it appears that the decrease in the protein content as a result of norgestrel administration might be due to its antiestrogenic profile.

Glycogen, a carbohydrate reserve, was also found to be diminished significantly in treated group. This lowering of the level of glycogen may be due to decreased rate of synthesis because of antiestrogenic action of the steroid as glycogen is an estrogen dependent substrate[8]. However the breakdown of glycogen does not appear to be through EMP pathway since the level of lactic acid was also lowered significantly. Similarly, no evidence is available in this study for anabolic activity, where lactic acid was being rapidly utilized through Krebs cycle at a rate higher than the normal. This contention is further supported by the values of acid soluble phosphorus which showed no change after treatment with this drug. Since the production of energy means trapping of Pi for the formation of ATP.

To sum up the position, it may be stated that long-term treatment with norgestrel in continuous low doses induced a general lowering of the metabolic status of the Fallopian tube, which may be one of the reasons of its contraceptive efficacy along with the other major mechanism of action like general retardation of metabolic activity of uterus and ovary[9–10], etc.

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1. Edgren, R. A., Jone, R. C., Clancy, D. P. and Nagra, C. L., *Jr. Reprod. Fertil.*, 1968, Suppl. 5, 13.
2. Sen, A. (late), Sanwal, P. C., Srivastava, A. K., Pande, J. K., Dasgupta, P. R. and Kar, A. B., *Contraception*, 1970, 2, 59.
3. Sanwal, P. C., Pande, J. K., Dasgupta, P. R., Kar, A. B., and Setty, B. S., *Steroids*, 1970.
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, 193, 285.
5. Montgomery, R., *Arch. Biochem.*, 1957, 67, 378.
6. Hawk, P. B., Oser, B. L. and Summerson, W. H., *Practical Physiological Chemistry*, McGraw-Hill Co., N.Y., 1954.
7. Lerner, L. J., Hilf, R., Turkheimer, A. R., Michel, I. and Engel, S. L., *Endocrinology*, 1966, 78, 111.
8. Gross, M., *Fertil. Steril.*, 1961, 20, 572.
9. Mears, E., Vessey, M. P., Andolsek, L. and Oven, A., *Brit. Med. J.*, 1969, 2, 730.
10. Foss, G. L., Sevendsen, F. K., Fotherby, K and Richards, D. J., *Ibid.*, 1968, 4, 489.

EFFECT OF ARHAR MOSAIC VIRUS ON NODULATION, NITROGEN VALUE AND NITROGEN FIXATION BY SANNHEMP (*CROTALARIA JUNCEA* L.)

SANNHEMP is an important leguminous crop which is mainly cultivated for green manure, fodder and for fixing atmospheric nitrogen to enrich the soil. Several plant viruses are reported to infect this crop in nature but their effect on its nitrogen value nodulation and nitrogen fixation has not been studied. The present communication reports our observations on arhar mosaic virus strains (ASM and AMM) infection on the above aspects of Sannhemp (*Crotalaria juncea* L.).

Arhar mosaic virus strains (ASM and AMM) were originally isolated from field grown arhar [*Cajanus cajan* (L.) Millsp.] and their culture maintained in arhar cv. S-8 in glass house. Both the strains infect *Crotalaria juncea* L. and produce mosaic mottling with reduced growth but ASM is more severe than AMM in its reaction. Strain ASM has a DEP 1 : 1000,000, TIP 80° C and longevity *in vitro* 11 days while the other strain AMM has a DEP 1 : 1000,000, TIP 60° C and longevity *in vitro* 16 days at room temperature (Min. 48–62, Max. 48–100° F). Both the isolates are infectious only to leguminous host. The inoculum of both the strains was prepared separately by macerating the young infected leaves in a mortar and squeezing the pulp through muslin cloth. The infective sap was diluted 1 : 10 with distilled water before use. Clay pots of 20 cm diameter containing 5 kg of sterilized mixture of sand, loam and compost (1 : 1 : 2) were taken. Sixty such pots having five seedlings of Sannhemp in each were divided into three sets of 20 pots. Seven-day old seedlings of first and second sets were inoculated separately with ASM and AMM strains, respectively, while the third set was left as healthy control. The plants were harvested after 45 days of inoculation. At the time of harvest data on growth, nodulation, nitrogen value of plant and nitrogen percentage of soil were recorded. The growth data was recorded as described by Singh and Bhargava[1] and nodulation by the methods given by Tu *et al.*[2]. Total nitrogen of composite dried plant or soil material was estimated by a colorimetric procedure described by Snell and Snell[3] and Misra[4], respectively, by using hilger pattern Biochemical Absorptiometer with filter No. 43. The nitrogen value of plants is the total sum of the nitrogen present in different parts of the plant and the nitrogen added or fixed to the soil which is obtained from the difference between the nitrogen present in the soil at the time of harvest and nitrogen of pot soil at the time of seeding.

TABLE I

Effect of arhar mosaic virus on growth, nodulation and nitrogen content of Sannhemp*

	Treatment				
	Healthy	ASM	**	AMM	**
Height of shoot (cm)	74·9	54·1	−27·8	55·6	−25·8
Length of root (cm)	26·5	24·7	−6·8	26·0	−1·9
Fresh wt. of shoot (g)	12·83	9·49	−26·0	−10·23	−20·3
Fresh wt. of root (g)	2·47	1·21	−51·0	1·31	−47·0
Dry wt. of shoot (g)	2·72	1·96	−27·9	1·65	−39·3
Dry wt. of root (g)	0·38	0·43	+13·2	0·38	00·0
No. of nodules/plant	24·0	4·0	−83·3	10·0	−58·3
Fresh wt./ nodule (g × 10^{-2})	0·200	0·260	+30·0	0·255	+27·5
Dry wt./ nodule (g × 10^{-2})	0·035	0·030	−14·3	0·030	−14·3
Volume/nodule (ml × 10^{-2})	0·250	0·280	+12·0	0·270	+8·0
% Total nitrogen in shoot	2·17	2·50	+15·2	2·35	+8·3
% Total nitrogen in root	1·90	1·98	+4·2	2·22	+16·8
% Total nitrogen in nodule	2·76	3·00	+8·7	2·98	+8·0
Nitrogen value (g)	0·08942	0·06060	−32·2	0·056151	−37·2
Total % soil nitrogen	0·68	0·60	−11·8	0·63	−7·4
Total nitrogen added to soil	0·46	0·38	−17·4	0·41	−10·9

* % nitrogen/100 mg dry wt.
** % increase (+) or decrease (−) over healthy plant.

Results presented in the table indicate that both the virus strains reduced the shoot height, root length, fresh and dry weight of shoot. Although the diseased root of sannhemp showed a reduction in fresh wt. but its dry wt. was higher in comparison to healthy ones. Both the strains reduced the nodule number per plant and their dry weight but increased the nodule size and fresh weight. Arhar mosaic virus increased total nitrogen content in shoot and root than their healthy counterparts, but the total nitrogen value per plant was higher in healthy than infected ones. Before seeding the pot soil contained 0·22% nitrogen but at the time of harvest percentage of soil nitrogen was increased. Maximum increase was noted with healthy sannhemp plants followed by AMM and ASM infected plants.

Arhar mosaic virus infection reduced the growth and fresh weight of sannhemp plants but increased the dry weight of the root in comparison to healthy plants. Stunting of growth is the most common symptom produced by the virus infection[5]. The virus infection decreased the nodule number and their dry weight but increased their size and fresh weight. A reduction in nodule number was also reported in white clover plants infected with clover phyllody virus[6,7], and soybean infected with soybean mosaic virus (SMV) and pod mottle virus (BPMV)[2]. The reduction in number of nodules was concomitantly associated with an increase in their fresh weight and size in virus infected plants. Tu *et al.*[2] suggested that reduced nodulation in infected plants was probably caused by viral multiplication leading to physiological changes of reduced photosynthesis, increased respiration and the imbalance of auxins and enzyme levels. The higher dry weight in root and lower in nodule of diseased plants can be explained as suggessed by Gibson[8] that it might be due to complex auxin relationship or competition in roots and nodules for carbohydrates. The increase or decrease in dry weight seems to be related to carbohydrate accumu-

lations. A higher percentage of nitrogen was found in the nodules of infected plants. Similar findings were also reported by Tu *et al.*[11] and Rajagopalan and Raju[9] in SMV infected soybean and in *Dolichos* enation mosaic virus infected *Dolichos lab lab,* respectively. It is believed that this higher nitrogen percentage in the nodules is due to insufficient utilization of nitrogen by infected plants. Arhar mosaic virus reduced the total nitrogen value of plants. According to Orlob and Arny[10] this decrease is due to the inhibition of protein synthesis or increased rate of degradation of proteins in infected plants.

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Gorakhpur, *February* 8, 1974.

1. Singh, R. and Bhargava, K. S., *Phytopath, Z.*, 1966, 55, 26.
2. Tu, J. C., Ford, R. E. and Quiniones, S. S., *Phytopathology,* 1970 *a,* 60, 518.
3. Snell, F. D. and Snell, C. T., *Colorimetric Method of Analysis,* D. Van Nostrand, New York, 1949, p. 2.
4. Misra, R., *Ecology Work Book,* Oxford IBH, Publishing, Co., New Delhi, 1968.
5. Bos, L., *Sympotoms of Virus Diseases in Plants,* 2nd Ed., Centre for Agricultural Publishing and Documentation, Wageningen, 1970.
6. Joshi, H. U. and Carr, A. J. H., *J. gen. Microbiol.,* 1967, 49, 385.
7. — and Jones, D. G., *Ibid.,* 1967, 47, 139.
8. Gibson, A. H., *Austral. J. biol. Sci.,* 1966, 9, 499.
9. Rajagopalan, N. and Raju, P. N., *Phytopath. Z.,* 1972, 73, 285.
10. Orlob, G. and Arny, D. C. *Phytopathology,* 1961, 51, 768.
11. Tu, J. C., Ford, R. E. and Grau, C. R., *Ibid.,* 1970 *b,* 60, 1653.

EFFECT OF SOME MYCOTOXINS ON THE INFECTIVITY OF TOBACCO MOSAIC AND TOBACCO RING SPOT VIRUSES

ALTHOUGH plant virus inhibitors have been found in a number of species of higher plants and fungi, relatively few have been characterized chemically[1,2,10]. In the earlier studies Rao and Raychaudhuri[6], Sharma and Raychaudhuri[8], used the crude culture filtrates of *Trichothecium roseum* and *Aspergillus niger* respectively and reported that they are inhibitory to Potato virus X. Recently Kang *et al.*[4] reported that the culture filtrate of *Aspergillus flavus,* containing aflatoxins, inhibited the infectivity of vegetable marrow mosaic virus upto 70 to 80%. Subbarayudu *et al.*[9] reported the effect of aflatoxins on cowpea mosaic virus infectivity.

With the end in view, we have isolated aflatoxins from the culture filtrates of *Aspergillus flavus* and separated by the method of Pons *et al.*[5]. Citrinin was extracted from the culture filtrate of *Penicillium citrinum*[7]. The T-2 toxin was obtained as a gift from Dr. O. Shottwell. The toxin solutions were prepared by dissolving a known amount of toxin in chloroform, to which later distilled water was added. After evaporating the chloroform, 500 and 1000 ppm toxin solution were prepared.

In the present studies, *Nicotiana tabacum* var. *xanthi-nc* was used as a local lesion host for tobacco mosaic virus (Johnson's No. 1 strain) and *Vigna sinensis* Savi for tobacco ring spot virus (Brinjal isolate). For testing the effect of these toxins, the standard inocula for these two viruses were prepared by communiting young infected leaves. The sap was filtered through double layered muslin cloth and diluted with distilled water to have 1 : 5 dilution which gave countable discrete local lesions on the above hosts. Different concentrations of each toxin were mixed with equal quantity of the virus inocula and incubated for 1 hr at room temperature (32° C). For control treatment, distilled water, from which chloroform was evaporated, was added to the virus inoculum, instead of toxin solution. After one hour incubation the inoculations were made by using half leaf method with inoculum wet cotton wad on the leaves previously dusted with celite. All the experiments were replicated three times and two independent experiments were conducted to confirm the results. The data obtained in both the experiments were averaged and presented in Table I.

It is quite obvious from the data given in the table that aflatoxins (B_1, B_2, G_1 and G_2), citrinin and T-2 toxin have inhibitory effect on tobacco mosaic virus and on tobacco ring spot virus. Aflatoxins, citrinin and T-2 toxin both at 500 and 1000 ppm inhibited the local lesion production of both the viruses and the percentage of inhibition ranged between 62·5 to 100% for TMV and 59·7 to 98·7% for TRSV respectively. In 1972, Kang *et al.*[4] reported 70 to 80% inhibition of vegetable marrow mosaic virus, with the culture filtrate of *Aspergillus flavus* containing aflatoxins. Citrinin also markedly inhibited local lesion formation on *Nicotiana glutinosa* leaves infected with TMV[11]. Earlier Rao and Raychaudhuri[6], Sharma and Raychaudhuri[8] used only the crude culture filtrate of fungus without isolating the actual inhibitory substance. But in the present studies the toxins were isolated and the studies reveal that the inhibitory substance present in the crude culture filtrate of these fungi may be aflatoxins in the case