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Studies on phyllosphere Fungi
IV. Effect of magnesium chloride on phyllosphere population of virus infected (PVX) and healthy plants of *Lycopersicum esculentum* Mill. cv. Best of all

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Abstract

Phyllosphere and phylloplane mycoflora of healthy and potato virus X (PVX) infected plants of *Lycopersicun esculentum* in relation to the treatment of different concentrations of magnesium chloride has been investigated. 250 ppm $MgCl_2$ level resulted to the maximum fungal population on the leaf surface of healthy and diseased plants. 125 ppm concentration of $MgCl_2$ on the other hand favoured the maximum fungal colonization on phylloplane region in both healthy and diseased plants. In both, healthy and diseased plants, 125 ppm concentration of $MgCl_2$ proved equally good for growth of plants and the chlorophyll content of the leaf. The variation in the leaf mycoflora in the present study seems to be governed by a number of factors operating simultaneously.

INTRODUCTION

Pretreatment of leaves of *Vicia faba* with substances that changed the permeability of the host plasma-membrane enhanced the susceptibility to attack by *Botrytis fabae* (Sol, 1966). Sol (1966, 1967, 1968) treated the leaves of *V. faba* with sucrose, potassium chloride lanthanum chloride and decenyl succinic acid and in all the cases reported an increase in lesions per unit area. Heuvel (1969, 1970) reported antagonistic effects of epiphytic leaf micro-organisms of leaves infected by *Alternaria zinniae*. Mishra and Srivastava (1971b) observed a decreased phyllosphere mycoflora of *Petunia hybrida* with an increased virus (CMV) infection. Pretreatment of leaves with suitable substances changes the physiological behaviour of leaf cells and consequently the parasitic and saprophytic leaf mycoflora are appreciably affected. The studies, however, regarding phyllosphere mycoflora with respect to virus infection and trace element treatment are still not properly undertaken.

Variation in the magnesium level may affect chlorophyll status of leaf and thereby the leaf environments which govern the microbial population of the leaf. With this assumption the present work has been undertaken to investigate the effect of magnesium on the leaf surface mycoflora, virus infection and the interrelationship between the latter two.

MATERIALS AND METHODS

Lycopersicum esculentum Mill. cv. Best of all raised in sand culture, was used in the present study. The virus infected plants were obtained by inoculating the Potato Virus X (PVX), maintained on *Nicotiana tabacum* cv. White Burley, in insect proof chamber. Nutrient solution with varying Mg Cl_2 levels, viz., 50, 125, 250, and 500 ppm was prepared by using Hoagland and Snyder four salt solution (See Mclean and Cook, 1958). One ml saturated solution of micro-elements per litre of above solution was added (Chesters and Street, 1948) and the pH was adjusted to 7 before use. 50 ml of above solution per plant was supplied through irrigation twice a week. On alternate weeks sterilized distilled water was also supplied to the plants. To control CU was supplied equal amount of the standard solution.

Ten days old seedlings of equal size of *L. esculentum* were transplanted in internally wax-coated earthen pots containing thoroughly washed sand. Ten plants were raised for each healthy (H) or virus infected (D) set. To get infected plants the PVX was inoculated on the aerial parts of the plants. The carborundum powder was dusted before inoculation on leaves and the inoculum was rubbed gently with fore--finger.

The phyllosphere and phylloplane mycoflora were assessed as described by Mishra (1971a). The nutrient medium in the isolation of fungi was potato — dextrose — agar medium. The phyllosphere and phylloplane population was expressed on the basis of per square cm area of the leaf in the former and the number of fungal species in the latter. Infection percentage of phylloplane was also calculated from the data obtained in the present study.

Chlorophyll concentration of leaf was determined by undirectional paper chromatography by using Whatman paper No. 24 and by densitometer. The substitute standards were prepared and calculations of chlorophyll a and b were established (Peach and Tracey, 1955) and were expressed in μ g/2 g fresh weight of the leaf.

The virus concentration in the sap of the host plant was obtained by counting the local lesions produced on the opposite leaves of *Chenopodium amaranticolor* Coste et Reyn. Growth data (length of fresh shoot, dry and fresh weight of the shoot), virus concentration, and chlorophyll concentration of each set were determined when the plants were 60 days old.

RESULTS AND DISCUSSION

Eighteen fungal species were obtained from phyllosphere of healthy and virus infected sets amongst which *Phycomycetes* were represented by 2 species, *Ascomy-*

cetes by 1 species. *Deuteromycetes* by 12 species and *Mycelia sterilia* by 3 species (Table 1).

Aspergillus terreus (50 D), *A. niger* (125 H), *Curvularia tetramera* (125 D), *Curvularia lunata* (250 H), black sterile fungus (250 D), *Mucor hiemalis* (500 H) and *Aspergillus flavus* (500 D) were most specialized isolates and could only be isolated from the sets mentioned in the brackets (Table 1). *Cladosporium epiphyllum* (500 — H, D), *C. herbarum* (CU—H, D, 50—H, D and 250—H, D), *Aspergillus sydowi* (125 H), and *A. fumigatus* (125 D) were dominant species. *Cladosporium herbarum* was least affected with the various levels of magnesium, used in the present study and was isolated throughout from all the sets (Table 1). The highest and lowest number of fungal species was exhibited by 125 and CU ppm Mg levels respectively in healthy sets whereas these values in diseased set were from the CU and 500 sets respectively (Fig. 1).

Nine fungal species were cultured from the phylloplane of healthy and diseased sets, kept for all the magnesium concentrations and control plants. *Phycomycetes*

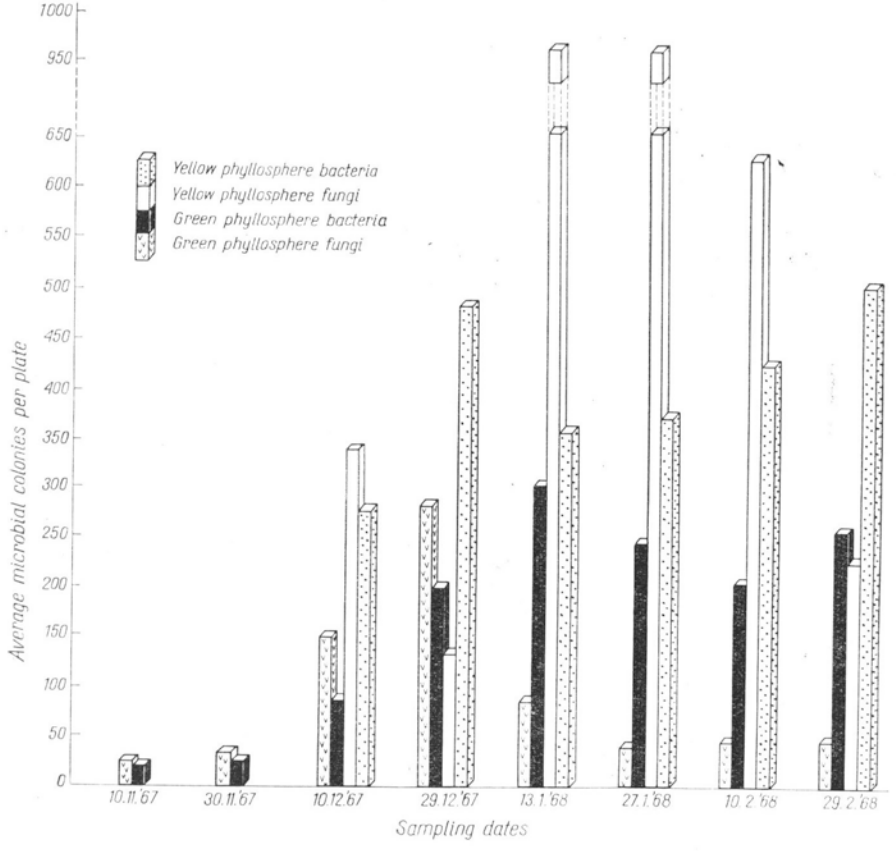

Fig. 1. Phyllosphere ($X10^2$) and phylloplane (infection percentage) fungal population of healthy (H) and virus infected (D) *Lycopersicum esculentum* when treated to different concentrations of $MgCl_2$

represented by 1 species, *Deuteromycetes* by 7 species and *Mycelia sterilia* by 1 species, were recorded. *Rhizopus nigricans* (=*R. stolonifer*) (CUD, 50—H, D, 125 H), *Aspergillus niger* (CUH) and *A. ustus* (125 D, 250—H, D, 500—H, D) were found to be dominantly associated species in the sets indicated in the brackets. No fungus was found to be specific to any particular treatment (Table 1).

Fungal population in phyllosphere region was appreciably affected by magnesium levels. In healthy control set the lowest fungal population was recorded. Increasing Mg^{++} level in healthy sets resulted in an increase in population up to 250 ppm Mg^{++} level and thereafter a decreasing tendency was observed. In diseased sets, the lowest fungal population was noted in control set. Like healthy one, in diseased set also, increasing Mg^{++} level brought to an increase in fungal population, reaching its maximum at 250 Mg level and a marked decrease was noted afterwards (Fig. 1). The number of fungal species, isolated from H set from CU — 500 Mg levels was found to be 3, 3, 7, 6 and 5 whereas at corresponding concentrations in D set these values were 2, 4, 7, 9 and 10 respectively (Table 1).

The phylloplane region exhibited the pattern, similar to phyllosphere and 3, 5, 6, 4, and 3 species were cultured from healthy plants treated with CU, 50, 125, 250 and 500 ppm Mg levels respectively, whereas 3, 3, 5, 5 and 6 fungal species were isolated from diseased plants of the corresponding concentrations of Mg^{++} respectively (Table 1). Infection percentage of phylloplane inoculum (leaf pieces) was found to be 80, 85, 90, 100 and 100 in CU — 500 Mg levels in healthy sets and 90, 90, 100, 100 and 100 in diseased sets of corresponding concentrations of Mg respectively (Fig. 1).

One hundred and twenty-five ppm concentration of magnesium proved the best for fungal colonization on leaf surface of healthy plants because highest number of fungal isolates could be isolated from phyllosphere and phylloplane regions of this set. On the other hand, in diseased tomatoes the pattern was reverse. In control set the number of fungal species was much less. With an increase in Mg^{++} level, the number of fungal isolates also increased up to 125 ppm and it decreased gradually afterwards, whereas, in virus infected sets, a continuous increase was always noted (Table 1).

The virus concentration — average number of local lesions produced on 20 leaves of *Chenopodium amaranticolor*) was recorded as 90, 270, 320, 200, 110 from CU to 500 ppm sets respectively. The shoot sap of diseased plants, irrigated with nutrient solution containing 125 ppm Mg^{++} level, yielded the maximum virus concentration (Fig. 2). The effect of various Mg^{++} levels in solution also affected the morphological set up of the healthy and diseased plants in a regular pattern. Maximum values for height, fresh and dry weight of shoot were recorded from 125 ppm set in healthy plants whereas minimum values were observed in the set where standard nutrient solution was supplied. In diseased plants, however, the maximum and the minimum values for fresh and dry weight of shoot were obtained from 125 and control sets respectively. Similar was the case with shoot height which was highest in 125 ppm and lowest in control in diseased plants (Fig. 2).

Table 1

Phyllosphere and phylloplane mycoflora of healthy and virus infected *Lycopersicum esculentum* in relation to magnesium treatment

Fungal species	Magnesium in level ppm									
	CU		50		125		250		5000	
	H	D	H	D	H	D	H	D	H	D
Rhizopus nigricans (=*R. stolonifer*)	—/+	—/++	—/++	—/++	—/++				+	+
Mucor hiemalis										+
Aspergillus nidulans			+			+		+		+
Phoma species		—/+	—/+	—/+		—/+	—/+	—/+		—/+
Aspergillus fumigatus			+		+	++		+		+
A. sydowi		+		+	++		+	+		+
A. flavus				—/+				—/+	—/+	+/+
A. terrus				+						
A. niger	—/++				+/+		—/+	—/+		
A. ustus	—/+	—/+	—/+		—/+	—/++	—/++	+/++	—/++	+/++
Penicillium sp. 1	+				+	+		+		
Cladosporium epiphyllum			—/+		+/+	+/+	+/+	—/+	++	++
C. herbarum	++	++	++	++	+	+	++	++	+	+
Curvularia tetramera						+				
C. lunata					—/+	—/+	+			—/+
Alternaria tenuis (=*A. alternata*)				+					+	
A. solani									—/+	—/+
White sterile fungus	+		—/+		—/+	+/+	+	+	+	+/+
Grey sterile fungus					+		+	+		
Black sterile fungus								+		
Total No. of fungi	3/3	2/3	3/5	4/3	7/6	7/5	6/4	9/5	5/3	10/6

Denotions: ++ dominance; + presence; — absence; Numerator Phyllosphere fungi; Denominator Phylloplane fungi
H — healthy, D — virus infected

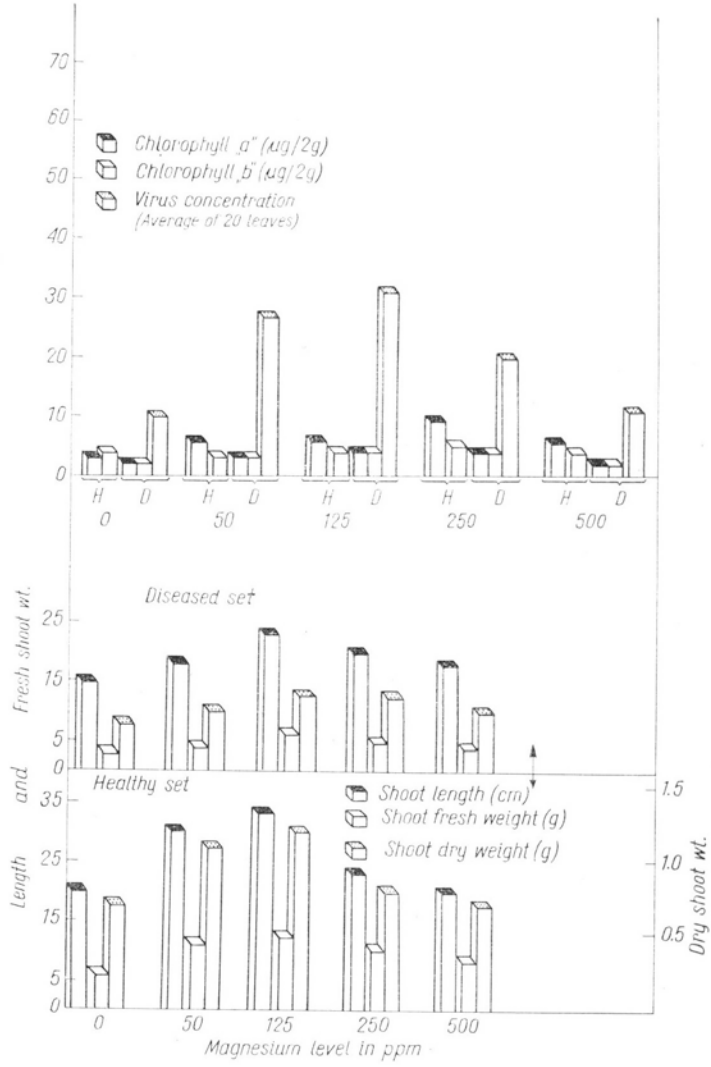

Fig. 2. Chlorophyll content, length, fresh and dry weight of shoot of healthy and diseased plants of *Lycopersicum esculentum* and virus concentration of diseased plants

In the healthy plants the maximum values for chlorophyll a and b were obtained in the sets 250 ppm respectively whereas minimum values for chlorophyll a and b were recorded from the set where no Mg was supplied and 50 ppm sets respectively. In infected plants, on the other hand, the maximum values for chlorophyll a and b were recorded from 125 and 250, and 125 ppm sets respectively. The minimum values in diseased plants for both the chlorophylls were imparted by control set alone (Fig. 2).

Appreciable variation in the phyllosphere and phylloplane is exhibited in mycoflora with respect to treatment of different levels of magnesium. Magnesium, an im-

portant constituent of chlorophyll, affects the chlorophyll content of the leaf. As it is evident from the results of present investigation, the chlorophyll content is directly proportional to the amount of magnesium applied to a certain degree (250) and 500 ppm in the case of healthy and virus infected plants respectively. The magnesium requirement of healthy and diseased plants thus differed. The growth of the plant is also affected by the treatment of magnesium (Fig. 2).

The phyllosphere and phylloplane mycoflora, which is governed by the plant and more particularly by the leaf condition, is favourably affected by the concentration of the magnesium suitable for the plant growth. The higher concentrations, unfavourable for the plants, also affected the leaf surface mycoflora adversely. The effect of magnesium on the phyllosphere and phylloplane mycoflora may be direct through the supply of the element in the form of nutrient to the organisms.

The virus infection is appreciably affected by the concentration of magnesium. 125 ppm favoured the maximum lesions and lower and higher concentrations affected the virus multiplication adversely. No clear correlation is observed between the virus lesions per unit area and the leaf surface fungi except that higher concentration of magnesium affected the virus and the leaf surface fungi nearly alike. Mishra and Srivastava (1971b), however, reported a decrease in phyllosphere fungi with an increase in the virus lesions on *Petunia hybrida*. This different condition in the present study is obviously due to the interpolation of one more factor i.e. magnesium supply to the plants which affected the growth of the plant and the virus multiplication and thereby the physiological set up of the plants.

Chlorophyll content and length and fresh and dry weight of shoot of the healthy and diseased plants were affected directly by the application of magnesium. 125 ppm Mg^{++} concentration proved much favourable for the growth of the plants in healthy and diseased sets whereas 250 ppm was best for chlorophyll content. Virus concentration was most suitably favoured by 125 ppm and the leaf surface fungi were the maximum in both H and D sets. In case of 250 ppm Mg^{++} level, chlorophyll, therefore, seems to affect the leaf surface fungi directly. This is but expected in view of the fact that leaf-exudation, the primary factor, responsible for leaf surface mycoflora is affected by photosynthetic activity of the cell and the permeability of the leaf tissue. The higher photosynthetic activity in presence of high chlorophyll content coupled with possible decrease in permeability of the leaf cells due to virus infection in diseased cells possibly affected the leaf surface flora alike and the uniform pattern in the fluctuation in the leaf surface mycoflora was encountered. Higher population in diseased set may possibly be due to the increased leakage from the leaf surface due to increase in the permeability of the diseased cells which favoured the growth of the saprophytes.

The condition, however, is more complex due to interaction of various factors in the present study and a clearer picture may be obtained if individual factors such as magnesium effect and the virus concentration were taken into consideration.

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Badania nad grzybami liściowymi

IV. Wpływ chlorku magnezu na populacje mikroorganizmów liści pomidorów odm. 'Best of all' roślin zdrowych i zainfekowanych wirusem (PVX)

Streszczenie

Celem przeprowadzonych doświadczeń było zbadanie mikroflory liściowej pomidorów odmiany 'Best of all' i zmian, jakie w niej zachodzą pod wpływem chlorku magnesu stosowanego w różnych stężeniach.

Badana była mikroflora pomidorów zdrowych oraz zainfekowanych wirusem ziemniaczanym X (PVX).

Maksymalną liczbę gatunków występujących na powierzchni liści zarówno roślin zdrowych, jak i zakażonych wirusem obserwowano przy użyciu 250 ppm $MgCl_2$, natomiast koncentracja 125 $MgCl_2$ sprzyjała największej kolonizacji grzybów.

Najlepszy wzrost liści jęczmienia, jak również największą zawartość chlorofilu w liściach, otrzymano przy użyciu 125 ppm $MgCl_2$, a uzyskane wartości były równie wysokie u roślin zdrowych, jak i porażonych wirusem.

Zmienność w mikroflorze liściowej badanych roślin zależy od licznych współdziałających czynników.