Using Some Growth Retardants for Inhibition of Maize Dwarf Mosaic Virus (MDMV)

Mohamed, E.F.
Botany Department, Faculty of Agriculture, Fayoum University, Egypt.
e-mail:emaddwidar@yahoo.com

Abstract: The current study was carried out to evaluate the effect of some growth retardants such as alar, ethrel, cycocel and paclobutrazol with different concentrations (50, 100, 150 and 200 ppm.) on maize dwarf mosaic virus (MDMV) *in vitro* and *in vivo*. *In vitro* experiment, growth retardants reduced the occurrence of mosaic symptoms on maize plants. These percentages were reduced to 40%. Paclobutrazol was the most effective treatment giving mosaic symptoms percentages reached to 48, 44, 44 and 40 % when used with concentrations of 50, 100, 150 and 200 ppm respectively. On a statistical basis, paclobutrazol caused a significant reduction in the mosaic symptoms percentages when used with a concentration of 50, 100, 150 and 200 ppm. While cycocel caused a significant reduction in the mosaic symptoms percentages, only, when used with a concentration of 200 ppm. So, paclobutrazol and cycocel was more effective in reducing the mosaic symptoms produced by MDMV on maize plants *than* other treatments. In pre-inoculation experiment, all tested growth retardants reduced the mosaic symptoms percentages. These percentages were reduced to 32%. Paclobutrazol was the most effective treatment giving mosaic symptoms percentages reached to 40, 36, 36 and 32 % when used with concentrations of 50, 100, 150 and 200 ppm respectively. On a statistical basis, paclobutrazol and cycocel caused a significant reduction in the mosaic symptoms percentages when used with a concentration of 50, 100, 150 and 200 ppm. While ethrel caused a significant reduction in the mosaic symptoms percentages, only, when used with a concentration of 200 ppm. In post-inoculation experiment, all tested growth retardants reduced the mosaic symptoms percentages (infectivity of MDMV). These percentages were reduced to 40%. Paclobutrazol was the most effective treatment giving mosaic symptoms percentages reached to 56, 52, 52 and 40 % when used with concentrations of 50, 100, 150 and 200 ppm respectively. On a statistical basis, paclobutrazol caused a significant reduction in the mosaic symptoms percentages, only, when used with a concentration of 200 ppm. Pre-inoculation treatment was more effective in reducing virus infectivity than post-inoculation treatment. [Journal of American Science 2010;6(9):5-13]. (ISSN: 1545-1003).

Key words: Maize dwarf mosaic virus (MDMV), potyviruses, alar, ethrel, cycocel, paclobutrazol and growth retardants.

1. Introduction

Maize (*Zea mays* L.) is one of the major cereal crops in Egypt cultivated in large area for human, animal, and poultry feeding. Maize plants are attacked by virus and virus-like diseases which damage the plants (Aboul-Ata and Ammar, 1989). Zeyen *et al*., (1987) reported that, in 1977 a sudden and dramatic epidemic of maize dwarf mosaic virus (MDMV) struck commercial fields of maize in Minnesota causing multi-million dollar losses. The epidemic was unusual in that the virus historically had been confined to the southern USA and Ohio River Valley with only occasional occurrences reported from states bordering Canada. An extensive 5-year study of the virus in Minnesota revealed a low incidence of the virus from 1978-81, and commercial fields badly infected in 1977 were not again infected. No evidence of wild host plants was found, neither was seed transmission in maize considered to be important, and MDMV strain ratios changed from year to year as did the distribution of infected fields. Concomitant studies on aphid retention have revealed that the virus can be retained by aphid vectors for more than 19 h, and that the 1977 epidemic was associated with a weather pattern that could be linked to potential aphid transport from the southern Great Plains of North America. All circumstantial evidence led to a long-distance aphid transport hypothesis. This may be worth considering whenever unexpected epidemics of aphid-transmitted non-persistent viruses occur in regions where the particular virus is not endemic, and may also explain the widespread distribution of certain aphid-transmitted non-persistent viruses. Maize dwarf mosaic disease caused by Maize dwarf mosaic virus (MDMV) decreases yield of maize plants on average by 2.4% for each 10% increase in MDM-diseased plants (Scott *et al.,* 1988). MDMV is one of the world's main viruses in maize producing areas. MDMV is spread worldwide wherever maize and sorghum are grown. MDMV has been described in North and South America, Europe, Africa, and Asia. In Europe, this disease was first described as the maize mosaic virus. MDMV infections have a detrimental effect on plant growth, flowering, grain yields and quality of the commercial crop (Hai and Zhan, 2003). MDMV belongs to potyviruses group (Shukla *et al.* 1994). MDMV particles were reported to be flexuous filamentous ranging from 725 to 750 nm in length and about 13 nm in width, and contain a coat protein of about 39 KDa (El-Morsi *et al.* 2003). McDaniel and Gordon (1989) reported that, viral nucleic acid was digested by RNase but not DNase and virions contained 6.6% RNA by weight as determined by UV absorbance. Liu *et al.* (2003) showed that, the full-length nucleotide sequence of a potyvirus causing the maize dwarf mosaic (MDM) disease in China was obtained by reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of the cDNA 5'-end (5'-RACE). The viral genome comprised of 9596 nucleotides except the polyA tail and encoded a putative polyprotein of 3603 amino acids. The terms growth retardant is used for all chemicals that slow cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects. Groups of growth retardants include quaternary ammonium carbamates and phosphonium compounds. One of the most widely used growth retardants is cycocel. Certain growth retardants have been shown to interact with auxin or with gibberellic acids. Cycocel effects on some phosphorylated glycolytic intermediates (Adedipe and Ormrod, 1970; Trevor and Leslie, 1974; Shiow and George, 1985; Graebe, 1987; Grossmann, 1990; Grossmann *et al*., 1994; El-Okazy, 2008; and Gonçalves *et al*., 2009). Paclobutrazol is a triazole compound used extensively in agriculture and horticulture as plant growth regulator. In ornamental crops, Paclobutrazol is used for reducing the size of plants, improving compactness, and increasing other functional aspects, such as the ability to resist both abiotic and biotic stresses (Alejandra *et al*., 2007).

The aim of the present work is to evaluate some growth retardants on maize dwarf mosaic (MDMV) infectivity *in vitro* and *in vivo* which not previously been reported in Egypt. .

2. Material and Methods

1. Virus isolate:

Virus was isolated and identified by Mohamed (2007). Virus inoculum was the crude sap obtained by trituration of frozen leaves of maize (*Zea mays* L.) plants seedlings showing mosaic and stunt symptoms. Inoculation of leaves was carried out by rubbing with finger after their being dusted with carborandum as described by Rawlins and Tompkins (1936).

2. Growth retardants:

Growth retardants used in these experiments were alar, ethrel, cycocel and paclobutrazol. Different concentrations of growth retardants were prepared separately in distilled water: 50, 100, 150 and 200 ppm. All experiments were repeated twice. Five replicates were used for each treatment.

3. Effect of some growth retardants on MDMV infectivity *in vitro:*

The effect of different concentrations of some growth retardants (50, 100, 150 and 200 ppm) on the occurrence of mosaic symptoms produced by MDMV on maize (*Zea mays* L.) plants was investigated. 1 ml of the expressed sap containing virus was added to 1 ml of each of used growth retardants concentrations (50, 100, 150 and 200 ppm), mixed well. Distilled water was used as a control. Virus- growth retardants mixtures and the control were inoculated onto maize plants leaves. Data obtained were statistically analyzed according to Steel and Torrie, (1960).

4. Effect of some growth retardants on MDMV infectivity *in vivo:*

4.1. Pre-inoculation treatment:

1 ml of each of used growth retardants concentrations was rubbed on leaves of maize (*Zea mays* L.), then they mechanically inoculated with MDMV infected sap (1ml/plant), 3 days after growth retardants treatments. Distilled water was used as a control.

4.2. Post-inoculation treatment:

The former steps in pre-inoculation were applied except that, virus infected sap was applied first followed by growth retardants treatments.

3. Results and Discussion

Plant growth retardants are a group of synthetic compounds that modify plant architecture, mainly by inhibition of gibberellin biosynthesis. Growth retardants have a more general inhibitory action on isoprenoid biosynthesis in plants (Samira *et al.,* 2009; Guo *et al.,* 2010; Henriksen *et al.,* 2010; Thomann *et al.,* 2010; and Weber and Baker, 2010). Thousands of new organic compounds are synthesized each year by organic chemicals. The usual fate of such compounds is that they are described in the literature and remain as chemical curiosities on the shelf in some laboratory. Their potential usefulness in biology is not realized unless they are screened against a number of different biological systems. A number of interesting compounds were found, some of which have proved to be of value in agriculture and medicine, and also have an effective influence against virus and biotic activity. It has been found that, a number of different kinds of compounds act as growth retardants. The chemical cycocel contain a quaternary ammonium group(nitrogen atom to which are attached four chemical groups) whereas phoshon contains aphosphonium group(phosphorus atom to which are attached four groups). Growth retardants have other biological effects besides retarding stem elongation. Leaves of treated plants are frequently darker green than in untreated plants. Flowering is also accelerated in some plants following treatment with a growth retardant. The reduction in plant height following the application of several growth retardants can be overcome by treatment with a gibberellin. There is no obvious structural similarity between growth retardants and gibberellins and it is difficult to visualize how they might interact in a competitive manner at some growth site.It is believed that some of dwarfing compounds inhibit steps in the pathways leading to the formation of gibberellins. The precise nature of the inhibitory effects of the growth retardants is not known, but there seem little doubt that, their ultimate effect is on the biosynthesis of gibberellin. No endogenous growth retarding substances have as yet been recognized in plants, although quaternary ammonium compounds, such as choline, are commonly present. These compounds are active in metabolic processes, particularly in lipid metabolism and the cell membranes (Noggle and Fritz, 2002). There are a number of synthetic compounds which prevent the gibberellins from exhibiting their usual responses in plants such as cell enlargement or stem elongation. Because these compounds prevent gibberellins promoted plant growth, they are called as antigibberellins or growth retardants or dwarfing compounds. The antigibberellins or growth retardants have complicated chemical structure and are usually refered to their common trade names. Most notable of these are cycocel, phosphon, ancymidol, alar, paclobutrazol and amo-1618. It is now known that, these growth retardants show antigibberellins effect mainly by blocking certain steps of gibberellin biosynthesis in plant so that gibberellin is not made available for participation in plant growth. The inhibitory effect of antigibberellins however, can be countered by application GA_3 (Jain, 2006).

1. Effect of some growth retardants on MDMV infectivity *in vitro:*

The effect of different concentrations of some growth retardants: 50, 100, 150 and 200 ppm on mosaic symptoms produced by MDMV on maize plants is presented in Table (1) and Fig.(1). All tested growth retardants reduced the mosaic symptoms percentages (infectivity of MDMV). These percentages were reduced to 40%. Paclobutrazol was the most effective treatment giving mosaic symptoms percentages reached to 48, 44, 44 and 40 % when used with concentrations of 50, 100, 150 and 200 ppm respectively. On a statistical basis, paclobutrazol caused a significant reduction in the mosaic symptoms percentages when used with a concentration of 50, 100, 150 and 200 ppm. While cycocel caused a significant reduction in the mosaic symptoms percentages, only, when used with a concentration of 200 ppm. So, paclobutrazol was more effective in reducing the mosaic symptoms produced by MDMV on maize plants than other treatments. different concentrations of some growth retardants (alar, ethrel, cycocel and paclobutrazol) *in vitro* treatment.

2. Effect of some growth retardants on MDMV infectivity in *vivo:*

The effect of different concentrations of some growth retardants: 50, 100, 150 and 200 ppm on mosaic symptoms produced by MDMV on maize plants is presented in Table (2) and Fig.(2). In pre-inoculation experiment, all tested growth retardants reduced the mosaic symptoms percentages (infectivity of MDMV). These percentages were reduced to 32%. Paclobutrazol was the most effective treatment giving mosaic symptoms percentages reached to 40, 36, 36 and 32 % when used with concentrations of 50, 100, 150 and 200 ppm respectively. On a statistical basis, paclobutrazol and cycocel caused a significant reduction in the mosaic symptoms percentages when used with a concentration of 50, 100, 150 and 200 ppm. While ethrel caused a significant reduction in the mosaic symptoms percentages, only, when used with a concentration of 200 ppm. So, paclobutrazol and cycocel was more effective in reducing the mosaic symptoms produced by MDMV on maize plants *than* other treatments. In post-inoculation experiment, all tested growth retardants reduced the mosaic symptoms percentages (infectivity of MDMV). These percentages were reduced to 40%. Paclobutrazol was the most effective treatment giving mosaic symptoms percentages reached to 56, 52, 52 and 40 % when used with concentrations of 50, 100, 150 and 200 ppm respectively. On a statistical basis, paclobutrazol caused a significant reduction in the mosaic symptoms percentages, only, when used with a concentration of 200 ppm. Pre-inoculation treatment was more effective in reducing virus infectivity than post-inoculation treatment.

Table (1): Effect of different growth retardants on mosaic symptoms produced by MDMV on maize plants *in vtiro* treatment.

Growth retardants	Number of plants	Control	Growth retardants concentration (ppm)			
			50	100	150	200
Alar	Inoculated plants	25	25	25	25	25
	Showing mosaic symptoms	25	19	18	18	17
	% showing infection	100	76	72	72	68
Ethrel	Inoculated plants	25	25	25	25	25
	Showing mosaic symptoms	25	17	16	16	15
	% showing infection	100	68	64	64	60
Cycocel	Inoculated plants	25	25	25	25	25
	Showing mosaic symptoms	25	16	14	13	12
	% showing infection	100	64	56	52	48*
Paclobutrazol	Inoculated plants	25	25	25	25	25
	Showing mosaic symptoms	25	12	11	11	10
	% showing infection	100	48*	44*	44*	40*
Mean concentration		100	64.00	59.00	58.00	54.00
L.S.D at 5%		51.16				

* Significant difference

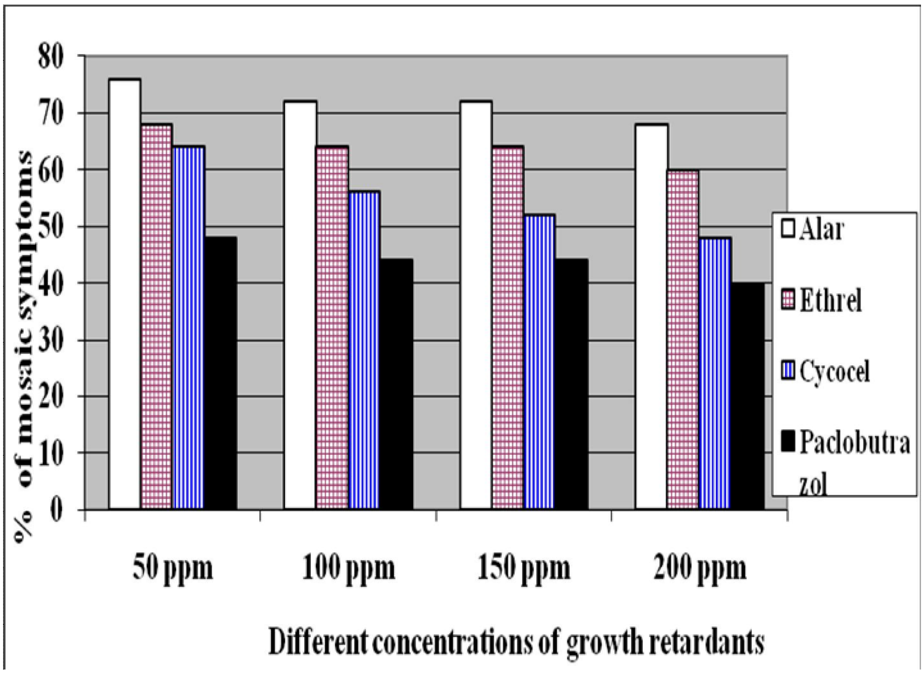

Fig. (1): % of mosaic symptoms produced by MDMV on maize plants under effect of different concentrations of some growth retardants (alar, ethrel, cycocel and paclobutrazol) *in vitro* treatment.

Table (2): Effect of different growth retardants on mosaic symptoms produced by MDMV on maize plants *in vivo* treatment.

Growth retardants	Number of plants	Control	Pre-inoculation Growth retardant concentration (ppm)				Post-inoculation Growth retardant concentration (ppm)			
			50	100	150	200	50	100	150	200
Alar	Inoculated plants	25	25	25	25	25	25	25	25	25
	Showing mosaic symptoms	25	16	16	15	14	20	20	19	18
	% showing infection	100	64	64	60	56	80	76	76	72
Ethrel	Inoculated plants	25	25	25	25	25	25	25	25	25
	Showing mosaic symptoms	25	14	14	13	12	18	17	17	16
	% showing infection	100	56	56	52	48*	72	68	68	65
Cycocel	Inoculated plants	25	25	25	25	25	25	25	25	25
	Showing mosaic symptoms	25	12	11	11	10	16	15	15	14
	% showing infection	100	48*	44*	44*	40*	64	60	60	56
Paclobutrazol	Inoculated plants	25	25	25	25	25	25	25	25	25
	Showing mosaic symptoms	25	10	9	9	8	14	13	13	10
	% showing infection	100	40*	36*	36*	32*	56	52	52	40*
Mean concentration		100	52	50	48	44	68	64	64	58.25
L.S.D at 5%			50.67				56.27			

*** Significant difference**

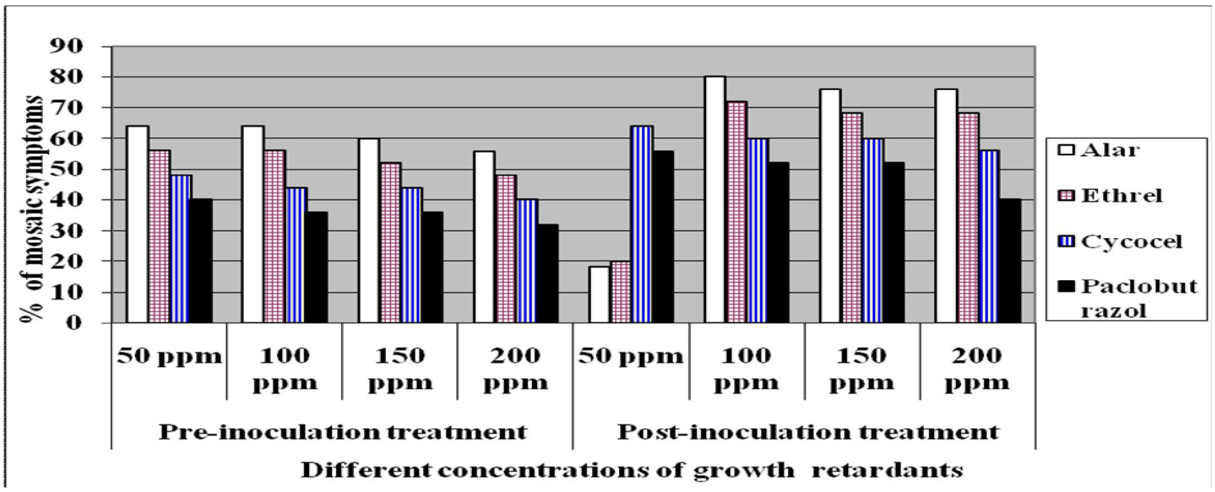

Fig. (2): % of mosaic symptoms produced by MDMV on maize plants under effect of different concentrations of some growth retardants (alar, ethrel, cycocel and paclobutrazol) *in vivo* treatment.

These results were agreement with that obtained by many investigators. Bailiss *et al.,* (1977) showed that, ethrel caused dwarfing in healthy and virus infected plants. It is suggested that ethrel stimulates the release of some substances which are directly involved in growth reduction. Benken *et al*., (1978) used ethrel to inhibit conidial germination of *Helminthosporium sativum* [*Cochliobolus sativus*] and suppress mycelial growth. Although the retardants possess no fungicidal properties, they suppressed sporulation of the pathogen. Hennighausen and Tiefenbach (1978) conducted that, cycocel was found to have neuromuscular blocking actions and, consequently, to lead to respiratory arrest in a situation of acute intoxication. The neuromuscular block has by all characteristics of a block by depolarisation. Acute toxicity of cycocel was found to differ by species as well, which was attributable primarily to differentiated sensitivity of various species to depolarising neuromuscular blockers. Hecht (1984) used ethrel, gibberellic acid, piperonylbutoxide, abscisic acid, kinetin, phytin acid and ribavirin against PVY in potato plants. Treatments decreased infectivity on average by 54.6%. Higher concentrations were more inhibitory than lower ones, with the exception of phytin acid. Treatments 6 h after inoculation were more effective. growth retardants may strongly retarded PVY translocation. Gabr *et al.,* (1985) conducted a field experiment to study the effect of various levels of cycocel and alar on the biochemical changes in tomato plants and fruits at different stages of growth. Chlorophyll a, b, total chlorophyll and carotenoids content of tomato plants increased by the application of cycocel or alar. The highest increase of concentration of chlorophyll a, b and carotenoids in tomato plants were found by spraying with 500 ppm alar or cycocel. Alar caused an increase in the percentage of total nitrogen at the different stages of growth. The concentration of P, K, Ca and Mg increased by the foliar spray of all treatments. Alar application at all used levels significantly increased the yield and also the weight of fruits. Highest plant productivity was obtained by using high concentrations of alar and cycocel. The concentration of juice, total soluble solids and vitamin C in tomato fruits increased at most of the levels added. Stein *et al.,* (1985) inoculated leaf tissue with ethrel, an ethylene-releasing compound. Estimation of resistance by size and number of local lesions was correlated with the amount of extractable virus as measured by ELISA, indicating that in the resistant tissue, virus replication, and not only the development of necrotic local lesions, is suppressed. An increase in a specific ribosomal fraction (R2), recovered by a 2-step procedure, was observed in tissues where resistance was most intense, i.e., between TMV stripes or after EMA injection. It is thought that this fraction may participate in maintaining the resistant state. Staszewicz (1988) reported that, potato plants were inoculated with potato Y potyvirus under field conditions and sprayed with ethrel at 100 ppm. PYV infection was then analyzed by ELISA. The results indicated that ethylene decreased the value of extinction and decreased PYV detection in potato leaves and tubers. Ali and Abdel-Moneim (1989) and Sahize and Ismail (2006) used cycocel and paclobutrazol for inhibition of fungal infection. Arora *et al.,* (1989) studied the incidence of tomato leaf curl virus (TLCV) in relation to varying concentrations of the 2 growth regulators applied at 4 different stages of plant growth. Cycocel [chlormequat] and alar [daminozide]. This treatment also reduced the percentage of plants affected by TLCV. Cycocel had most effect in reducing the number of virus-infected plants. Kumar and Bharti (1991) found that, application of cycocel decreased total soluble protein and free proline under stress conditions. Visedo *et al*.,(1991a), showed that, leaves treated with ethrel *in vitro* showed a senescence response, but this was followed by a necrosis that displayed an isoenzyme pattern highly similar to that of necrotic lesions induced by plum pox potyvirus (PPV) infection. It is concluded that an accelerated senescence process is involved in the induction of changes in the isoenzyme patterns of expression during the hypersensitive response of *Chenopodium foetidum* to PPV infection, and ethylene could participate in this process. Visedo *et al*.,(1991b) suggested that, ethrel could be involved in the senescence reaction produced by the virus infection. Phatak *et al.,* (1994) used cycocel for effects on the multiplication of a strain of cowpea mosaic comovirus in callus tissue derived from the stem of infected cowpea plants. Viral infectivity was reduced in callus tissues treated by cycocel. Seon *et al.,* (1999); Zhai *et al.,* (2004) and Zhu *et al.,* (2005) used paclobutrazol, alar and cycocel for virus elimination from different geographical regions using meristem tip culture. The virus-free seedlings were inspected using biological test, ELISA and SEM [scanning electron microscopy] methods. Sapatnekar and Sawant, (2001) reported that, three substances such as gibberellic acid (GA), naphthalene acetic acid (NAA) and Cycocel [chlormequat], each at 50 ppm concentrations were sprayed to tomato inoculated with tomato spotted wilt virus (TSWV) to determine the effect of growth regulators on the infectivity of the virus *in vivo*. The 3 substances were given at varying number of sprays and at different time intervals: one spray one week before inoculation, one spray one week after inoculation, 2 sprays each at one week interval given before inoculation, 2 sprays each at one week interval given after inoculation, and 2 sprays each at one week interval given before and after inoculation. The unsprayed inoculated plants served as the control. All the growth regulators inhibited TSWV infectivity *in vivo* even sprayed once either before or after inoculation. Maximum inhibition was noticed when NAA was sprayed twice before and after inoculation at weekly intervals. There was substantial increase in growth in respect of height, and fresh weights of shoots and roots. Virus assay from sprayed infected plants carried out on *Nicotiana glutinosa* indicated the inhibitory property of GA, NAA and Cycocel. Antony *et al.,* (2003) reported that, an experiment was conducted in India, to study the effects of chemical sprays and weather on virus infestation and yield. The treatments comprised 50 ppm salicylic acid; 100 ppm cycocel [chlormequat]; 6% kaolin; water; and no spray (control) at 30 days after sowing.

The inhibitory effect of growth retardants on the virus activity may be due to their effect on host susceptibility, or to their inhibitory on virus replication, or to a stimulation of some substances involved in virus localization in the host plant (Van Loon, 1979). Suppression of growth by paclobutrazol occurs because the compound blocks three steps in the terpenoid pathway for the production of the hormone gibberellin by binding with and inhibiting the enzymes that catalyze the metabolic reactions. One of the main roles of gibberellins is the stimulation of cell elongation. When gibberellin production isinhibited, cell division still occurs, but the new cells do not elongate. The result is shoots with the same numbers of leaves and internodes compressed into a shorter length. However, recent research has demonstrated that blocking a portion of the so-called terpenoid pathway causes shunting of the accumulated intermediary compounds above the blockage. The consequence is increased production of the hormone abscisic acid and the chlorophyll component phytol, both beneficial to plant growth and health. The unique structure of paclobutrazol that allows it to bind to an iron atom in the enzymes essential for the production of gibberellins also has the capacity to bind to enzymes necessary for the production of steroids in fungi as well as those that promote destruction of abscisic acid. The consequence is that paclobutrazol treated trees have greater tolerance to environmental stresses and resistance to fungal diseases. Morphological modifications of leaves induced by treatment with paclobutrazol such as smaller stomatal pores, thicker leaves, and increased number and size of surface appendages on leaves may provide physical barriers to some fungal, bacterial, and insect infestations (William, 2008).

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