Effect of Nickel on Multiplication of Potato Virus X in Tomato Plant

Nutritional changes in the condition under which the plants are grown affect the growth of the host-plant as well as the concentration of the virus in them. A survey of the literature reveals that a number of studies have been carried out whith micro and macronutrients in several host virus combinations which cover mainly multiplication of viruses in relation to host growth. Such studies with nickel are lacking. The experiments described here were performed to determine the effect of nickel nutrition on host growth, potato virus X multiplication and nitrogen and free amino acid levels in tomato plants.

The methods of raising the seedlings of tomato (*Lycopersicum esculentum* Mill. Cv. Marglobe) and preparing the potato virus X inoculum were the same as described by SINGH and SINGH[1]. Tomato seedlings of uniform size were selected, washed with tap water and then thoroughly rinsed in distilled water. After washing, each of them was transplanted singly in 10 cm (paraffin coated) clay pot filled with $^1/_2$ kg of purified quartz sand. 50 ml of nutrient solutions of Hoagland and Snyder (see MCLEAN and COOK[2]) containing 0.5 ml of micronutrient (CHESTER and STREET[3]) and miliequivalents (meq) of nickel sulphate, pH 7, were added to each pot twice a week. During the intervening period, the necessary amount of distilled water was also added to the pots to avoid drying of the plants. After 4 days of transplantation, the tomato plants were inoculated with potato virus X using 600 mesh of carborundum as abrasive. The leaves of control plants were also rubbed with carborundum mixed with distilled water. The growth of the plants, fresh and dry weights and virus concentration were noted after 40 days. At this time, the total nitrogen and free amino acids were also estimated in tomato leaves. The total nitrogen and amino acids estimations were done from oven-dried (93 °C) leaf material. For nitrogen, the sample was digested by the micromethod described by DONEEN[4] and the contents was estimated by using a colorimetric technique described by SNELL and SNELL[5] using a Hilger Pattern Biochemical Absortiometer. Separation, identification and quantitative estimation of amino acids was carried out by paper chromatographic technique[6], using Whatman No. 1 chromatographic paper. Amino acids from experimental samples were identified by comparing with standards which were run simultaneously. The quantity of each amino acid was estimated by Densitometer Type CM 11/5 Sr. no. 111 of Toshniwal.

Test plants were grown separately in nutrient solutions containing different levels of nickel (0, 7, 14, 21, 28 and 35 meq). Growth response to the various nickel levels showed a gradual increase in height, fresh and dry weights from Ni 0 up to Ni 14 meq level, above which growth decreased (Table). The growth of tomato plants at these levels was normal and the leaves had green coloration with brilliant mosaic symptoms. Systemic symptoms appeared approximately at the same time at all the Ni levels except at Ni 35. Symptoms expression, however, varied with the treatment. Plants grown at Ni 35 level showed effects of excess nickel. The effect of nickel excess started after the 3rd week of nutritional treatment, and it became progressively more severe with time. Plants developed chlorosis of the young leaves with a severe necrosis of the leaves and petioles. During the experi-

[1] R. SINGH and R. SINGH, Indian J. Microbiol. *3*, 61 (1971).
[2] R. C. MCLEAN and R. T. COOK, *Plant Science Formulae* (McMillan and Co. Ltd., New York, USA 1951).
[3] C. G. C. CHESTERS and H. E. STREET, Ann. appl. Biol. *35*, 443 (1948).
[4] L. D. DONEEN, Pl. Physiol. *7*, 711 (1932).
[5] F. D. SNELL and C. T. SNELL, *Colorometric Method of Analysis* (D. Van Nostrad, New York 1949), vol. 2.
[6] I. SMITH, *Chromatography and Electrophoretic Techniques* (William Heinemann Medical book Ltd., London 1960).

Effect of nickel nutrition on tomato plant growth, total nitrogen, total free amino acids (both on dry wt. basis) and potato virus X concentration

Nickel (meq)	Treatment	Stem height (cm)	Fresh shoot wt. (g)	Dry shoot wt. (g)	Nitrogen (%)	Free amino acids (%)	Local lesions [c]	
0	Healthy	29.5	7.44	0.491	3.28	1.010		
	Diseased	21.9	3.94	0.245	4.00	1.025	91	
7	Healthy	29.8	9.69	0.578	3.52	1.300		
	Diseased	23.1	4.40	0.314	4.02	1.415	99	
14	Healthy	34.1	10.31	0.656	4.95	1.795		
	Diseased	26.7	5.81	0.326	4.85	1.715	174	
21	Healthy	30.3	6.64	0.572	4.35	1.565		
	Diseased	26.7	5.00	0.230	4.25	1.480	132	
28	Healthy	27.2	6.30	0.528	4.29	1.310		
	Diseased	20.0	2.00	0.228	4.22	1.238	129	
35	Healthy	24.2	5.75	0.481	4.21	1.233		
	Diseased	18.5	1.58	0.218	4.19	1.118	122	
F calculated value							F tabulated value 1%	5%
	Inoculated	105.7[a]	21.53[a]	326.00[a]	1.12	1.16	16.26	6.61
	Treatment	18.5[a]	2.51	7.37[b]	6.23[b]	38.63[a]	10.97	5.05
C.D.	1% level	4.322	5.644	0.1128	1.0080	0.938		
	5% level	2.756	3.599	0.0719	0.6427	0.619		

[a, b] Significant at 1% and 5% level. [c] Average number of local lesions, produced on 20 leaves of *C. amaranticolor*.

mental period, 25% of plants died. The plants which were grown at Ni 21 and Ni 28 levels had also developed slight chlorosis with necrotic lesions on the leaves, petiol and stem. Such lesions were more in tomato plants grown at Ni 28 than those at Ni 21 level. The stem of these plants were slightly bent at one side. The disease symptoms were very mild mosaic with severe chlorosis of young leaves at Ni 28 and Ni 35 level, but at Ni 21 level the disease symptoms consisted of mild chlorosis with mosaic.

The results given in the Table show that levels of nickel which increased vegetative growth also increased the potato virus X concentration (up to Ni 24). Excess of Ni caused stunting of the plants. Increase in level of Ni caused a proportionate stunting of the plants. Its effect on virus concentration, however, was not proportionate. It has also been observed that the nitrogen and free amino acid contents were higher in healthy tomato leaves than in diseased at each treatment. The higher content of nitrogen and amino acid were noted in healthy tomato plant leaf grown at Ni 14. Twelve amino acids viz. arginine, aspartic acid, glycine, glutamic acid, alanine, threonine, methionine, valine, prolin, tryptophan, isoleucine and leucine were observed in healthy and diseased tomato leaves. The concentration of each amino acid varied in the healthy and diseased leaves growing at the same level, but the number remained the same[7].

Zusammenfassung. Der Effekt von Nickel (Ni) auf die Vermehrung des Kartoffelvirus in der Tomatenpflanze (Lycopersicum esculentum Mill cv. Marglobe) wurde untersucht. In Abhängigkeit vom Nickel-Spiegel nahm sowohl das vegetative Wachstum der Pflanze, der Virus, wie auch der Gesamtgehalt an Stickstoff und freien Aminosäuren zu.

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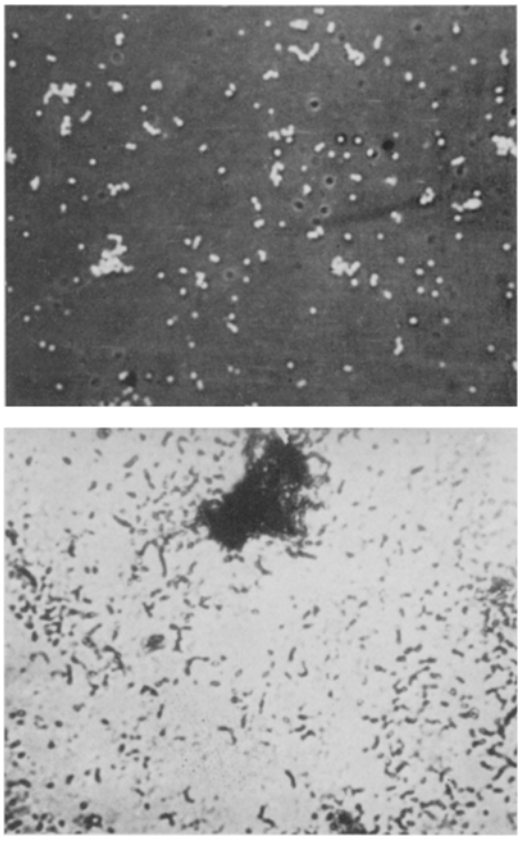
Endosymbiotic Microorganisms in *Cletus signatus* Walker (Coreidae: Heteroptera)

Symbiosis between insects and microorganisms is well known[1]. The bug *Cletus signatus* Walker harbours its bacterial symbiotes in the mycetome, which is associated with the last section of the midgut, the epithelial cells of which evaginate to form 2 rows of caeca[2]. Studies dealt with here were conducted to discover various characteristics of symbiotic microorganisms of *C. signatus*, their mode of transmission to progeny, elimination leading to aposymbiotic insects and their probable functions.

Symbiotes were coccoid rod-shaped bacteria both in culture media and tissue (Figures A and B), gram-positive, capsulated, spore-forming and motile. They hydrolysed starch, gelatin and casein and utilized sucrose, glucose and mannose without producing gas, but did not utilize xylose, arabinose and lactose. Citrate test, reduction of nitrates, production of ammonia from peptone, Voges Proskauer test, methyl red test, methylene blue reduction were positive, while indole test and lecithinase tests were negative. They fixed nitrogen in JENSEN's[3] nitrogen-free medium (5.6 mg/g sugar in 14 days). They were fatal to guinea-pigs in hypodermal injection of a heavy dose. On the basis of above characteristics the bacteria were identified as very closely related to *Bacillus cereus* Frankland, and Frankland[4] with certain differences, and were tentatively named *Bacillus cereus* var. *signatus*. Serological tests conducted confirmed that the cultured bacterium was similar to those found in various tissues.

Transmission of bacteria was found to occur through the ovaries, since the smears of ovaries, eggs and haemolymph showed the presence of bacterial symbiotes. The pathway of transmission is thus from mycetone to haemolymph and then to ovaries and developing oocytes. Symbiotes were also present in the smears of haemolymph of male bugs, malpighian tubes of both male and female bugs, and testes, although in much less numbers. The cause of such a distribution is not known at present. BUCHNER's[1] generalization that transmission in heteropterons having mycetones in the intestine is always external does not hold true in this case.

Cultured symbiotes were found autotrophic to all essential vitamins, when reared on a vitamin-free medium.



Photomicrographs showing a) cultured symbiotes; b) symbiotes in smear of mycetomic tissue; ×500.

[1] P. BUCHNER, *Endosymbiosis of Animals with Plant Microorganisms*, engl. edn. (Wiley Interscience, New York 1965), p. 909.
[2] M. K. NARULA, Doctoral Thesis, Indian Agricultural Research Institute, New Delhi–12, India (1969).
[3] H. L. JENSEN, Proc. Soc. appl. Bact. *14*, 89 (1951).
[4] R. S. BREED, E. G. D. MURRAY and N. R. SMITH, *Bergey's Manual of determinative Bacteriology*, 7th edn. (1957), p. 1094.