre of 1.9535. The WMV titre was equally high at WR_{75} using sprinkler irrigation (1.475) (Table 5).

There was enhancement of WMV titres in most of the treatments especially Sugar baby that received WR_{50} using sprinkler and basin irrigation with titres of 2.879 and 1.954 respectively. Nevertheless, the titres of CMV was also enhanced in few cases especially in Kaolak at WR_{50} with titres of 0.934 and 0.957for basin and sprinkler methods respectively and also at WR_{100} using sprinkler method (1.002) (Table 5).

Table 4. Reaction of watermelon to single and mixed virus infections

			Single infection		Mixed infection
Water regime	Type of irrigation	Variety	CMV	WMV	CMV +WMV
50%	Sprinkler	1	+	+	+
		2	+	+	+
	Basin	1	+	+	+
		2	+	+	+
75%	Sprinkler	1	+	+	+
		2	+	+	+
	Basin	1	-	+	-
		2	-	+	-

100%	Sprinkler	1	+	+	+
		2	+	+	+
	Basin	1	-	+	-
		2	-	+	-

+ = present, - = absent, 1= Kaolak, 2= Sugar baby

Table 5. Effect of different water regimes on virus infections in watermelon

Water regime	Type of irrigation	KAOLAK		SUGAR BABY	
		CMV (A_{405nm})	WMV(A_{405nm})	CMV(A_{405nm})	WMV(A_{405nm})
50%	Basin	0.934^{ab}	0.645^{bc}	1.105^{b}	1.954^{b}
	Sprinkler	0.957^{ab}	0.860^{abc}	1.285^{a}	2.879^{a}
75%	Basin	0.661^{bc}	0.967^{ab}	0.419^{d}	0.847^{c}
	Sprinkler	0.877^{ab}	1.039^{a}	0.984^{bc}	1.475^{b}
100%	Basin	0.479^{c}	0.606^{c}	0.455^{d}	0.675^{c}
	Sprinkler	1.002^{a}	0.764^{abc}	0.928^{c}	0.788^{c}
Healthy plant		0.389^{d}	0.282^{d}	0.389^{e}	0.282^{d}

Mean values followed by the same letter are not significantly different at 5% level of probability according to Duncan's multiple range test.

4. Discussion

This work reveals the importance of adequacy of water in production of watermelon. Water deficit contributed to disease spread which leads to yield loss. Plant growth was best when basin irrigation was at WR_{100} while WR_{50} led to poor growth of plants irrespective of irrigation type. The reason for this is because the 100% irrigation water supply was the optimum water requirement for watermelon, as a result, the plants were able to get enough water required for their physiological activities and this was translated to improved growth. Sani et al. (2008) reported that taller plants were produced when full consumptive use irrigation was used as compared to plants which received less amounts of water under the prevailing environmental conditions.

Complete yield loss of watermelon was reported in this experiment. The presence of viruses on the field could be responsible for the loss. Lin et al. (2012) reported that watermelon is prone to attack by several viruses which often results in destructive yield loss. Reduction in yield has also been observed by Gishumu et al. (2008).

This investigation revealed the natural occurrence of viruses (CMV and WMV) in watermelon plants grown on the field in the dry season of humid sub-humid ecology. This could be attributed to the fact that watermelon belongs to the family cucurbitacea, hence, its susceptibility to cucurbit viruses. The susceptibility of cucurbits to CMV and Potyviruses have been reported by many authors (Choi et al., 2000; Damicone et al., 2007; Lecoq, 2003).

Results on the effect of CMV on watermelon showed that both varieties were susceptible to CMV infection at $WR5_{50}$. This could be due to the fact that CMV is a common virus which causes disease of crops on the field in which watermelon is one of them. Roossinck (2002) reported that CMV is one of the most widely spread virus in the world infecting over 1,000 plant species belonging to more than 85 families. Also, Zitter and Murphy (2009) reported that Cucumber mosaic virus (CMV) is one of the important viruses in temperate, tropic and sub-tropic regions of the world which could cause crop losses on average of 10-20%. Kaolak was not susceptible to CMV infection at WR_{75} and WR_{100} using basin irrigation. It can be explained that at optimum conditions of growth, Kaolak may show some levels of resistance to CMV infection. This statement corroborated the report of Jerry, 2016 which stated that all cucurbit species are susceptible to CMV but watermelon is rarely infected.

The two watermelon varieties were susceptible to WMV, the susceptibility might be due to the fact that Potyviruses are the major genus in the Potyviridae family and infect a broad range of host plants. It was reported by ICTV (2010) that potyviruses infect more than 2000 plant species of 550 genera and 81 families.

The ELISA result showed that both viruses were present in the leaves of the watermelon varieties. This implies that CMV and WMV were able to infect the varieties. Infections of cucurbit species by either potyviruses such as Zuchinni yellow mosaic virus (ZYMV) or Watermelon mosaic virus (WMV) or by CMV are very common and cause considerable damage worldwide in severe epidemics in cucurbit fields, either in single or double infections (Wang *et al*., 2002).

Virus diseases are one of the factors that affect plant growth and yield parameters. However, virus elimination can be achieved through proper management including supplying of required water during irrigation and control of insect vectors. The use of resistant cultivars and favourable climatic conditions are also important for plant growth. Therefore, disease resistance watermelon varieties and taking preventive cautions are suggested as means of economic control of diseases.

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