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IN MEMORIUM

DR. J. P. VARMA

M. Sc., Ph. D.

We learnt with profound sorrow the passing away of Dr. Jagdish Prasad Varma, Professor and Head, Department of Plant Pathology, Haryana Agricultural University and the founder Secretary, Indian Virological Society on Feb. 1, 1984. The science of Virology in general and Plant Virology in particular has lost an illustrious scientist.

Dr. Varma was born on June 2, 1932 in district Moradabad, U. P. He obtained his M. Sc. degree in 1956 from Agra University and Ph. D. in 1982 from Lucknow University. Throughout his distinguished career within and outside the country, he made a permanent imprint on the heart of his fellow colleagues and students. He was recipient of prestigious Alexander von-Humboldt Stiftung Fellowship in West Germany and had wide experience of attending national and international conferences in the disciplines of Virology and Plant Pathology. His desire and long craving for bringing together the scientists of Medical, Veterinary and Plant and other areas of Comparative Virology on a common platform, was instrumental in the birth of Indian Virological Society in December 1983. Dr. Varma was an efficient teacher and a devoted research worker who was held in high esteem and loved by one and all for his noble qualities, affable nature and pleasing demeanour. He had abiding interest in the discipline of Plant Virology. In his passing away the Indian Virological Society has lost an able scientist and organiser. We convey our heartfelt condolences to the members of the bereaved family.

NARAYAN RISHI

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CONTENTS

Inaugural Address-Second National Virology Conference <i>Dr. S. P. Raychaudhuri</i>	1
Mosquito Inoculation and Immunofluorescence Technique for Studies on Dengue Viruses <i>V. Dhanda and M. A. Ilkal</i>	6
Epidemiological Studies of Viral Diseases of Livestock in Haryana State <i>R. S. Chuahan, R. C. Kulshreshtha and R. K. Kaushik</i>	10
Isolation and Characterization of ovine Enteroviruses <i>N. C. Jain and S. K. Batra</i>	17
Occurrence of a Strong Virus Interfering Agent in Susceptible Plants Sprayed With <i>Pseuderanthemum Atropurpureum Tricolor</i> Leaf Extract <i>H. N. Verma and M. M. Abid Ali Khan</i>	26
Studies on a Virus Disease of white Button Mushroom (<i>Agaricus Bisporus</i> (Lange) Sing.) in India <i>R. P. Tewari and S. J. Singh</i>	35
Aphid Transmission Characteristics of Potato Virus Y ^N <i>M. N. Singh and S. M. Paul Khurana</i>	42
Studies on the Transmission of Papaya Mosaic Virus by <i>Rhopalosiphum Maidis</i> (Fitch.) <i>S. S. Cheema and R. S. Reddy</i>	49
Manipulation of Planting and Haulms Cutting Dates for Better Health Standards in Seed Potato Crops <i>A. K. Verma and K. S. Vashisth</i>	54
Effect of Yellow Vein Mosaic on Physiology of Okra <i>J. K. Johri and B. Padhi</i>	61
Studies on the Effect of some Alternative Cultural Methods on the Incidence of Yellow Vein Mosaic Virus (YVMV) Disease of Okra (<i>Abelmoschus esculentus</i> (L.) Moench.) <i>M. A. Khan and S. Mukhopadhyay</i>	69
Occurrence of a new Virus like Disease of Grapevine in Haryana <i>J. P. Singh, Suneel Sharma and J. P. Verma</i>	73
Evaluation of Rough Lemon Strains Against Citrus Tristeza Virus and Citrus Exocortis Viroid <i>S. S. Cheema, S. P. Kapur and R. S. Dhillon</i>	76
Purification, Electron Microscopy and Serology of Strains A and F of Sugarcane Mosaic Virus <i>Narayan Rishi and Sushma Rishi</i>	79

Recovery of Foot-and-Mouth Disease Virus in Bovine Kidney Cell Culture System	87
<i>Ravindra Sharma, Arvind Kumar, K. L. Ahuja and S. Prasad</i>	
Effect of Insecticides on Yellow Mosaic of Moth (<i>Vigna Aconitifolia</i> (Jacq) Marechel)	92
<i>G. S. Rathore and J. P. Agnihotri</i>	
The Detection of Rinderpest Antigen by Single Radial Haemolysis Technique	95
<i>R. C. Joshi, Umesh Chandra and R. P. Bansal</i>	
Occurrence of Wound Tumor and Woody Gall Virus Diseases of Citrus in India	97
<i>L. N. Dubey</i>	

INAUGURAL ADDRESS*

SECOND NATIONAL VIROLOGY CONFERENCE

Haryana Agricultural University, Hisar

December 19, 1983

Dr. C. M. Singh, Dr. R. P. S. Tyagi Professor R. K. Grover, distinguished Delegates, Guests, Ladies and Gentlemen.

May I express my sense of deep appreciation for the invitation extended to me to be amongst you today on the occasion of the Virology Conference. I am indeed happy that this conference is being held at the Haryana Agricultural University in association with Indian National Science Academy and the Department of Science and Technology, Government of India. This reminds me of the group discussion on virology held at N. B. R. I., Lucknow, Feb. 8-9-1980 which received full support and encouragement from the then Director, N.B.R.I., Dr. T. N. Khoshoo, who is now Secretary to the Govt. of India, department of environment. The success of that group discussion resulted in bringing out the full proceedings in printed form, which have been most helpful to the virus research workers, not only in India but in other countries as well. Dr. B. P. Singh needs to be congratulated for this publication.

Viruses were recognised at the end of the last century and the search for viruses was linked to trying to find "a black cat in a dark cellar without being certain, that it is there." The science of virology has evolved in less than a century to become paramount in studies regarding life itself. The viruses known to be associated with diseases of men, animals, plants and bacteria have attracted the attention of persons from many branches of life sciences. Not only it encompasses applied aspects and has economic impact but it has role in growth of other branches of agriculture, medical, veterinary and other life sciences such as molecular biology, molecular biochemistry, genetics, genetic engineering, etc. Within the field of virology, some major groups are recognizable. Depending upon the model host, the groups are separable into plant, animal, human and bacterial viruses. The discovery of tobacco mosaic gave birth to the science of Plant Virology.

Virology has been recognized as the pioneer science as it has made salient contributions to general virology by establishing the physical and chemical nature of viruses and by ascertaining the infectious nature and reconstruction of viruses *in vitro*. It continues to contribute toward the understanding of the biology of the virus itself.

Having originated as a branch of human and animal pathology on one hand, plant pathology on the other hand the science of virology, developed at first in response to practical needs, has reached a point where progress is dictated at least as much by the logic of its internal development as by the demands of applied areas. In India, there has been increasing recognition, of need for joint efforts and

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for cross fertilization of ideas between plant, animal, human and bacterial virologists as well as mycoplasmalogists.

Like all sciences, virology has not developed in a straight path, but rather by a slow accumulation of empirical knowledge. Some diseases now known to be caused by viruses have been recognized for thousands of years. A Chinese description of a pestilence dating from the 10th century B.C. apparently refers to small pox. Yellow fever was known for centuries in tropical Africa. Plant virus diseases such as potato leaf roll, have been traced to record of several hundred years ago, and tulips with the ornamentally appreciated colour variegation known as tulip break, caused by a virus, have been cultivated since the 16th century. The transmissibility of small pox has been known for centuries, and vaccination against small pox by extracts containing vaccinia virus was introduced as medical practice by Jenner at the end of the 18th century. The transmissibility of tobacco mosaic by mechanical inoculation of sap of infected plants was demonstrated by Mayer in 1886. Later Loeffler and Frosch in 1898-1899 showed transmission of foot-and-mouth disease. These followed an intense period of discovery of virus pathogens.

The early years of virology saw the development permitting the microscopic visualization of virus particles or intracellular inclusions. The work on the size and properties of virus particles contributed to the development of modern techniques of ultrafiltration, ultracentrifugation, purification, crystallization and chemical characterization of virus particles. Research on cellular pathology of virus infections stimulated tissue culture and chick embryo techniques as means of investigating viruses in simplified systems. The discovery in 1910 of a virus that produces malignant tumors in chickens and the generalization of this discovery to a whole group of fowl cancers opened the way for the realization that viruses are a major agent of neoplastic transformation both in animals and in plants.

The discovery of bacteriophages and the deliberate use of bacteriophages as models for the study of virus cell relation provided perhaps the most important single factor for the present integration of virology. Recognition of specificity of host-virus relations determined by genetic and development factors assumed importance to conquer the virus diseases of man, animals and plants. Epidemiological studies highlighted the role of insect and other vectors in the transmission of many virus diseases of animals and plants and the clarification of the role of latent infections of reservoir hosts in perpetuating virus diseases. Mutability in viruses established the nature of viruses as independently evolving and, therefore, taxonomically independent genetic systems. Serological properties of viruses established their nature as chemically specific, host-independent structural elements and provided a basis for diagnostic tests for virus diseases.

More recently work on yellow diseases mostly transmitted by leaf hoppers has now proved their association with Mycoplasma/Spiroplasma/Rickettsia. New

pathogenic entities-viroids-causing plant diseases have also been discovered. It is extremely difficult to culture their organisms and study in full detail for proper understanding of these pathogens. Kunkel's classical work proved that chemotherapy and heat therapy are useful in controlling such diseases of yellows type which are mostly transmitted by leaf hopper vectors.

Maintenance of pure cultures of viruses and insect vectors is most important and attempts should be made to establish a culture collection of viruses as well as a sera bank.

In medical and veterinary field, the science of virology has contributed immensely to human and animal welfare. The global eradication of small pox in 1980 is a singular example of practical use of viral vaccines and of international cooperation and now deadly disease small pox of man does not figure on the face of the world.

In Asia, for the first time, a classical work on a plant virus which is transversally transmitted upto 6 generations of a leaf hopper was clearly demonstrated by Fukushi in Japan.

In India the first authentic proof of tobacco leaf curl and its transmission by white flies was established in 1937-41 by Dr. B. P. Pal *et al.*, Researches on plant viruses were initiated in a methodical way by Dr. B. N. Uppal in 1939-40 in Poona. During more than the last 4 decades many plant viruses and virus diseases, their insect vectors etc. have been described in India and a beginning was made on MIO's associated with plant diseases. Resistant sources in many crop plants have been identified. Work has also been done on tissue culture in relation to plant viruses, heat and chemotherapy as well as cross protection studies for control of plant viruses. Plant quarantine is most essential to control introduction of viruses transmitted through bulbs, tubers, rhizomes etc. besides those which are transmitted through seeds. We do not have properly organised seed certification organisation. These points need consideration of the Virologists of India. Many virus diseases are now known to be transmitted by fungi and nematodes but no work seems to have been undertaken on this aspect in India. We have no knowledge of the effects of pollution and toxicology vis-a-vis virus affected plants and insect vectors. I sincerely hope that all these points will be kept into consideration during discussions.

Viruses have been considered ideal models for modern basic research in biological sciences. The developments that are now taking place in modern biology including virology hold great promise for the improvement of plant, animal and human health in developing countries. They hold promise to find new solutions to many intractable tropical communicable diseases. With new DNA technology, mass production of pure candidate antigens and antibodies through recombinant,

DNA and monoclonal antibody techniques has become possible for improved diagnosis, treatment and control.

The recombinant DNA technology has revolutionised the field of virology and has brought hope for the control of human, animal and plant diseases. Vaccinia which was instrumental to the world wide eradication of small pox is now being considered as a potent carrier for integrating genes of many bacteria, viruses and parasites and scientists are planning to provide a single vaccine against many infectious diseases.

Problem of genetic diseases and of cancer have assumed increasing importance in developing countries. Here too, the new biology and researches in virology will offer exciting possibilities. Cancer caused by viruses in animals such as leukine in cattle and fowl, Mareks disease in poultry, provide an excellent animals model for cancer research in man. It will be exciting to mention that researches in animals have already provided an efficient vaccine against Marek's disease, a malignant disease of poultry. This brings hope of development of an effective vaccine against cancer in man in future.

There has been debate about basic and applied research relevant to developing countries. One can understand in the present context that basic research is directed towards increase in knowledge in science while applied research is directed towards practical application of science. Without the back up provided by basic research, the techniques and methodologies of applied research will not advance. It may be the intimate association of basic and applied research which leads to success. The lesson that one draws from all this is that no sustained development is possible for developed or developing countries without basic research.

With several Universities, both of traditional nature and for agricultural research, it should be possible to train virologists so that the baffling problems especially those of national importance may be solved. For this purpose, what is needed is to include virology in the syllabus in all the departments where plant science is studied. Unfortunately in many leading universities, this science is normally missing. Very fascinating results could be obtained, if some trained scientists work in these areas.

In extension side it is equally poor and the knowledge imparted to the field workers is restricted to association of fungle disease symptoms like smuts, rusts, rots, etc. It is equally important to bring to their notice the necessity of conducting thorough survey in the field for viral infection which sometimes is very difficult because certain crops carry virus without symptoms. In plant quarantine stations, it is most important to have properly equipped laboratories for checking presence of viruses. It is gratifying to note that the plant protection Directorate has recently

strengthened their staff at seaports and airports and we hope, methods will be devised in the near future to stop introduction of any virus infected seeds or planting material before they are allowed to enter in our agricultural land. Domestic quarantine is also important.

During the two days of the conference it is supposed to discuss about recently discovered viruses and virus diseases, host virus interaction and management of viral diseases, new techniques for rapid detection and diagnosis of viruses and recent advances in virus-vector relationship. It is indeed a very interesting field of research which by basic studies some day may bridge the gap between human, animal and plant diseases which may have the same pathogens involved in such maladies. For cross-fertilization of ideas a conference like this involving plant, animal and human virologists, will be very useful and the virologists may think a permanent platform or organization whereby they may meet at regular intervals to review the progress of research in virology in India. I am sure with the talent available and with good will and cooperation of all present here and other virologists who could not join us this time you will be able to come to some conclusions which when followed will make a new era in Virology. This will be possible only by interdisciplinary team work at National and International levels.

In this seminar arranged at a very short time by untiring efforts of Dr. S. P. Varma and his colleagues, several papers have been included in four sessions including almost all aspects of Virology. Besides two special lectures, the general meeting and plenary session, full discussion will take place about collaboration work which is possible only through a common forum. We hope that fifty scientific papers listed in the programme will create lot of interest amongst the participants.

I thank you once again, for giving me this opportunity to share my thoughts and understanding with you on Viruses and Virus Diseases. I wish all success in endeavours of the virologists who have assembled here from all over the country.

With these words, I have great pleasure in inaugurating this conference and wish your deliberations a success.

MOSQUITO INOCULATION AND IMMUNOFLUORESCENCE TECHNIQUE FOR STUDIES ON DENGUE VIRUSES

V. DHANDA AND M. A. ILKAL

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SUMMARY

The difficulties associated with studies on dengue viruses by conventional mouse inoculation technique and the sophisticated requirements of a tissue culture laboratory prompted us to adapt the newly developed mosquito inoculation and immunofluorescence techniques in this laboratory. The technique is now being successfully used for different types of studies, which include primary isolation of dengue viruses from field collected material (both human sera and mosquito specimens), xenodiagnosis and titration of the virus. The technique is found useful for both laboratory and field studies on vectors as it facilitates the rapid detection of viral antigen in individual mosquito by immunofluorescence. It has been found to be more sensitive than the conventional methods currently in use for the isolation of dengue viruses.

Dengue fever, which, in the recent past has caused extensive epidemics in several parts of the country, is caused by four types of dengue viruses. These viruses are difficult to study in the laboratory by the currently available techniques of mouse inoculation and tissue culture system. These difficulties have been fully explained by Rosen and Gubler (1974), who, for the first time utilized the mosquito inoculation technique for the propagation of dengue viruses. Later, Kuberski and Rosen (1977) employed immunofluorescence technique for the detection of dengue viruses in mosquitoes infected by parenteral inoculation. Since then many workers (Gubler and Rosen, 1976; Gubler *et al.*, 1979; Jumali *et al.*, 1979; Thet-Win, 1982; Watt *et al.*, 1982) have employed these techniques for studies on dengue viruses.

The purpose of the present communication is to report the results of the experiments conducted on dengue viruses using this technique as adapted in this laboratory.

MATERIAL AND METHODS

Aedes aegypti mosquitoes used for the propagation of dengue viruses were obtained from the laboratory colony being maintained at this Institute.

The mosquito inoculation technique was essentially similar to that described by Rosen and Gubler (1974). The inoculated mosquitoes were incubated at 28 to 29 °C and 85 to 90% R.H. The incubation period varied from 10 to 14 days. During this period they were maintained on 10% glucose solution. The incubation period for mosquitoes infected by oral route generally varied from 15 to 20 days. After incubation, the head of the mosquitoes was removed with the help of a

scalpel and placed on a glass slide. Head squash was prepared by placing a small cover glass over the head and gently pressing with a pencil eraser. All the chitinous material was removed from the slide with forceps. The squashes were allowed to dry and fixed in chilled acetone (approx. 4 °C) for 10 minutes. The fixed slides were stored at sub-zero temperature for a few days before using them for fluorescent antibody test. The viral antigen was detected on the slides by indirect fluorescent antibody (IFA) test. The hyperimmune serum to dengue viruses was prepared in rabbits. The immune serum was absorbed at 4 °C overnight with crude *Aedes aegypti* mosquito suspension prepared in phosphate buffered saline (pH 7.2) mixed with acetone extracted mouse liver powder to remove the non-specific reactants in the serum. The anti-rabbit immune serum conjugated with fluorescein isothiocyanate was procured from M/s Nordic Immunological Laboratory, U. K. The identification of the virus was done by complement fixation (CF) test on infected mosquito suspension.

RESULTS

Processing of Field Collected Sera

Serum specimens collected in the acute phase of illness from cases during the suspected dengue epidemic in Rahuri in Maharashtra in 1979 were tested by this technique. These sera were earlier processed by mouse inoculation technique (N. I. V., unpublished data). The comparative results of virus isolation by mosquito inoculation technique are given in Table 1. Out of 24 sera specimens tested, dengue virus was isolated from 13 specimens by mosquito inoculation technique, against 8 isolated in infant mice. The 5 serum specimens from which the virus was not isolated in infant mice protected the surviving mice when challenged with dengue virus.

TABLE 1

Comparative results of dengue isolation by mosquito inoculation and infant mouse inoculation techniques from Rahuri specimens

No. of specimens processed	Mosquito inoculation		Infant mouse inoculation		
	Positive by FA	Identification by CF test	Virus isolation	Identification by CF test	Positive by cross challenge test
24	13	3 Dn type 1	8	5 Dn type 1	5
		1 Dn type 2		2 Dn type 1	
				or 2	
		1 Dn type 1 or 2		1 Dn type not identified	
		8 not typed			

Diagnosis by Feeding Mosquitoes on Patients

Aedes aegypti mosquitoes were fed on two patients, both laboratory personnel, suspected to be suffering from dengue fever. One of the patients acquired the infection in the laboratory after he was accidentally bitten by an experimental mosquito (Ilkal *et al.*, 1984). The other had a naturally acquired infection. In the first case head squashes of the mosquitoes were prepared on 13th and 20th days after infective blood meal. One out of 9 mosquitoes tested on the 13th day and 4 out of 8 tested on 20th day were found positive in IFA test. In the second case the head squashes were prepared only on the 20th day and 8 out of 16 mosquitoes were found positive. In the first case the virus was identified as dengue type-2 by complement fixation test performed on infected mosquito suspension in the third passage. The typing has not yet been done in the second case.

Virus Assay

Virus assay was done on blood-virus mixture used for studies on vector competence of mosquitoes. Some of these specimens were also assayed in infant mice by i. c. route. The comparative results showing the titre of the virus by mosquito inoculations and infant mice are given in Table 2. It is interesting to note that the titre of the virus isolated in mosquitoes, which had undergone only 1 mouse brain passage was higher in the mosquito assay system as compared to mouse. On the other hand, the titre of the laboratory established strain of virus was generally higher in mice than in mosquitoes.

TABLE 2

Comparative results of different strains of Dengue-1 virus assay done in blood-virus mixture by infant mouse inoculation and mosquito inoculation techniques.

Experiment No.	Delhi strain isolated by mosquito inoculation (1 mouse brain passage)		Hawaiian strain-laboratory established (90 mouse brain passage)	
	In mice	In mosquitoes	In mice	In mosquitoes
1	4.4	5.2	5.7	5.6
2	3.7	4.1	5.2	5.2
3	5.1	5.7	5.3	4.4
4	—	—	5.5	4.7

Note : Virus titres are expressed as dex LD₅₀/0.02 ml in infant mouse and dex LD₅₀/0.2 ul in mosquito.

DISCUSSION

Since the work of Kuberski and Rosen (1977) who first described this technique, it has been considered as one of the most sensitive techniques for studies on dengue viruses. During our study, we have found it immensely useful for laboratory studies as well as for processing field material. We have found it more sensitive than the conventional infant mouse inoculation technique. We have also found this technique suitable for processing some of the field specimens which may be contaminated and present problems in other assay systems. Above all, the technique is economical and the only major requirement is a fluorescent microscope. Maintenance of a mosquito colony should not present much problems as this is simpler and more economical than either maintaining a mouse colony or a tissue culture laboratory.

It should, however, be necessary to sound a word of caution as the mosquitoes infected by inoculation are capable of transmitting the virus. The safety can be increased by using male mosquitoes instead of females, but the survival of male mosquitoes for the duration of the experiment is usually a problem. Some laboratories are now using *Toxorhynchites*, a non-biting mosquito (Rosen, 1981; Thet-Win, 1982; Watt *et al.*, 1982). But the slow breeding and protracted life history of this mosquito make it rather difficult to get them in sufficiently large numbers for experimental purposes.

REFERENCES

- Gubler, D. J. and Rosen, L. 1976. A simple technique for demonstrating transmission of dengue virus by mosquitoes without the use of vertebrate hosts. *Amer. J. Trop. Med. and Hygiene*. **25** : 146-50.
- Gubler, D. J., Suharyono, W., Lubis, I., Eram, S. and Sulianti Saroso, J. 1979. Epidemic dengue hemorrhagic fever in rural Indonesia. I. Virological and epidemiological studies. *Amer. J. Trop. Med. and Hygiene*. **28** : 701-10.
- Ilkal, M. A., Dhanda, V., Rodrigues, J. J., Mohan Rao, C. V. R. and Mourya, D. T. 1984. Xenodiagnosis of laboratory acquired dengue infection by mosquito inoculation and immunofluorescence. *Indian J. Med. Res.* **79** : 587-90.
- Jumali, Sanarto; Gubler, D. J., Nalim, S., Eram, S. and Sulianti Saroso, J. 1979. Epidemic dengue hemorrhagic fever in rural Indonesia. III. Entomological studies. *Amer. J. Trop. Med.* **28** : 717-24.
- Kuberski, T. T. and Rosen, L. 1977. A simple technique for the detection of dengue antigen in mosquitoes by immunofluorescence. *Amer. J. Trop. Med. and Hygiene*. **26** : 533-37.
- Rosen, L. 1981. The use of *Toxorhynchites* mosquitoes to detect and propagate dengue and other arboviruses. *Amer. J. Trop. Med. and Hygiene*. **30** : 177-83.
- Rosen, L. and Gubler, D. 1974. The use of mosquitoes to detect and propagate dengue viruses. *Amer. J. Trop. Med. and Hygiene* **23** : 1153-60.
- Thet-Win. 1982. Detection of dengue virus by immunofluorescence after intracerebral inoculation of mosquitoes. *Lancet* **1** : 53-54.
- Watt, D. M., Harrison, B. A., Nisalak, A., Scott, R. McN and Burke, D. S. 1982. Evaluation of *Toxorhynchites splendens* (Diptera : Culicidae) as a bioassay host for dengue viruses. *J. Med. Entomol.* **19** : 54-59.

EPIDEMIOLOGICAL STUDIES OF VIRAL DISEASES OF LIVESTOCK IN HARYANA STATE

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SUMMARY

Investigation of various diseases in different species of livestock, as caused due to various etiological agents including viruses, was conducted. During this investigation the important viral diseases diagnosed on the basis of clinicopathological picture and serological testing, in cattle and buffaloes, were rinderpest and giant cell pneumonia; in sheep and goats, blue tongue and sheep pox; in pigs, swine fever and swine pox and in camels, rinderpest and camel pox.

Amongst various infectious/contagious diseases, viral diseases are more deadly to the livestock population. The viral diseases have been gaining more importance because of the import of exotic animals and cross breeding programmes in livestock in our country, as the exotic and crossbred animals are more susceptible to different infections as compared to the indigenous stock. A few of the old existing diseases are emerging, while some of the viral diseases are considered getting entrance to our country through exotic germplasm. In the Haryana state the investigation of various diseases in different species of animals was conducted and the present communication is to report the prevalence of important viral diseases recorded.

MATERIAL AND METHODS

All the epidemics of diseases among the different species of livestock reported to this department were attended and the diseases were confirmed after applying the required laboratory tests. Complete epidemiological data including the detailed history, clinical symptoms and rate of morbidity and mortality were collected and analysed.

RESULTS AND DISCUSSION

The viral diseases recorded in different species of animals were as follows :

Rinderpest in Cattle and Buffaloes

Rinderpest is an acute, highly contagious disease of ruminants caused by paramyxovirus and characterized by high fever and focal erosive lesions mainly confined to the mucosa of the gastrointestinal tract (Blood *et al.*, 1979) and it is a highly fatal disease, which causes a loss of about Rs. 30 crores to India annually (Singh, 1980). This disease was considered to be totally eradicated from

our country including Haryana state. But it attacked the livestock population of Delhi and Haryana during the years 1981 and 1982 as detailed in Table 1.

TABLE 1

Details of outbreaks of rinderpest in cattle and buffaloes in Haryana state

S. No.	Species	Place	App. Mortality (%)	Period
1.	Buffaloes	College of Vety. Sciences, HAU, Hisar	25	July, 1981
2.	Cattle and buffaloes	Village Palla (Ballabhgarh area)	30	Sep., 1981
3.	—do—	Delhi area	10	Dec., 1981
4.	—do—	Chitkana (Distt. Sonapat)	2.7	Sep., 1982

Recurrence of rinderpest after total eradication has been reported in Nigeria also by Nawathe and Lamorde (1982).

In affected cattle and buffaloes the clinical manifestations observed were typical of rinderpest and were characterized by pyrexia (105-107 °F), anorexia, lacrimation and congestion of buccal mucosa. There were discrete necrotic patches alongwith bran like deposits in oral cavity. Severe diarrhoea and in some cases dysentery was also recorded. On postmortem of both cattle and buffaloes, haemorrhages particularly in subcutaneous tissues and other visceral organs including endocardium and spleen were noticeable. In large intestine at caecocolic junction, there was a zone of haemorrhages running transversly across the colonic mucosa, producing a characteristic stripped appearance known as "Zebra markings". Subscapular, mediastinal and mesenteric lymphnodes showed haemorrhages and oedema. The clinical manifestations and gross lesions recorded in the present study were in confirmation to those as reported by Scott (1964) and Maurer *et al.* (1955). The disease was confirmed by agar gel diffusion and counter-immunoelectrophoresis tests, using reference antisera, obtained from the Indian Veterinary Research Institute, Mukteswar (Kumaon) as described by Bansal *et al.* (1981).

Rinderpest in Camels

During the course of field investigation of camel diseases, a few camels showed clinical signs of diarrhoea, dullness, listlessness, weakness, off feed and excessive salivation. The buccal mucosa was inflamed and on innerside of lips and cheeks some circular or elliptical ulcers, undergoing healing, were notice-

able. The faeces were watery in consistency and foul smelling. Rectal temperature was 104 °F. On cultural examination, no organisms of pathological significance were isolated from blood and faecal samples. Transmission experiments, however, failed to reproduce the disease in buffalo calves.

In order to determine the susceptibility of camels to experimental rinderpest infection, two healthy young camels 8-12 months old, were inoculated with 10 ml of 10% suspension of spleen, collected from a buffalo calf, which was previously inoculated with virulent rinderpest virus. One animal was given subcutaneous and the other intravenous inoculation. These camels were kept under observation and the body temperature was recorded daily in the morning and evening. Blood was also collected for hematological studies. Both the camel-calves showed a febrile reaction on 4th day of inoculation which lasted for three days. Body temperature rose upto 105 °F and gradually came down to normal. Except for slight hyperaemia of visible mucous membranes and mild diarrhoea, no distinct symptoms of lesions of rinderpest could be noticed. No significant change in the total and differential leucocytic count was observed. Likewise Singh and Ata (1967) inoculated virulent strains of rinderpest virus in camels and recorded slight thermal reaction and no other significant sign; however they detected high level of neutralizing antibody titre in the sera of inoculated camels.

Blood, collected from experimentally inoculated camels at the height of febrile reaction, was inoculated into two susceptible buffalo-calves. It gave rise to the development of typical symptoms and lesions of rinderpest in these calves within six days of inoculation. Taylor (1968) isolated rinderpest virus from the blood of a camel inoculated I/V with virulent Kabete 'O' strain, but he failed to reproduce the disease in camels or cattle from infected blood of inoculated camels.

To find out the development of internal lesions, if any, one of the camels was slaughtered on 21st day of post inoculation. No appreciable lesion, gross or microscopic, could be observed in any of the visceral organs, except slight congestion of mucosa of the lips, small intestine and rectum. No attempt was made to confirm the disease by employing any serological test.

Giant Cell Pneumonia

Two reports of mortality in buffalo-calves (3-8 months old) of both sexes were diagnosed to be associated with giant cell pneumonia. The morbidity and mortality patterns recorded were as given in Table 2.

TABLE 2

Outbreaks of giant cell pneumonia in buffalo-calves

S. No.	Population at risk	No. of animals affected	Morbidity rate (cause specific) (%)	Number died	Case fatality rate (%)
I	126	30	23.81	12	40
II	100	15	15	7	46.67

Clinical symptoms manifested by the sick calves were anorexia, nasal discharge, hyperpyrexia (103-105°F), mild diarrhoea, dyspnoea, harsh hacking cough and death usually within 24-72 h. Treatment of the ailing animals with sulphamezathine and antibiotics did not yield any encouraging results.

Cultural examination of blood and intestinal contents did not reveal any organism of pathological significance.

Postmortem examination of dead calves revealed areas of consolidation involving apical, cardiac and anteroventral portion of diaphragmatic lobes of the lungs. At places, there were small greyish white nodules raised above the pleural surface. A dirty white to greyish thick exudate was seen on incising the bronchi.

Microscopically, there was infiltration of mononuclear cells alongwith some polymorphs in the alveoli and bronchi. The most characteristic histologic feature was the presence of syncytial giant cells, which were either lying free in the lumen or were still attached to the alveolar walls. There was proliferation of septal cells which resulted into pseudo-epitheliazation of the alveoli. A moderate to heavy peribronchial and perivascular lymphoid cell infiltration was observed in some areas. Staining of the sections with Giemsa's stain, a few acidophilic pleomorphic cytoplasmic inclusions simulating the viral inclusions were detected in some of the alveolar and bronchiolar epithelium. Interstitial mucosa revealed infiltration of large number of lymphocytes and eosinophils. Arora and Kalra (1973) and Verma *et al.* (1982) also recorded similar gross and microscopic lesions in lungs of buffalo calves died of giant cell pneumonia.

Blue Tongue

Blue tongue is an arthropode borne viral disease of sheep. Though cattle and goats may harbour the virus, but generally fail to show pronounced clinical symptoms (Blood *et al.*, 1979).

The classical and acute fulminating types of outbreaks diagnosed during the years 1975 to 1982 revealed the clinical symptoms comprising rise of body temperature ranging from 104 to 107 °F for about 5-8 days; a few sheep in acute fulminating type died during febrile period. There was an increase in the respiratory rate. Typical mucosal lesions in the mouth and the coronary bands and hooves were observed in the classical form. Affected sheep showed least inclination to move and the hooves and bulbs of the foot pads were painful on pressure. Gingivitis and haemorrhagic streaks of oral mucosa, soft palate and nasal mucosa were observed with stringy salivation and frothy mucopurulent deposits over the nose. Oedema of lips, tongue and necrotic shallow ulcers were seen on labial mucous membrane and sides of tongue. In addition, during the year 1982, in the epidemics of blue tongue oedema of the head, ears and lower part of the face extending down the neck (bottle jaw appearance) and torticollis were also observed in a few adult sheep. Morbidity rate recorded was 10% in adults and 20% in weaner lambs and mortality rate was 0.55 to 1.10 per cent in all the age groups.

Postmortem examination revealed purpling of skin and intramuscular haemorrhages, congestion, cyanosis and shallow necrotic ulcers on gums and occasionally on dorsal surface and sides of tongue and oedema of epiglottis. Dental pad and soft palate showed petechial haemorrhages. Multiple petechial and ecchymotic haemorrhages were seen on the epicardial surface and on the base of pulmonary artery and papillary muscles of the heart. Petechial haemorrhages were seen in the abomasum and spleen. In a few sheep, haemorrhagic enteritis was also evident.

The disease was confirmed by the demonstration of blue tongue antibodies in the sera of the affected sheep employing the technique of micro agar gel precipitation test, using the reference polyvalent blue tongue antigen procured from the Arthropod-Borne Animal Disease Research, Denver Federal Center, Denver, Colo, U.S.A. Sodhi *et al.* (1981) reported prevalence rate of blue tongue 9.3% in Corriedale and 4% in local sheep and goats in Punjab by using micro agar gel precipitation test.

Sheep Pox

Sheep pox outbreaks caused 24 and 14.80 per cent morbidity and mortality, respectively among the affected flocks. The symptoms observed were anorexia, thick nasal discharge, coughing, dyspnoea and rise in body temperature. The lesions of sheep pox, present on various parts of the body, which were more pronounced on the face, abdomen, caudal region and inside the legs, were seen at different stages of development such as papules, pustules and scabs.

Postmortem examination revealed typical gun shot like nodules in the lungs. Histopathologically, proliferation of epithelial lining cells of bronchioles and the alveoli was noticeable. Alveolar walls were thickened due to infiltration of mononuclear cells and the alveoli were filled with proteinous exudate. Inclusion bodies, however, in skin sections could not be demonstrated. The disease was further confirmed by the demonstration of typical pock lesions on chorio allantoic membrane of the embryonated eggs. Sapre and Kalia (1980) reported an outbreak of sheep pox in Rajasthan.

Camel Pox

Camel population in the different parts of this state was found affected due to pox to the extent of 5-18 per cent. Both benign and malignant forms of the disease were observed. Initially the lesions appeared as hard intracutaneous nodules, leading to the formation of sequestrum. The early lesions in the oral cavity were characterized by the of vesicles, which later ruptured, resulting in raw bleeding ulcers. Nasal mucosa was much inflamed and showed membranous plaques and mucopurulent nasal discharge. The lesions were also present in the eyes of three camels causing keratitis and corneal opacity.

Histopathological examination of skin revealed ballooning of the epidermal cells. The epidermal cells in the pustular stage of the lesions manifested degenerative changes, alongwith the infiltration of large number of polymorphs. Mild infiltration of lymphocytes, macrophages, polymorphs and a few plasma cells was noticeable in the dermis and subcutis, particularly around the dermal vessels. However, no distinct pock like inclusion could be observed in tissue sections. Tantawi *et al.*, (1974) recorded similar lesions of a camel pox, including cytoplasmic eosinophilic inclusion bodies in infected chorioallantoic membrane.

Swine Pox

Three outbreaks of swine pox among growing pigs were investigated in the state. The affected animals were listless, off feed and had slight rise of temperature. The characteristic pock lesions were seen on the surface of ears, face, neck, inner side of legs and on the belly. The microscopic picture was highly suggestive of swine pox with evidence of intracytoplasmic inclusions in the skin epithelium. Olufemi *et al.*, (1981) diagnosed swine pox on the basis of symptoms and lesions in Nigeria.

Swine Fever

Swine Fever is considered to be under control in India and its vaccine production has also been discontinued. But 12 outbreaks of swine fever during the period 1979-82 have been diagnosed in the Haryana state. During these outbreaks in all the cases examined characteristic gross and microscopic lesions of swine fever were observed.

REFERENCES

- Arora, R. G. and Kalra, D. S. 1973. Giant cell pneumonia in buffalo calves. *Trop. Anim. Hlth. Prod.* **5** : 110-13.
- Bansal, R. P., Uppal, R. K., Oberoi, M. S. and Joshi, R. C. 1981. Quick diagnosis of rinderpest by detection of antigen by counter immunoelectrophoresis. *Indian J. Anim. Sci.* **51** : 766-69.
- Blood, D. C., Henderson, J. A. and Radostitis, O. M. 1979. Rinderpest. In *The Veterinary Medicine*, 5th edn. pp. 626-30. The E. L. B. S. and Bailliere Tindall, London.
- Maurer, F. D., Jones, T. C., Easterday, B. and Detray, D. 1955. The Pathology of Rinderpest. *Proc. Amer. Vet. Med. Assoc.* pp. 201-11.
- Nawathe, D. R. and Lamorde, A. G. 1982. Recurrence of Rinderpest in Nigeria. *Vet. Record.* **111** : 203.
- Olufemi, B. E., Ayoade, G. O., Ikede, B. O., Akpavie, S. O. and Nwufoh, K. J. 1981. Swine Pox in Nigeria. *Vet. Record.* **109** : 278-80.
- Sapre, M. V. and Kalia, O. P. 1980. Incidence of sheep pox outbreak in Rajasthan. Autovaccination on large scale. *Wool and Woollens of India.* **17** : 38-40.
- Scott, G. R. 1964. Rinderpest. *Advances in Veterinary Sciences.* **9** : 113-224.
- Singh, I. P. 1980. Personal communication.
- Singh, K. V. and Ata, F. 1967. Experimental rinderpest in camels. A preliminary report. *Bulletin of Epizootic Diseases of Africa.* **15** : 19-23.
- Sodhi, S. S., Oberoi, M. S., Sharma, S. N. and Baxi, K. K. 1981. Prevalence of blue tongue virus precipitating antibodies in sheep and goats of Punjab, India. *Zentralblatt fur veterinarmedizin.* **28** : 421-23.
- Tantawi, Kh., Gorshenina, V. and Rida, I. 1974. Cultivation of Camel Pox virus in developing chick embryo. *Veterinariya Moscow.* **4** : 51.
- Taylor, W. P. 1968. The susceptibility of one humped camel (*Camel dromedaries*) to infection with Rinderpest virus. *Bulletin of Epizootic Diseases of Africa.* **16** : 405-10.
- Verma, P. C., Kalra, D. S. and Kaushik, R. K. 1982. Some studies on Giant Cell Pneumonia in buffalo calves (*Bubalus bubalis*). *Haryana Veterinarian.* **21** : 54-56.

ISOLATION AND CHARACTERIZATION OF OVINE ENTEROVIRUSES

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SUMMARY

Thirty eight cytopathic agents were isolated in primary lamb kidney cell culture (LKCC) from rectal swabs collected from 42 sheep belonging to Bhiwani, Jind and Hisar districts in Haryana State and Jaipur district in Rajasthan State. The viral isolates produced typical polio-virus like cytopathic effects (CPE) in LKCC. Six of the isolates selected at random, at least one representing each of the different geographical regions, were studied in detail for their physico-chemical properties. These were resistant to ether and chloroform, stable at pH 3.00 and inactivated at 50°C in 30 min but their infectivity was stabilized in the presence of molar magnesium chloride ($MgCl_2$) at 50°C for one h. The size of two of these isolates as determined by millipore filtration technique, ranged between 0.025-0.050 μm and their growth was not inhibited in the presence of 50 $\mu g/ml$ of BUDR. The isolates grew well in McCoy (Human Synovial cells) and BHK-21 (Baby hamster kidney cells) with CPE similar to that in LKCC. All these isolates appear to be antigenically related to each other, to goat enterovirus G178 and bovine enterovirus ECBO 100/60 on the basis of cross-serum neutralization test.

Enteroviruses are one of the commonest microbial agents inhabiting the intestinal tract of men and animals. The isolation of these agents from diseased animals, though previously considered to be insignificant because of uncertainty of these agents to produce disease, is being viewed seriously now-a-days. These viruses have been isolated from almost all species of domestic animals, viz., cattle (Kunin and Minuse, 1958; Bogel, 1968; Jain *et al.*, 1978) and buffaloes (Nath *et al.*, 1970). There are a very few reports of isolation of these agents from sheep (McFerran *et al.*, 1969; Rao *et al.*, 1972). This paper reports the isolation, characterization and comparison of the enterovirus isolates from sheep with diarrhoea/nasal discharge and healthy incontacts belonging to different geographical regions and their antigenic relationships with that of goat bovine enteroviruses.

MATERIAL AND METHODS

Rectal swabs were collected from 42 sheep with diarrhoea/respiratory tract infection and healthy incontacts (Table 1). These swabs were brought to the laboratory on ice and processed according to the method of Cliver and Bohl (1962).

TABLE 1

Number of faecal swabs collected and isolation made from sheep of Haryana and Rajasthan

Location of animals	Number of samples collected	Clinical condition	Laboratory number	Number of virus isolates
1. Haryana				
(i) Isarbal (Bhiwani)	3	Diarrhoea	82Sh1-82Sh3	2
(ii) Kamopura (Jind)	3	Diarrhoea	82Sh12-82Sh14	3
(iii) Khaider (Hisar)	18			18
(iv) Haryana Veterinary Vaccine Institute (Hisar)	10*	Apparently healthy	77ShPL1-77ShPL10	10
2. Rajasthan				
(i) Central Sheep & Wool Research Institute, Avikanagar, Jaipur	8	Pneumonia & Diarrhoea	82Sh4-82Sh11	5
	42			38

* Pool of 4 swabs each

Primary lamb kidney monolayers (LKCC) grown in 160 ml milk dilution bottles according to the method of Youngner (1954), were used for the isolation of the virus. The growth medium consisted of MEM Eagle's with Hank's Salts (Bios, Bombay) supplemented with 10% inactivated sheep serum and antibiotics (Penicillin-200 I.U., streptomycin-100 µg per ml of medium).

A set of two bottles of LKCC having confluent growth were inoculated with one ml per bottle of each sample. After adsorption for an hour at 37°C, the bottles were fed with maintenance medium and incubated at 37°C for a week. One third number of bottles were mock infected to serve as controls. These bottles were examined microscopically for any viral cytopathic effects every day. Three to five blind passages were made before considering a sample negative for virus isolation.

Cultivation in Continuous Cell Lines

Six of the viral isolates, 82Sh2, 82Sh5, 82Sh9, 82Sh13, 77Sh2 and 77ShPL6 selected at random were adapted to grow in continuous cell lines of McCoy (Human Synovial cells) and BHK-21 (Baby hamster kidney cells) grown in 15 X 125 mm glass tubes and 110 ml culture bottles. McCoy cells were grown in MEM Eagle with Hank's salts and nonessential amino acids (Bios, Bombay), BHK-21 cells were grown in BHK-21 medium (Glasgow modification, Bios-Bombay) with 10% tryptose phosphate broth. Both the media were supplemented with 10% inactivated goat serum and antibiotics. Maintenance medium did not contain any serum.

The cell line culture bottles having confluent growth were inoculated with 0.5 ml of LKCC grown virus at 5th passage level. The CPE produced by these agents were studied by inoculation of virus onto cells grown on coverslips followed by staining with hematoxylin and eosin (H & E). A suitable number of coverslips were mock infected to serve as controls. The virus titre in each cell line was determined after the 5th passage.

Biophysical and Biochemical Characterization

The type of nucleic acid in the genome of the six viral isolates was determined by studying the effect of 50 µg/ml of 5-Bromo-2' deoxyuridine (BUDR) on their replication (Burke *et al.*, 1968). Equine herpesvirus-type-1 strain EA 26 (Jain *et al.*, 1976) and goat enterovirus G178 (Jain *et al.*, 1982) were included as DNA and RNA virus controls, respectively. Their sensitivity to 20% diethyl ether (Andrews and Horstman, 1949), 4.8% chloroform (Feldman and Wang, 1961), thermal inactivation at 50°C for 30 min, cationic stabilization with M MgCl₂ against heat inactivation

(Wallis and Melnick, 1962) and pH inactivation at pH 3.00 (Hamparian *et al.*, 1963) was also studied.

The particle size of two of the viral isolates (82Sh2 and 82Sh5) adapted in McCoy cell line was studied by millipore filtration technique. The cell culture harvested virus suspension was passed through millipore membranes of 0.025 and 0.050 μ m pore size. The filtrate was titrated for residual virus infectivity.

Serological Characterization

Antigenic relationship among the size isolates was studied by cross-serum neutralization (Cross-SNT) using constant virus variable serum dilution method.

Antisera for cross-SNT were produced in rabbits weighing 1.5-2.0 kg. Following pre-immunization bleeding from heart, each rabbit was injected with one ml of virus harvest (titre $\log_{10}^{4.74}/0.1$ ml) mixed with equal volume of Freund's Complete Adjuvant (Difco) intramuscularly in hind limbs at four different sites. Second, third and fourth injections of 1.5, 2.0 and 2.5 ml virus alone were injected intravenously at weekly intervals. This was followed by test bleeding from the heart. Booster injection of 2.5 ml virus alone was given intravenously two weeks after 4th injection. Rabbits were bled for antiserum two weeks after booster injection. Two rabbits were immunized against each isolate. All the sera were inactivated at 56°C for 30 min prior to use in cross-SNT.

Equal volume of two fold serial dilution (10 through 640) of each antiserum in HBBS was mixed with 100 CCID₅₀/0.1 ml of homologous and heterologous virus. This mixture, after incubation at 37°C for one h in a water bath was inoculated onto 3 tubes of McCoy cells. After adsorption for 1 h, 1.8 ml of maintenance medium was added to each tube. Necessary controls included were cell culture tubes mock infected with HBSS, infected with 100 CCID₅₀/0.1 ml virus and uninfected McCoy cells. The antigenic relationship among sheep virus isolate 82Sh2, goat enterovirus-G 178 and bovine enterovirus-ECBO 100/60 were also studied by cross serum neutralization test.

RESULTS

Thirty eight cytopathic agents were recovered from 42 rectal swabs from sheep with diarrhoea, respiratory infection and healthy incontacts (Table 1). The CPE in LKCC, BHK-21 and McCoy cells included retraction of cytoplasmic processes, rounding of the infected cells and increased refractivity of the cytoplasm. These cells were ultimately sloughed off from the glass surface. The CPE appeared at

a few foci in the beginning. But later on it involved all the cells of the monolayer. During first passage it took more than 48 h for the CPE to be completed but later on this period was reduced to less than 24 h post-infection.

The replication of six sheep viral isolates and ECBO 100/60 was not inhibited in the presence of 50 $\mu\text{g/ml}$ of BUDR (Table 2) while that of EA 26-a DNA virus, was inhibited upto 5 $\log_{10}\text{CCID}_{50}$. This indicated that all the six viral isolates have RNA genome. It is apparent from Table 2 that there was no reduction in titer after treatment of these agents with ether and chloroform, indicating their resistance to lipid solvents. The isolates were stable in acidic pH and when subjected to heat treatment (50°C for 30 min) lost their infectivity. But they were stabilized in presence of M MgCl_2 . The isolates 82Sh2 and 82Sh5 retained their infectivity after passing through membrane filter of 0.050 μm porosity but this infectivity was lost after passing through 0.025 μm membrane filters. This indicated that the size of these viruses is between 0.025 and 0.050 μm .

The results of cross-SNT of sheep isolates are given in Table 3. All the six isolates inspite of giving poor titres with homologous antisera cross reacted with heterologous antisera. The studies on the relationship of enteroviruses of different species by cross-SNT indicated that the isolate 82Sh2 when tested with antisera against 82Sh2 and G178 gave titres of 1:40 and 1:20, respectively and titres of 1:40 with ECBO 100/60 was the same as observed with homologous antiserum of 82Sh2 virus (Table 4), thus indicating presence of common antigens.

DISCUSSION

The isolation of enteroviruses from rectal swabs of sheep conforms to the reports of McFerran *et al.* (1969); Rao *et al.* (1972) and Raghavan and Dutt (1975). None of the six viral isolates was inhibited by BUDR indicating the presence of RNA in the viral genome. The size and physico-chemical properties of the viral isolates are similar to that of enterovirus isolates reported by McFerran *et al.* (1969) and McFerran *et al.* (1971). The isolates exhibited typical poliovirus-like CPE in primary LKCC, BHK-21 and McCoy cells. Similar type of CPE in primary LKCC has been reported by McFerran *et al.* (1971).

Cross serum-neutralization tests revealed that these isolates from sheep of different geographical regions were antigenically related, though the existence of antigenic variation in enterovirus isolates of bovine (McFerran *et al.*, 1971), and porcine (Knowles and Buckley, 1980) has been reported. The isolate 82Sh9 gave less neutralizing titre (20) when tested against homologous antiserum than to

TABLE 2
Physico-chemical characters of sheep viral isolates

Isolates	Infectivity titer (Log_{10} CCID ₅₀ /0.1 ml) after treatment with							Size range (μm)
	Control	BUDR*	Ether	Chloroform	Heat	MgCl ₂	pH	
82Sh2	4.50	4.50	4.50	4.50	0.00	4.50	4.50	0.025-0.050
82Sh5	3.74	4.25	3.74	4.25	1.50	3.74	4.50	0.024-0.050
82Sh9	5.25	5.25	4.50	5.25	0.00	5.25	4.74	N.D.**
82Sh13	4.74	5.25	4.50	4.50	0.00	4.50	4.74	N.D.
77Sh2	5.50	6.25	4.74	5.25	1.50	2.25	5.50	N.C.
77ShPL6	5.25	4.50	4.74	4.50	1.50	4.50	4.50	N.C.
ECBO 100/60	8.76	8.76	—	—	—	—	—	—
EA 26	9.50	4.50	—	—	—	—	—	—

* 5-Bromo-2-deoxyuridine

** N.D.— Not done

Ovine Enteroviruses

TABLE 3

Antigenic relationship among different viral isolates of sheep by cross serum neutralization test

Virus*	Antiserum (Titre)					
	82Sh2	82Sh5	82Sh9	82Sh13	77Sh2	77ShPL6
82Sh2	40	40	20	20	40	10
82Sh5	20	40	20	40	40	10
82Sh9	20	20	20	20	40	10
82Sh13	10	20	20	40	40	10
77Sh2	10	10	20	20	40	10
77ShPL6	10	20	10	20	20	10

* Using 100 CCID₅₀/0.1 ml virus

TABLE 4

Antigenic relationship among virus isolates of sheep, goat and cattle by cross-serum neutralization test

Virus*	Antiserum titre (Reciprocal dilution)			
	82Sh2	G178	ECBO	100/60
82Sh2	40	20	40	
G178	20	20	20	
ECBO 100/60	40	20	80	

*Using 100 CCID₅₀/0.1 ml of virus

heterologous antiserum (anti-77Sh2) which gave neutralizing titre 40. This difference may be due to the individual variations of the rabbits used for the production of antisera against the isolates.

Interestingly, sheep, goat and bovine enterovirus isolates were found to cross react in serum-neutralization test using hyperimmune serum against each isolate (Table 4). These observations indicated that sheep, goat and bovine enterovirus may have common antigen(s). The enteroviruses are generally believed to be host-specific; however, many animal species have antibodies that neutralize enteroviruses from another species (McFerran, 1962; Nath *et al.*, 1971a; Nath *et al.*, 1971b). These cross reactions have been considered non-specific (Dunne, 1975) although cross reactions between enteroviruses have not been thoroughly studied. There seems to be no earlier report on sharing of antigen (s) among bovine, sheep and goat enteroviruses and requires detailed studies. The simultaneous isolation of antigenitically related enteroviruses from diseased and in contact healthy sheep from regions wide apart indicated their common prevalence. This warrants further studies on their pathogenicity in natural host.

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REFERENCES

- Andrews, C. H. and Horstmann, D. M. 1949. The susceptibility of viruses to ethyl ether. *J. Gen. Micro. biol.* **3** : 290-91.
- Bogel, K. 1968. Bovine rhinoviruses. *J. Amer. Vet. Med. Assoc.* **152** : 780.
- Burke, C. N., Luginbuhl, R. E. and Williams, L. F. 1968. Avian adeno-like viruses—characterization and comparison of seven isolates. *Avi. Dis.* **12** : 483-505.
- Cliver, D. O. and Bohl, E. H. 1962. Neutralization of bovine enteroviruses by colostrum, milk and blood serum. *J. Dairy Sci.* **45** : 926-32.
- Dunne, H. W. 1975. Procine enteroviruses. In: *The Diseases of Swine*, 4th edn., pp. 353-68. Edited by H. W. Dunne and Leman, A. D. Iowa State University Press, Ames.
- Dunne, H. W., Huang, C. M. and Lin, W. J. 1974. Bovine enteroviruses in the calf: An attempt at serologic, biologic and pathogenic classification. *J. Amer. Vet. Med. Assoc.* **164** : 736-40.
- Feldman, H. A. and Wang, S. S. 1961. Sensitivity of various viruses to chloroform. *Proc. Soc. Exp. Bio. and Med.* **166** : 736-40.
- Hamparian, V. V., Hilleman, M. R. and Kelter, A. 1963. Contributions to characterization and classification of animal viruses. *Pro. Soc. Exp. Bio. and Med.* **112** : 1040-50.

Ovine Enteroviruses

- Jain, N. C., Batra, S. K. and Kulshrestha, R. C. 1982. A note on neonatal diarrhoea in kids. *Indian J. Com. Micro. Immū. and Inf. Diseases.* **3** : 50-52.
- Jain, N. C., Manchanda, V. P., Garg, D. N. and Sharma, V. K. 1976. Isolation and characterization of EHV-1. *Vet. Rec.* **99** : 57.
- Jain, N. C., Tanda, H. K. L., Prasad, S. and Chandiramani, N. K. 1978. Isolation and characterization of enteroviruses from calves. *Indian J. Exp. Biol.* **16** : 282-85.
- Knowles, N. J. and Buckley, L. S. 1980. Differentiation of porcine enterovirus serotypes by complement fixation. *Res. Vet. Sci.* **29** : 113-15.
- Kunin, C. M. and Minuse, E. 1958. The isolation in tissue culture, chick embryo and suckling mice of filterable agents from healthy dairy cattle. *J. Immunol.* **80** : 1-11.
- Mcferran, J. W. 1962. A substance in the sera of man and animals neutralizing a bovine enterovirus. *J. Pathol. and Bacteriol.* **83** : 83-88.
- Mcferran, J. B., Nelson, R., McCracken, J. M. and Ross, J. G. 1969. Viruses isolated from sheep. *Nature* **221** : 194-95.
- Mcferran, J. B., Nelson, R. and Knox, E. R. 1971. Isolation and characterization of sheep adeno viruses. *Arch. ges. Virusforsch.* **35** : 232-241. Cited : *Vet. Bull.* **42** (1972) : 1835.
- Nath, N., Balaya, S. and Mohapatra, L. N. 1970. Isolation of bovine enteroviruses. *Indian J. Med. Res.* **58** : 1328-35.
- Nath, N., Balaya, S. and Mohapatra, L. N. 1971a. Bovine enterovirus neutralizing substance in human. *Indian J. Anim. Sci.* **41** : 808-10.
- Nath, N., Balaya, S. and Mohapatra, L. N. 1971b. Human enterovirus neutralizing substance in bovine sera. *Indian J. Anim. Sci.* **41** : 811-13.
- Rao, K. M., Saraswat, K. C. and Pathak, R. C. 1972. Isolation of enteroviruses from sheep. *Indian J. Biol.* **10** : 453-54.
- Raghavan, R. and Dutt, N. S. 1975. A note characterization of three cytopathic viral agents isolated from pneumonic lungs of sheep. *Indian J. Anim. Sci.* **45** : 109-12.
- Wallis, C. and Melnick, J. L. 1962. Cationic stabilization. A new property of enteroviruses. *Virology* **16** : 504-06.
- Youngner, J. S. 1954. Monolayer tissue culture. I. Propagation and standardization of suspensions of trypsin dispersed monkey kidney cells. *Proc. Soc. Exp. Biol. and Med.* **85** : 202-5.

OCCURRENCE OF A STRONG VIRUS INTERFERING AGENT IN SUSCEPTIBLE PLANTS SPRAYED WITH *PSEUDERANTHE-MUM ATROPURPUREUM TRICOLOR* LEAF EXTRACT

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SUMMARY

Extract from *Pseuderanthemum atropurpureum tricolor* Radlk. (PAE) leaves, when sprayed over susceptible plants, produced some strong virus interfering agent (VIA) in the leaves of treated plants. The leaf juice from treated plants (R-juice) neutralized virus infectivity. VIA was present in plants showing resistance following extract treatment. No such virus interfering activity was detected in the leaf juice from non-treated plants.

Maximum virus neutralizing activity was observed in the R-juice extracted, 24 to 48 h after treatment with the PAE. Virus neutralizing activity was checked following application of Actinomycin-D in treated leaves, on incubating the juice with trypsin for 1 h, on heating the R-juice at 90°C, on diluting the juice to 1/40 and storage for 12 days. The virus interfering agent present in the juice from treated plants could not be sedimented at 1, 20, 000 g, was precipitated by ammonium sulphate and ethanol, adsorbed on charcoal and celite, and was active between pH 6-8. The various characteristics suggest that VIA may be proteinaceous in nature.

Occurrence of some antiviral agent in virus infected plants is well established now (Antignus *et al.*, 1977; Loebenstein, 1963, 1972; Loebenstein *et al.*, 1966; Mozes *et al.*, 1978; Nienhaus and Babovic, 1978; Ross, 1964; Sela *et al.*, 1964). Similarly, some virus interfering substances which neutralize virus infectivity *in vitro* have also been reported to be present in plants treated with certain plant extracts or their purified products and occurrence of these substances was co-related with disease resistance in plants (Gianinazzi, 1982). Further for synthesis of these interfering substances, host mediation is required (Verma and Awasthi, 1980; Verma *et al.*, 1979c, 1979d).

In present investigations, production of a strong virus interfering or neutralizing agent in several hypersensitive and systemic hosts following treatment with particularly clarified leaf extract of another plant, *Pseuderanthemum atropurpureum tricolor* has been described. Some of the characteristics of the virus interfering agent (VIA) induced in *Cyamopsis tetragonoloba* plant following treatment with *P. atropurpureum tricolor* leaf extract have been described.

MATERIAL AND METHODS

The details of raising the test host plants, maintenance of virus culture, preparat-

ion of virus inocula and *P. atropurpureum tricolor* leaf extract (PAE) were more or less same as described earlier (Verma and Awasthi, 1979; Verma *et al.*, 1979; Mukerjee *et al.*, 1981; Mukerjee, 1977).

Experimental Procedure

Preparation of virus interfering Agent (VIA). Lower leaves of young vigorously growing source plants were rubbed or sprayed with partially clarified extract of *P. atropurpureum tricolor* leaf extract at different intervals, upper non-treated leaves were harvested and marcerated with distilled water in sterilized mortars. The sap obtained was filtered through muslin cloth and centrifuged at 3,000/g for 15 min. The supernatant, thus, obtained was designated as R-juice. By exactly the same procedure the upper non-treated leaves from control plants (where basal leaves were treated with distilled water) were harvested and extract prepared was designated as NR-juice. The R and NR juice were mixed in equal volumes with virus aliquots. Virus titre, in the mixtures and controls (Virus+DW) was evaluated by inoculating the mixtures on some suitable local lesion and systemic hosts.

Test for antiviral activity of VIA or VNA. The activity of VIA or VNA was tested *in vitro* against several viruses on their respective hypersensitive hosts. The treatments were distributed on Latin square pattern (Gibbs and Harrison, 1976). The per cent decrease in virus infectivity was calculated by the formula.

$$\frac{\text{Lesions on control leaves} - \text{Lesions on treated leaves}}{\text{Lesions on control leaves}} \times 100$$

The data were analysed statistically for the significance of the results (Snedecor, 1961). The experimental details for the study of biophysical characteristics of virus interfering agent were same as described earlier (Verma and Awasthi, 1979, 1980).

Effect of actinomycin-D on the systemic induced resistance and production of virus interfering agent (VIA). Actinomycin-D (Merck sharp and Dhome, USA) 20 µg/ml in distilled water was applied at different time intervals following extract treatment onto the same leaves of *C. tetragonoloba* to which PAE had been applied earlier. An equal number of leaves in control sets was treated with actinomycin D alone, extract alone or distilled water. After 24 h of treatment, the upper non-treated leaves from half of the plants of each set were harvested separately and juice extracted and partially clarified by centrifugation. These R and NR juices were incubated with equal amount of sunnhemp rosette virus aliquot separately

and inoculated on *C. tetragonoloba* for estimating virus titre. The remaining plants were inoculated with virus to find out the degree of induced resistance and its reversal by AD.

Effect of trypsin. Partially clarified VIA containing juice from *C. tetragonoloba* (After DEAE-cellulose stage) was mixed with equal amount of trypsin solution (250 µg/ml) (Servafein biochemica, Germany) in phosphate buffer, pH 7.6. The pH of mixture was maintained in between 7 and 8. The mixtures were incubated at 37°C for 0.1 and 2 h. Controls consisted of mixture, in which 2 ml buffer was added instead of trypsin solution. The antiviral activity of the samples was tested *in vitro* separately on *C. tetragonoloba* against sunnhemp rosette virus.

RESULTS

The resistant juice (R-juice) extracted from treated and non-treated upper leaves of *C. tetragonoloba* plants, contained a potent virus interfering agent (VIA). No such virus neutralizing activity was detected in the NR-juice (non-resistant juice) obtained from distilled water treated plants. The antiviral activity of the R-juice was recorded 6 h after PAE treatment, and maximum antiviral activity was exhibited by the juice extracted from the leaves, which had been treated 24 to 48 h earlier with PAE, VIA could be extracted from treated plants upto 144 h (Table 2).

VIA or VNA extracted from *C. tetragonoloba*, *L. esculentum*, *N. tabacum* var. NP31, *D. stramonium*, *D. metel*, *C. melo* var. momordica L, *L. siceraria* and *C. junca* was very potent (Table 1).

TABLE 1

Comparison of the virus neutralizing activity of leaf juices from non-treated plants (NR-juice) and *Pseuderanthemum atropurpureum tricolor* leaf extract (PAE) sprayed plants (R-juice)

Plant species	Average number of local lesions ^a	
	NR-juice	R-juice
<i>Cyamopsis tetragonoloba</i>	152	0
<i>Cucumis melo</i>	163	0
<i>Crotalaria juncea</i>	149	0
<i>Datura stramonium</i>	139	0
<i>Lagenaria siceraria</i>	140	0
<i>Lycopersicon esculentum</i>	138	0
<i>Nicotiana rustica</i>	122	0
<i>Nicotiana tabacum</i>	162	0
Var. NP-31		
<i>Nicotiana glutinosa</i>	123	42
<i>Chenopodium amaranticolor</i>	129	80

^aVNA activity was estimated by mixing clarified juices from non-treated and treated plants with sunnhemp rosette virus (SRV) and inoculation of mixture on 10 leaves of *Cyamopsis tetragonoloba*.

Occurrence of Virus Interfering Agent

TABLE 2

Production of some virus interfering or neutralizing agent VIA or VNA in upper leaves of *C. tetragonoloba* following treatment of lower leaves with PAE

Time interval between PAE treatment and preparation of leaf extract (h)	Average number of local lesions $\pm$ SEM ^a		
	Distilled water	NR-juice	R-juice
0	156 $\pm$ 7	150 $\pm$ 8	129 $\pm$ 6
6	162 $\pm$ 11	146 $\pm$ 7	48 $\pm$ 2
18	171 $\pm$ 5	152 $\pm$ 10	36 $\pm$ 5
24	150 $\pm$ 10	128 $\pm$ 8	0 $\pm$ 0
48	142 $\pm$ 9	124 $\pm$ 6	0 $\pm$ 0
72	184 $\pm$ 12	169 $\pm$ 12	24 $\pm$ 3
144	152 $\pm$ 6	136 $\pm$ 4	54 $\pm$ 8

^aVNA activity was estimated by mixing clarified juice from non-treated and treated plants with SRV and inoculation of mixture on 10 leaves of *C. tetragonoloba* $\pm$ standard error of the mean.

The R-juice from *C. tetragonoloba* plant showed broad spectrum antiviral activity against different viruses however, the per cent decrease or increase in VIA or VNA activity varied with different viruses (Table 3). Thus, VIA activity was

TABLE 3

Interference of viruses infection with VNA, produced in *C. tetragonoloba* leaves following treatment with PAE extract, in homologous and heterologous test hosts

Test host : Virus	Average number of local lesions ^a	
	NR-juice	R-juice
A. Homologous host		
<i>C. tetragonoloba</i> : SRV	152	0
B. Heterologous hosts		
<i>N. glutinosa</i> : TMV	137	0
<i>N. rustica</i>	82	0
<i>C. amaranticolor</i> : TMV	258	19
<i>D. metel</i> : TMV	63	0
<i>V. sinensis</i> : SRV	68	0
<i>C. amaranticolor</i> : SRV	138	0
<i>C. amaranticolor</i> : CGMMV	271	0

^aVNA or VIA activity was estimated by mixing clarified juice from non-treated and treated plants with viruses and inoculation of mixture on respective hosts.

not virus specific. Production of VIA or VNA was sensitive to metabolic inhibitor, actinomycin-D and no virus interfering activity was detected in the R-juice if AD was applied soon after PAE-treatment. VIA production however continued uninterrupted if AD was applied 12 h after PAE treatment (Fig. 1). Virus neutralizing activity of R-juice was lost following incubation with trypsin (250 μ g/ml), for 30 min at 45 °C (Fig. 2).

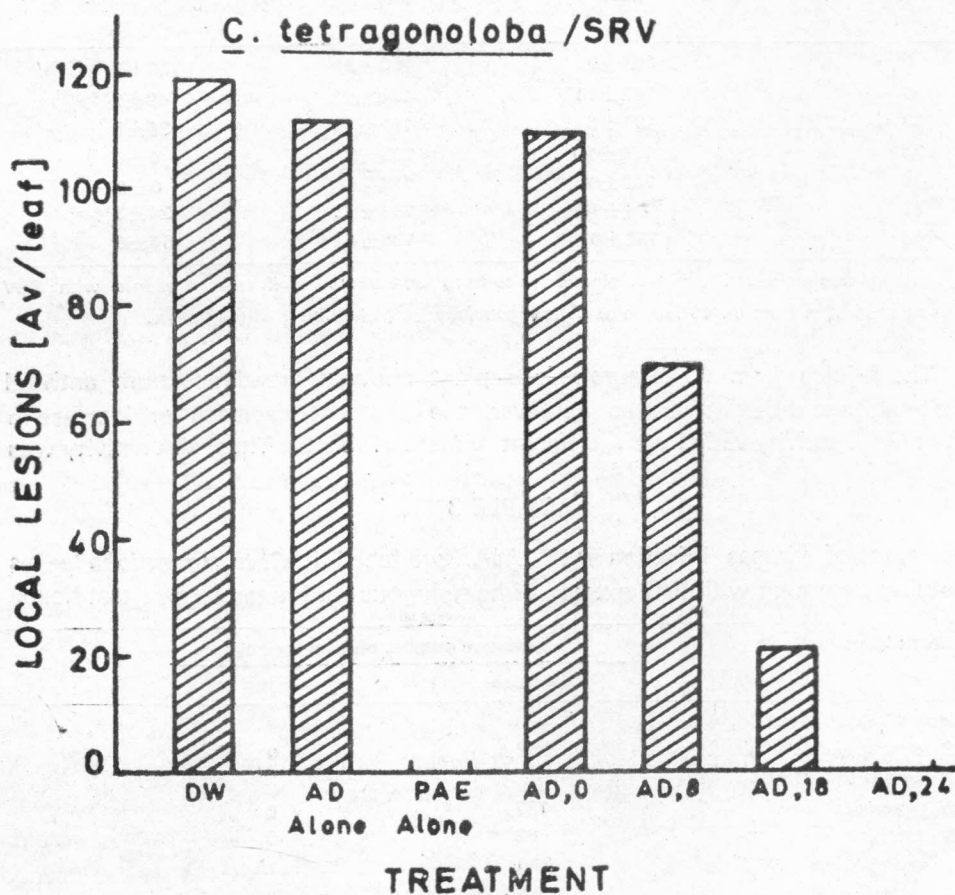


Fig. 1. Virus neutralizing activity of *C. tetragonoloba* leaf extract as affected by application of actinomycin D at different intervals following PAE treatment.

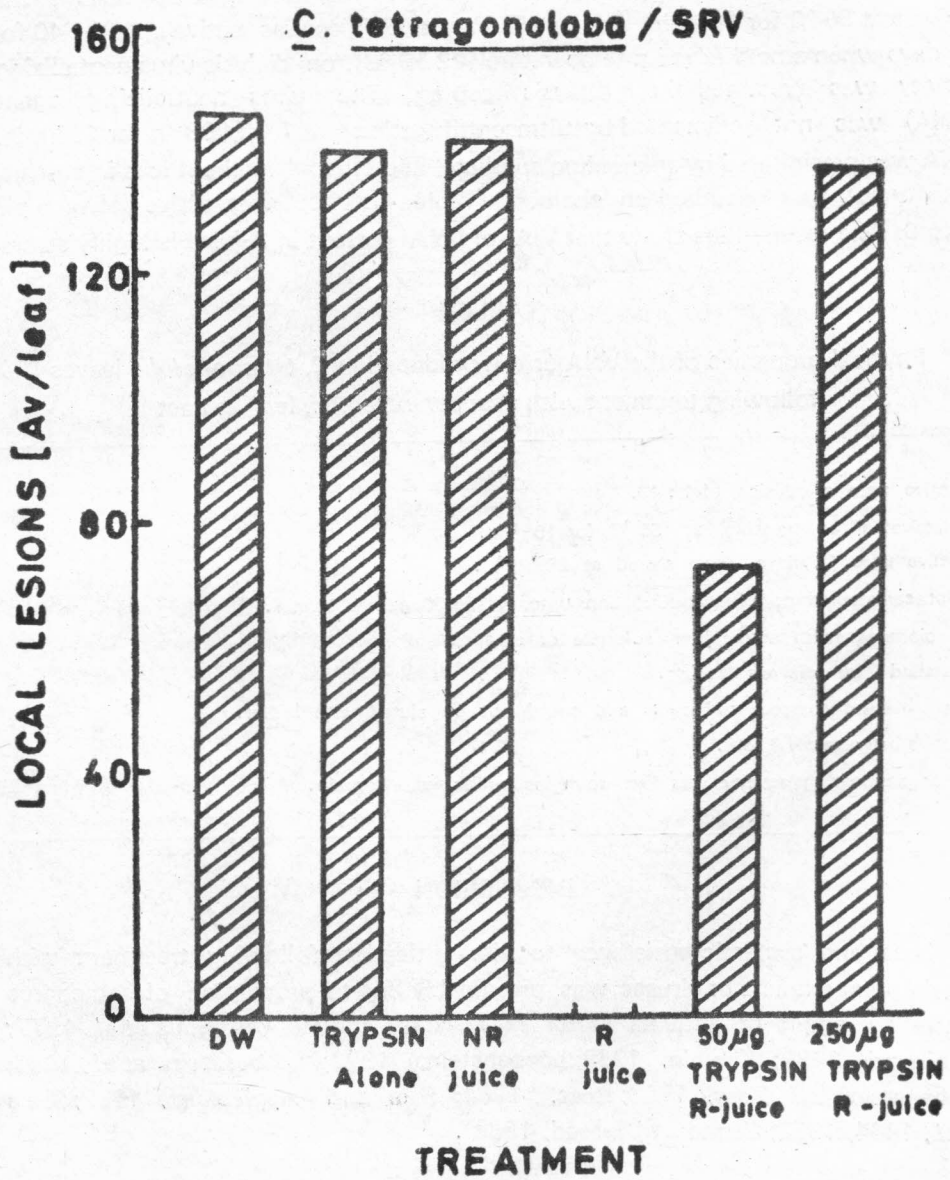


Fig. 2. Effect of trypsin on the activity of virus neutralizing agent obtained from PAE treated *C. tetragonoloba* leaves.

The virus neutralizing activity of the R-juice was destroyed by heating the R-juice at 90 °C for 10 min, and on diluting it, R-juice was active upto 1 : 40 for 12 days when stored at room temperature (22 ± 6 °C), on dialysis virus neutralizing activity was retained in dialysis tubing. The virus neutralizing agent (VNA) was not sedimented by ultracentrifugation at 1,20,000 g for 120 min. VNA was precipitated by ammonium sulphate and ethanol without loss in antiviral activity but was adsorbed on charcoal (Table 4). VNA was active between pH 6 to 8. The results thus show that VIA or VNA present in R-juice is highly stable.

TABLE 4

Physical properties of the VNA or VIA produced in *C. tetragonoloba* leaves following treatment with *Pseuderanthemum* leaf extract

-
- Active upto a dilution of 1 : 40.
 - Inactivated on heating at 90 °C for 10 min.
 - Active upto 12 days, when stored at 25-35 °C.
 - Not sedimented on ultracentrifugation upto, 1,20,000 g for 120 min.
 - Precipitated with ammonium sulphate or ethanol.
 - Retained in dialysis tubing.
 - Adsorbed on charcoal and celite and could not be eluted with buffer.
 - Active between pH 6 to 8.
 - Virus neutralizing activity was lost upon incubation with trypsin.
-

DISCUSSION

Local and systemic resistance to virus infection following treatment with certain plant extracts or viruses was presumably due to production of some virus interfering agent or antiviral factor in resistant tissues (Antignus *et al.*, 1977; Gianinazzi, 1982; Kimmin, 1969; Loebenstein, 1972; Loebenstein *et al.*, 1966; Loebenstein and Ross, 1963; Ross, 1964; Sela and Applebaum, 1962; Sela *et al.*, 1964, 1978; Verma and Prasad, 1983).

In the present work also it was found that when leaves of host plants were treated with *P. atropurpureum tricolor* leaf extract, they developed resistance at site as well as at remote site. When leaves from treated plants showing resistan-

ce were crushed and clarified extract, mixed with virus inocula, the virus infectivity was markedly decreased. On the contrary, if the leaves from untreated (control) plants were crushed and juice mixed with virus inocula, no decrease in virus infectivity was observed. Obviously, inhibitor treatment in leaves induced the formation of some potent virus neutralizing agent, which is either absent or present in non-detectable, concentrations in untreated plants.

The synthesis of the virus interfering agent (s) occurred a few hours after treatment with PAE and could be checked by application of actinomycin-D, simultaneously with the inhibitor. Since AD acts at the transcription level, it would appear that the synthesis of VIA or VNA is under control of cellular DNA.

More or less similar results were recorded for inhibitor present in *Boerhaavia diffusa* (Verma and Awasthi, 1980) and *D. metel* (Mukerjee *et al.*, 1981) Maximum synthesis of VIA, however, in this case occurred between 24 to 48 h after inhibitor treatment. Physical characteristics of the VIA were not very different. Since virus interfering activity was destroyed following incubation with trypsin, the nature of virus neutralizing agent (VNA) may be proteinaceous. Possibly, like vertebrates, cells of plants may produce some macro molecular substance(s) which interfere with the replication of plant viruses and enable the plant cells to resist and to recover from virus infection like interferon induced virus inhibitory or antiviral protein (Isaacs, 1963). VIA may have a direct action on virus particles or may block some steps leading to the synthesis of new virus particles.

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REFERENCES

- Antignus, Y., Sela, I. and Harpaz, I. 1977. Further studies on the biology of an antiviral factor (AVF) from virus infected plants and its association with the N-gene of *Nicotiana* species. *J. Gen. Virol.* **35** : 107-16.
- Gianinazzi, S. 1982. Antiviral agents and inducers of virus resistance analogies with interferon. In. *Active Defence Mechanisms in Plants*, edited by R. K. S. Wood, Plenum Publishing Corporation, New York. pp. 275-98.
- Gianinazzi, S. and Kassanis, B. 1974. Virus resistance induced in plants by polyacrylic acid. *J. Gen. Virol.* **23** : 1-9.

H. N. VERMA AND M. M. ABID ALI KHAN

- Gibbs, A. and Harrison, B. 1976. *Plant Virology: The principles*. Edward Arnold Publishers Ltd., London.
- Issacs, A. 1963. Interferon. *Adv. Virus Res.* **10** : 1-35.
- Kimmins, W. C. 1969. Isolation of a virus inhibitor from plants with localized infections. *Can. J. Bot.* **47** : 1879-86.
- Loebenstein, G. 1963. Further evidence on systemic resistance induced by localized necrotic virus infections in plants. *Phytopathology* **53** : 306-08.
- Loebenstein, G. 1972. Localisation and induced resistance in virus infected plants. *Ann. Rev. Phytopath.* **10** : 177-206.
- Loebenstein, G., Rabina, S. and Van Praagn, T. 1966. Induced interference phenomena in virus infections. In: *Viruses in plants*, (eds. A. B. R. Beemster and J. Dijkstra) pp. 151-57. North Holland Pub. Co., Amsterdam.
- Loebenstein, G. and Ross, A. F. 1963. An extractable agent, induced in uninfected tissues by localized virus infections that interferes with infection by tobacco mosaic virus. *Virology* **20** : 507-17.
- Mozes, R., Antignus, Y., Sela, I. and Harpaz, I. 1978. The chemical nature of an antiviral factor (AVF) from virus infected plants. *J. Gen. Virol.* **38** : 241-49.
- Mukerjee, K., Awasthi, L. P. and Verma, H. N. 1981. The inhibitory activity of an interfering agent, extracted from the leaves of host plants treated with *Datura* leaf extract, on plant virus infection. *Z. Pflanzenkr. Pflanzensch.* **88** : 228-34.
- Ross, A. F. 1964. Systemic resistance induced by localized virus infections in bean and cowpea. *Phytopathology* **24** : 1436.
- Sela, I. and Applebaum, S.W. 1962. Occurrence of an antiviral factor in virus-infected plants. *Virology* **17** : 543-48.
- Sela, I., Harpaz, I. and Birk, Y. 1964. Separation of a highly active antiviral factor from virus infected plants. *Virology* **22** : 446-51.
- Sela, I., Hauschner, A. and Mozes, R. 1978. The mechanism of stimulation of the antiviral factor (AVF) in *Nicotiana* leaves (The involvement of phosphorylation and the role of the N-gene), **89** : 1-6.
- Snedecor, G. W. 1981. *Statistical Methods*. Allied Pacific Private Limited, Bombay.
- Verma, H. N. and Awasthi, L. P. 1979a. Antiviral activity of *Boerhaavia diffusa* root extract and the physical properties of the virus inhibitor. *Can. J. Bot.* **57** : 926-32.
- Verma, H. N. and Awasthi, L. P. 1980. Occurrence of a highly antiviral agent in plants treated with *Boerhaavia diffusa* inhibitor. *Can. J. Bot.* **58** : 2141-44.
- Verma, H. N., Awasthi, L. P. and Mukerjee, K. 1979c. Induction of systemic resistance by antiviral plant extracts in non-hypersensitive hosts. *Z. Pflkrankn. Pfl. Schutz.* **86** : 735-40.
- Verma, H. N., Awasthi, L. P. and Saxena, K. C. 1979. Isolation of the virus inhibitor from the root extract of *Boerhaavia diffusa* inducing systemic resistance in plants. *Can. J. Bot.* **57** : 1214-17.
- Verma, H. N. and Prasad, V. 1983. *Recent Advances in Plant Pathology*, (eds. A. Husain et al.), Print House, Lucknow, India. pp. 312-23.

STUDIES ON A VIRUS DISEASE OF WHITE BUTTON MUSHROOM (*AGARICUS BISPORUS* (LANGE) SING.) IN INDIA*

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SUMMARY

White button mushroom (*Agaricus bisporus*) is prone to virus disease which causes serious problems in its successful cultivation. During the survey of a mushroom farm some trays showed abnormal fruiting bodies and low yield. Most of the cultures raised from such fruiting bodies showed slow growth with browning in centre and strand formation was lacking. On electron microscopy spherical virus particles of ~35 nm were observed. However, no such particles were noticed from the similarly processed healthy mushrooms. The virus infected crop produced 1.91 kg/tray in comparison to 5.62 kg/tray in healthy crop showing a loss of 65.96 per cent.

The white button mushroom *A.bisporus* (Lange) Sing.) is most popular cultivated mushroom and is widely grown in many countries viz., U.S.A., France, Taiwan, The Netherlands, United Kingdom, China etc. on large scale. Unfortunately this mushroom is prone to virus disease which causes serious problems in its production. Mushroom virus disease has been reported from various conditions viz., U.S.A. (Kneebone *et al.*, 1962), U. K. (Gandy, 1962). The Netherlands (Dielman van Zaayen and Temmink, 1968), Denmark (Hansen and Block, 1967) and Australia (Paterson, 1968). Though the incidence of the disease was noticed as back as 1948 in U.S.A. (Sinden and Hauser, 1950) and later on in Europe (Anonymous, 1957) its causal agent was unknown for a long time. However, Gandy and Hollings (1962) and Hollings (1962) established the viral nature of the disease. In India, mushroom virus has not been reported so far and it is possible that its incidence might have been overlooked.

MATERIAL AND METHODS

Recently during the survey of mushroom farm in Bangalore (Karnataka) abnormal fruiting bodies were noticed in some trays and yield was low. On examination of fruiting bodies and rhizomorphs no visible pathogens were noticed hence it was suspected either due to carbon dioxide or due to viral infection as stalks were elongated. When the change in position of trays did not improve the crop it showed the possibility of virus infection. The symptoms were: sporocarp loosely standing on casing soil, stipe slightly bent and long with small cap opening at

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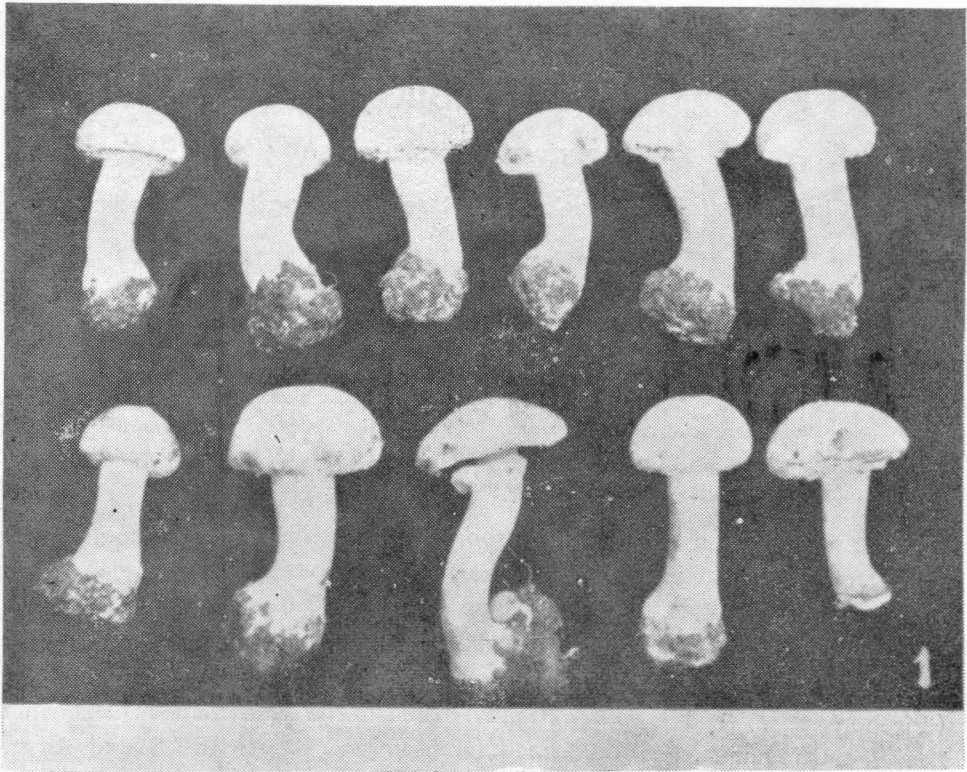


Fig. 1. Virus infected White button mushrooms.

early stage (Fig. 1), mushrooms light in weight, barren zones in trays showed disintegration of mycelium from casing soil and compost, and slow and poor yield was noticed. When compost from suspected trays was inoculated in trays with normal crop it produced abnormal mushrooms in 3-4 weeks. This showed the possibility of disease being transmitted. Studies on mycelial growth and electron microscopy were done to confirm the viral nature of the disease.

For electron microscopy diseased and healthy mushrooms were macerated in 0.05 M phosphate buffer (pH 7.2) in a blender separately and centrifuged at 10,000 g for 30 min. To the supernatant 6% polyethylene glycol (6000 MW) was added to precipitate the virus. The suspension was centrifuged at 30,000 g for 1h and the pellet was dissolved in 0.05 M phosphate buffer. This was further centrifuged at 10,000 for 15 min. This formed the partially purified virus preparation and was used for electron microscopy.

RESULTS

Tissue cultures were raised from diseased and healthy mushrooms separately and were observed for their growth for three weeks period. The growth pattern of

Virus Disease of White Button Mushroom

diseased cultures showed variations, whereas healthy cultures showed vigorous and fluffy mycelial growth with strands radiating from the centre. In comparison to healthy cultures' growth, the mycelial growth in diseased cultures was (a) very slow, browning in centre 20% (Fig. 2); (b) moderate suppressed growth with light

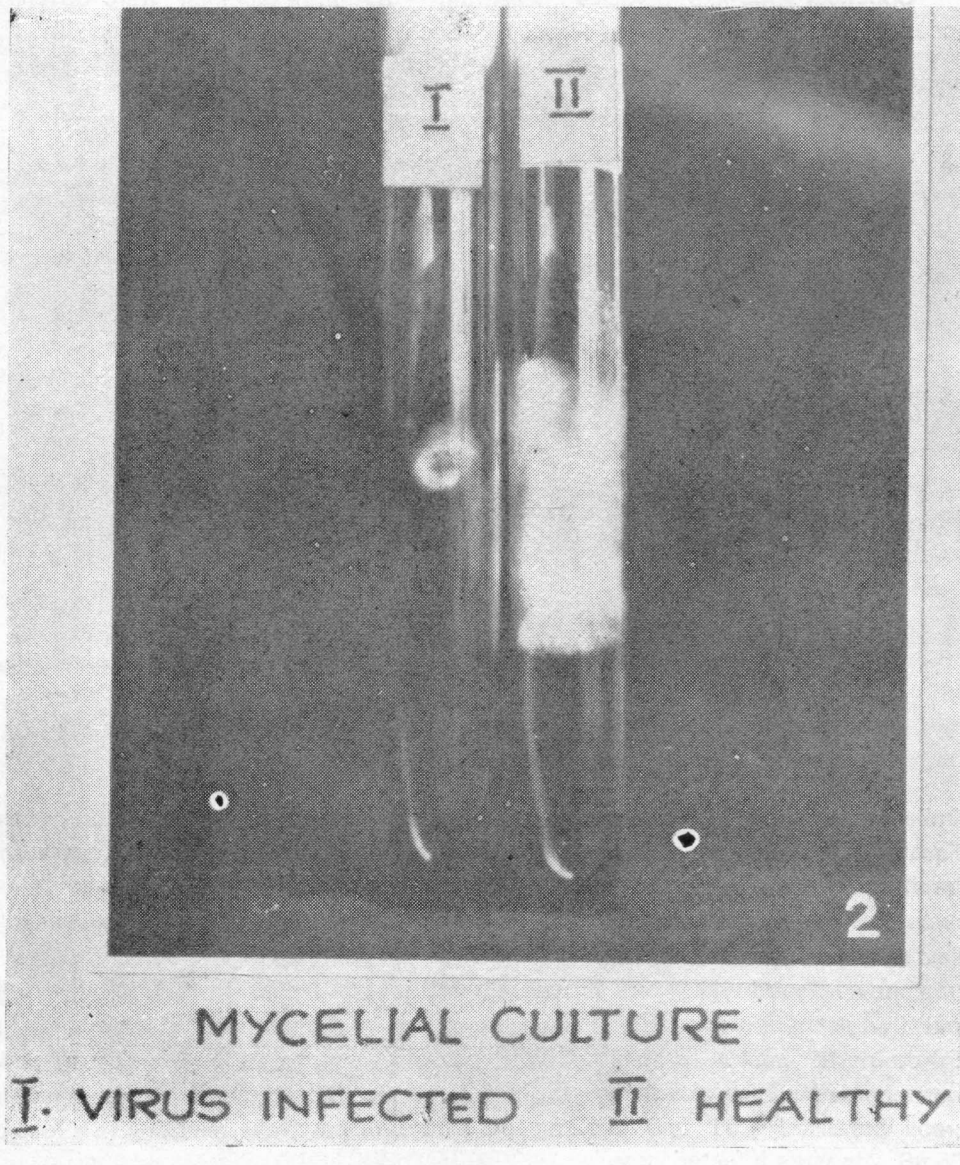


Fig. 2. Diseased (I), and Healthy (II) Mushrooms Culture,

browning in centre 40%; (c) moderate growth with poor strand formation, little browning 18%; (d) normal growth but poor strand formation and no browning 12%; and (e) normal growth with strand formation as healthy cultures 10 per cent.

Spherical virus-like particles measuring ~ 35 nm in diameter were observed under electron microscope (Fig. 3). Such particles were not seen in the

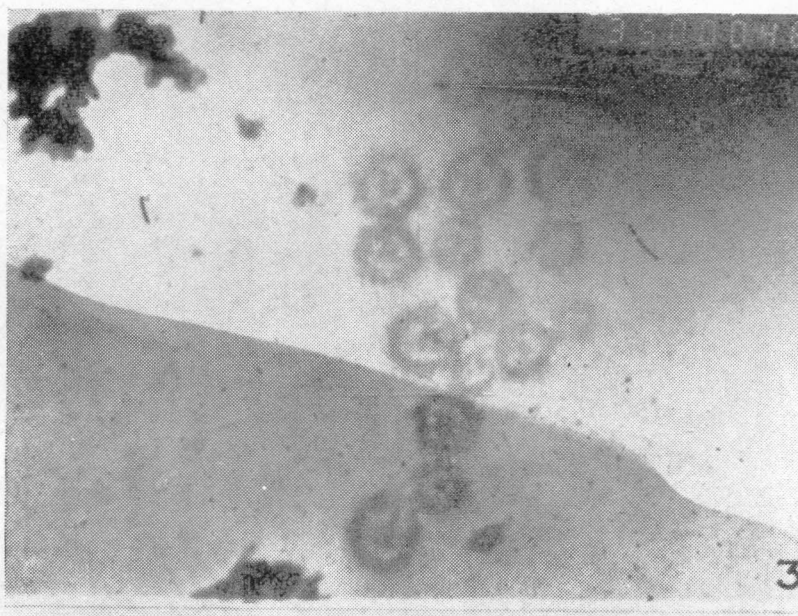


Fig. 3. Virus particles extracted from diseased White button mushroom.

similarly processed healthy mushroom. This confirmed the viral nature of the disease. The weekly yield performance of infected and healthy crops is presented graphically in Fig 4. In virus infected crop about 25% trays showed clear symptoms of virus disease. Patches of barren zones were noticed in casing soil. Rest of trays were showing normal mushrooms but growth was slow. First week crop showed good yield but in subsequent weeks it was low. However, in IX and X week there was sudden increase resulting in highest yield of the crop (44.0 kg) during IX week. In healthy crop, pattern was normal and maximum yield was recorded in II week and lowest in VIII week. The virus infected and healthy crops produced 1.91 and 5.62 kg per tray, respectively resulting in a loss of 65.96% in virus infected crop.

Virus Disease of White Button Mushroom

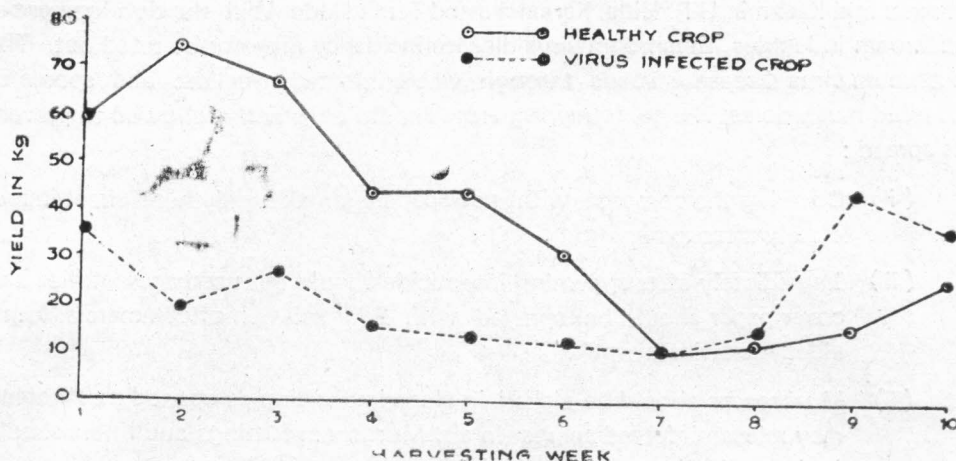


Fig. 4. Cropping pattern in healthy (65 trays) and virus infected (103 trays) crops.

DISCUSSION

A very serious disease of white button mushroom was observed in the United States of America during 1948, its causal agent was unknown and was named as 'La France disease' (Sinden and Hauser, 1950). Later on, it was noticed in Europe with some common symptoms and different names were given due to variations in symptom viz., Brown disease, Watery stipe (Gandy, 1960a), disease (Kneebone *et al.*, 1962) and die-back (Gandy and Hollings, 1962). Gandy (1960b) found that the disease was caused by an infectious agent transmitted by hyphal anastomosis. Gandy and Hollings (1962) and Hollings (1962) confirmed the viral nature of disease. van Zaayen (1979) reported that the mushroom virus disease resulted in a total crop loss of 4.5% in The Netherlands during 1967. The present information showing crop loss of 65.96% clearly indicates that the mushroom virus disease may be a limiting factor in successful mushroom production.

In U K., five kinds of virus particles have been reported and numbered as mushroom virus 1 to 5 (Hollings, 1962). However, most frequently observed particles are 25 nm (mushroom virus 1), 19 nm X 50 nm (mushroom virus 3) and 34 nm (mushroom virus 4). The virus particles ~ 34 nm in diameter have been found to occur in large numbers. In present studies also virus particles observed seem to be from this group. van Zaayen (1979) reviewed in detail virus diseases of mushrooms and emphasized a common term 'mushroom virus disease.'

In India, white button mushroom cultivation is hardly two decades old. However, its cultivation is becoming more and more popular in Himachal Pradesh,

Jammu and Kashmir, U.P. Hills, Karnataka and Tamil Nadu. With the development of mushroom industries, mushroom virus disease incidence may not be ruled out. The mushroom virus disease spreads through viable infected mycelium and spores of diseased mushrooms, Hence, following steps should be strictly followed to prevent its spread :

- (i) Covering the compost with newspapers or thin polythene sheet immediately after spawning.
- (ii) Immediately after spawning insecticide should be used against flies and cover paper should be sprayed with 2% solution of commercial formaldehyde twice a week.
- (iii) Mushrooms should be picked in closed condition as opened mushrooms may release infected spores in air. Mushroom cuttings and litters should be immediately removed and destroyed.
- (iv) Once crop is over cooking out for 12 h at 70 °C should be followed to destroy mycelium, spores and harmful organisms in compost.
- (v) Trays should be sprayed with 5% formaldehyde solution before compost filling.
- (vi) Disinfection of rooms, corridors platforms and surrounding areas with 5% formaldehyde solution should be strictly followed.

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REFERENCES

- Anonymous. 1975. It is 'La France'. MGA (Mushroom Growers Association) Bulletin **96** : 407-09.
- Dielman-van Zaayen, A. and Temmink, J. H. M. 1968. A virus disease of cultivated mushrooms in the Netherlands. *Neth. J. Pl. Path.* **74** : 48-51.
- Gandy, D. G. 1960a. Watery stipe of cultivated mushrooms. *Nature Lond.*, **185** : 482-83.
- Gandy, D. G. 1960b. A transmissible disease of cultivated mushrooms (Watery stipe). *Ann. Appl. Biol.* **48** : 427-30.
- Gandy, D. G. 1962. Studies on die-back of mushrooms. *Mushroom Sci.* **5** : 468-79.
- Gandy, D. G. and Hollings, M. 1962. Die-back of mushrooms : A disease associated with a virus. *Rep. Glasshouse Crops Res. Inst.* **1961** : 103-08.

Virus Disease of White Button Mushroom

- Hansen, H. P. and Block, B. 1967. Champignon virose i Denmark (Mushroom virus in Denmark). *Horticultura* **21** : 179-81.
- Hollings, M. 1962. Viruses associated with a die-back disease of cultivated mushrooms. *Nature*, Lond. **196** : 962-65.
- Kneebone, L. R., Lockard, J. D. and Hager, R. A. 1962. Infectivity studies with X-disease. *Mushroom Sci.* **5** : 461-67.
- Sinden, J. W. and Hauser, E. 1950. Report on two new mushroom diseases. *Mushroom Sci.* **1** : 96-100.
- van Zaayen, A. 1979. *Mushroom Viruses in Viruses and Plasmids in Fungi*. Edited by Paul A. Lemke. Marcel Dekker Inc., New York and Barel. pp. 239-324.

APHID TRANSMISSION CHARACTERISTICS OF POTATO VIRUS Y^N

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SUMMARY

Potatoes are universally afflicted by a large number of viruses and 11 viruses have been reported infecting potatoes in India. During periodical surveys in 1982 in and around Shimla/Kufri, a few isolates of PVY^N (causing veinal necrosis in Samsun NN tobacco) were established from mild mottled plants of Kufri Chandramukhi at Shimla/Kufri. Even though its incidence was very low, it may become widespread and important in future due to its mild symptoms as the virus has already assumed importance in Netherlands. Aphid transmission characteristics of this virus are relatively less worked upon. Studies were, therefore, undertaken to observe its transmission by *Myzus persicae* Sulz. and *Aphis gossypii* Glov. Since *A. gossypii* proved to be a poor vector, the virus-vector relationship between PVY^N and *M. persicae* alone was worked out.

MATERIAL AND METHODS

Colonies of aviruliferous aphids/clones of *M. persicae* and *A. gossypii* were maintained by frequent subculturing on healthy virus-free/immune plants of cabbage and chilli, respectively, throughout the investigations. PVY^N was maintained by frequent subculturing by sap/aphid inoculations on *Nicotiana tabacum* var. White Burley. Test plants of tobacco var. White Burley (3-4 week old, having 3-4 leaves) were transplanted singly in 10 cm earthen/plastic pots and maintained in an insect-proof glasshouse. All transmissions were tried to and from tobacco var. White Burley, unless mentioned otherwise. For aphid transmission only viviparous apterous adults were used. They were starved for 1h before they were allowed to feed for 5 min on a detached virus infected tobacco leaf. Thereafter such (viruliferous) aphids were transferred in groups of five aphids to each test plant for 15 min inoculation feeding unless otherwise stated. Later the aphids were killed by spraying 0.1% Metasystox. The test plants were maintained in an insect-proof glasshouse for 4-6 weeks. All experiments were repeated at least twice. For each replicate in an experiment, a minimum of 5-10 plants were used. Adequate controls were always maintained by exposing them to equal number of non-viruliferous aphids. None of the plants under control developed infection/symptoms.

RESULTS

Influence of the Number of Aphids

A varying number of viruliferous aphids of *M. persicae* clone H. V. III was released on to the test plants. The data (Table 1) indicate that even single aphid per plant was able to transmit the virus. The per cent of infection ranged between 20 to 76.6 per cent. The number and amount of transmission increased with an increase in number of inoculative aphids/plant. The highest rate of transmission of PVY^N was obtained with either 5 or 10 aphids/plant.

TABLE 1
Influence of the number of aphids on PVY^N transmission

Aphids plant	Trial			%Avg. Transm.
	I	II	III	
1	3*	1	2	20.0
2	4	3	3	33.3
5	7	8	8	76.6
10	7	8	6	70.0

* No. of plants infected/10 inoculated/trial

Effect of Pre-acquisition Fasting

Aphids, pre-starved for different periods, i. e., either 0, 0.5, 1, 2 or 4 h, were allowed an acquisition feeding for 5 min and thereafter transferred either singly or in batches of five per plant for effecting transmission.

Even single unstarved aphids were able to transmit the virus to a low extent. But aphids pre-starved for 1 h resulted in maximum transmission. Prolonged pre-acquisition fasting beyond 1 h (e. g. 2-4 h; Table 2) resulted in a decline in their ability to acquire the virus for transmission.

TABLE 2
Effect of fasting on PVY^N transmission

Fasting (h)	Trial			% Avg. Transm.
	I	II	III	
0.0	1*	1	1	10.0
0.5	6	4	5	50.0
1.0	8	7	7	73.3
2.0	7	7	7	70.0
4.0	6	6	6	60.0

* No. of plants infected/10 inoculated in each trial

Acquisition-Threshold Period

To determine the virus acquisition-threshold period (after 1 h pre-acquisition fasting), the aphids were allowed acquisition feeding by individuals for 15, 30, 60 sec (under observation) and 2, 5 and 15 min or virus-source leaves followed by 15 min transmission (inoculation) feedings on test plants.

The data (Table 3) indicate that aphids acquired the virus from the infected plants even within 15 sec feeding. But the percentage of transmission was maximum when acquisition access for 5 min was allowed. Further increase in the acquisition access was responsible for a corresponding decrease in virus acquisition/transmission.

TABLE 3
Acquisition threshold for PVY^N transmission

Acqu. Threshold	Trial				% Avg. Transm.
	I	II	III	IV	
15 sec	1*	0	0	0	5.0
30 "	1	0	0	1	10.0
60 "	2	2	1	1	30.0
2 min	2	2	3	2	45.0
5 "	3	3	4	4	70.0
15 "	3	3	3	3	60.0

* No. of plants infected/5 inoculated/trial

Inoculation Threshold Period

Pre-starved aphids, that were allowed 5 min acquisition access, were transferred in batches of five aphids/plant for different periods of inoculation access (Table 4). After the desired period of inoculation access (as being watched under a stereo binocular), the aphids were removed manually.

TABLE 4
Effect of inoculation feeding on the transmission of PVY^N

Inoculation Feeding	Trial			% Avg. Transm.
	I	II	III	
0.5 min	2*	1	2	13.3
1 "	3	4	3	33.3
2 "	4	5	5	46.6
5 "	6	6	6	60.0
15 "	6	7	7	66.6
24h	7	6	6	63.3

* No. of plants infected/10 inoculated/ trial

The aphids were able to poorly transmit the virus within 30 sec of inoculation access but not in 15 sec. Inoculation access of 15 min or more resulted in a higher rate of virus transmission (Table 4). There was, however, no increase in the per cent of transmission by further increasing the inoculation access time.

Retention of Virus by Aphids

The retention of the virus by an aphid, i. e., the number of plants an individual viruliferous aphid can infect was determined by feeding an aphid on a series on five plants for either 1 or 2 min on each of the first four plants and left overnight on the last plant in series.

The data (Table 5) revealed that in neither case, the viruliferous aphids caused transmission on more than 4 test plants in a series. However, the shorter inoculation feeding of 1 min, at times, resulted no infection on the first test plant. Nonetheless, feeding for 2 min had positive transmissions on the first and even second test plants in the series. Thence, it is derived that the aphids were not able to retain the virus beyond the initial feeding of few minutes, i. e., virus is being carried non-persistently by the vector.

TABLE 5
Persistence of PVY^N in aphids

Transm. Feeding (min)	Trial	Series of Plants*				
		1	2	3	4	5
1	I	+	—	—	—	—
	II	+	+	—	—	—
	III	—	+	+	—	—
2	I	+	+	—	—	—
	II	+	—	+	—	—
	III	+	—	—	—	—

* Infection by single aphid

Interaction between the Vector, Virus-source and Test Plants

To elicit information on the interaction between the vector and virus-source-test hosts of PVI^N, a total of 16 combinations were tried with four plant species as the donor test plants.

The data revealed that tobacco var. White Burley proved to be the best both as virus-source and test plants (Fig. 1). It was followed by potato var. Kufri Jyoti

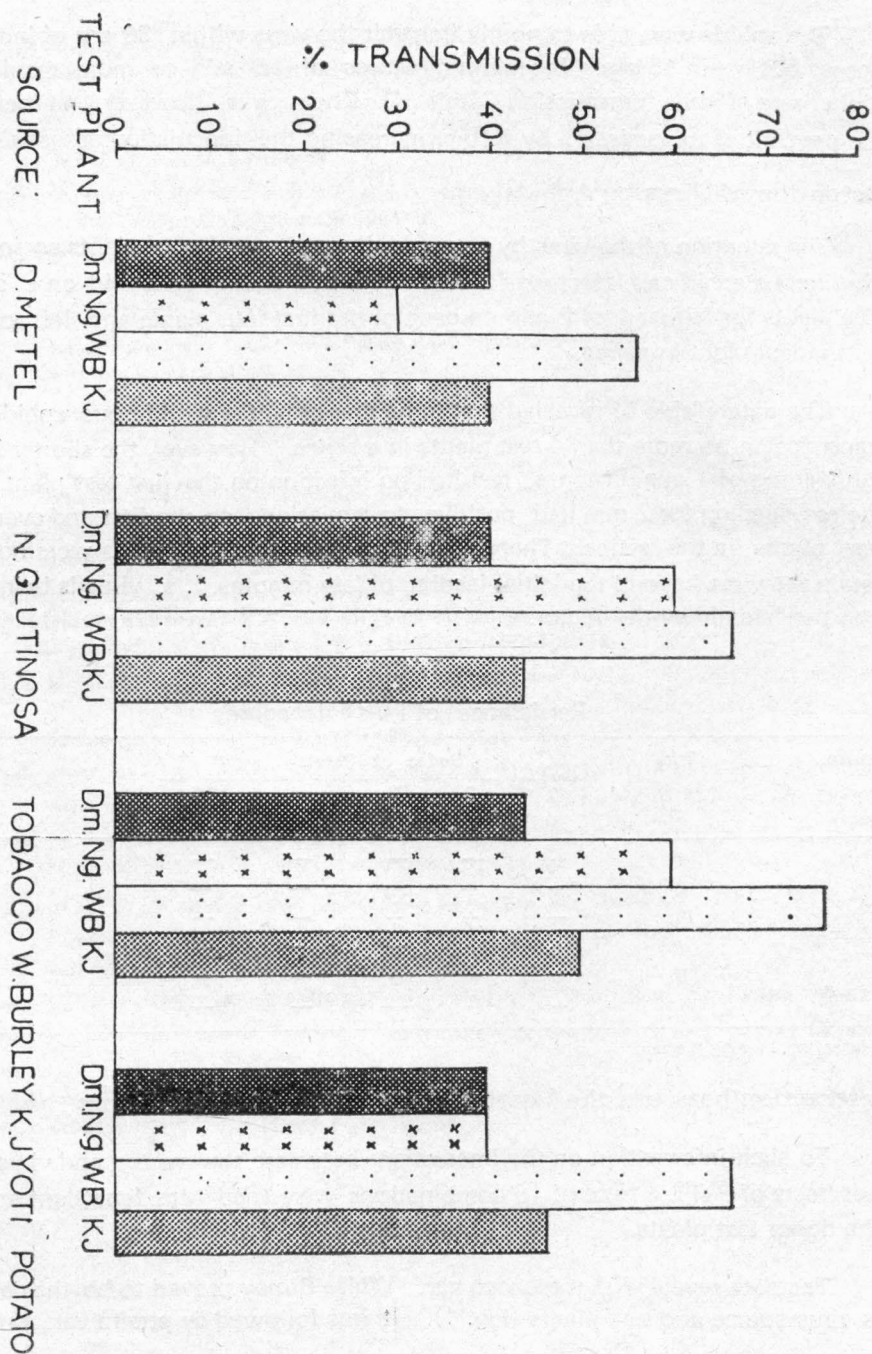


Fig. 1. Effect of source/test plants on the aphid (*M. persicae*) transmission of PVY^N (*test plants D. m. = *Datura metel*, N. g. = *Nicotiana glutinosa*, WB = tobacco var. White Burley, KJ = Potato seedling of Kufri Jyoti).

which proved to be a moderate type of host. On the other hand, *Datura metel*, an ideal host for PVY^O (Singh *et al.*, 1982, 1983), proved to be the poor virus-source and test plant.

DISCUSSION

Present investigations have confirmed *M. persicae* to be a potent vector of PVY^N. Short exploratory probes of virus-source tissues are effective for non-persistent viruses (Hashiba and Misawa, 1969; Watson and Plumb, 1972; Singh *et al.*, 1981). PVY^N was acquired and transmitted in as short as 15 and 30 sec. The acquisition- and transmission-thresholds for PVY^N isolate were similar to that observed earlier (Van Hoof, 1980) and within the known range for a number of other non-persistent viruses (Singh *et al.*, 1983a,b). Further prolonged transmission feeding did not increase the virus transmission as also reported for a number of other poty viruses (Bhargava and Khurana, 1970; Khurana *et al.*, 1973; Singh *et al.*, 1982). Variations in rate of transmission due to varying virus-source and or test plant species as well as number of inoculative aphids/plant are in close agreement with reports for strains of cucumber mosaic virus (Bhargava, 1951), ragi mosaic virus (Khurana *et al.*, 1973), cowpea banding mosaic virus (Sharma and Verma, 1977), brinjal mosaic virus (Seth and Raychaudhari, 1977) and also potato virus Y^O and M (Wetter and Volk, 1960; Bode and Weidemann, 1971; Kostiw, 1978; Syller, 1976; Singh *et al.*, 1979, Singh *et al.*, 1983a, b). It can be concluded that PVY^N transmission largely depends upon the preacquisition fasting, short acquisition and transmission feedings.

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Sincere thanks are due to Dr. G. S. Shekhawat, Head, Pl. Path. Division and Dr. B. B. Nagaich, ex-Director, CPRI, Shimla for their encouragement and ruthless criticism/suggestions.

REFERENCES

- Bhargava, K. S. 1951. Some properties of four strains of cucumber mosaic virus. *Ann. Appl. Biol.* **38** : 377-88.
- Bhargava, K. S. and Khurana, S. M. Paul. 1970. Insect transmission of papaya viruses with special reference to papaya mosaic virus. *Zbl. Bakt. Abt.* **124** : 688-96.
- Bode, O. and Weidemann, H. L. 1971. Untersuchungen zur blattlausübertrag-barbeit von kartoffel-M-Und-S-Virus. *Potato Res.* **14** : 119-29.
- Bokx, J. A. de. (ed.) 1972. Viruses of potatoes and seed-potato production. PUDOC, Wageningen, pp. 233.

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REFERENCES

- Bhargava, K. S. 1951. Some properties of four strains of cucumber mosaic virus. *Ann. Appl. Biol.* **38** : 377-88.
- Bhargava, K. S. and Khurana, S. M. Paul. 1970. Insect transmission of papaya viruses with special reference to papaya mosaic virus. *Zbl. Bakt. Abt.* **124** : 688-96.
- Bode, O. and Weidemann, H. L. 1971. Untersuchungen zur blattlausübertrag-barbeit von kartoffel-M-Und-S-Virus. *Potato Res.* **14** : 119-29.
- Bokx, J. A. de. (ed.) 1972. Viruses of potatoes and seed-potato production. PUDOC, Wageningen, pp. 233.

M. N. SINGH AND S. M. PAUL KHURANA

- Hashiba, T. and Misawa, T. 1969. Studies on the mechanism of aphid transmission of stylet-borne virus. II. A comparison between probing and feeding for the virus transmission. *Tohoku J. Agril. Res.* **20** : 97-106.
- Khurana, S. M. Paul and Singh, M. N. 1983. Additions to the viral-spectrum of potatoes in India. *Indian J. Pl. Pathol.* **1** : 159-62.
- Khurana, S. M. Paul, Raychaudhuri, S. P. and Sundram, N. V. 1973. Further studies on eleusine mosaic in Delhi. *Indian Phytopathol.* **26** : 554-59.
- Kostiw, M. 1978. Transmission of four isolates by *Myzus persicae* Sulz and *Aphis nasturtii* Kalt. Proc. 7th-Trin. Conf. EAPR, Warsaw, Poland, 26 June-1 July, 1978. pp. 246-47 (Abstr.).
- Lal, S. B. and Khurana, S. M. Paul, 1983. Detection and identification of viruses and mycoplasmas and evaluation of yield losses. In Potato Production, storage and utilization. (ed. B. B. Nagaich), CPRI, Simla. pp. 334-48.
- Nagaich, B.B., Shekhawat, G.S., Khurana, S.M. Paul and Bhattacharayya, S.K. 1974. Pathological problems of the potato cultivation in India. *J. Indian Potato Assoc.* **1** : 32-44.
- Seth, M. L. and Raychaudhuri, S. P. 1977. Investigations on the relationship of a new brinjal mosaic virus and its vector, *Aphis gossypii* Glov. *Acta Botanica Indica* **5** : 133-38.
- Sharma, S. R. and Varma, A. 1977. Factors affecting transmission of cowpea banding mosaic virus by aphids. *J. Entomol. Res.* **1** : 29-35.
- Singh, M. N., Khurana, S. M. Paul, Nagaich, B. B. and Agrawal, H. O. 1981. Probing behaviour of *Myzus persicae* clones and transmission of potato virus Y. Proc. 5th Intern. Virology Confr., Strasbourg (France), August 2-8, P 19/03, pp. 214 (Abstr.).
- Singh, M. N., Khurana, S. M. Paul, Nagaich, B. B. and Agrawal, H.O. 1982. Efficiency of *Aphis gossypii* pisum in transmitting potato viruses leaf roll and Y. In. Potato in Developing Countries, (eds. B. B. Nagaich et al.) Indian Potato Assoc. CPRI, Shimla, pp. 289-93.
- Singh, M. N., Khurana, S. M. Paul, and Nagaich, B. B. 1983a. Evidence on heredity variations in the virus transmission efficiency of the aphid clones. *Z. Pflanzenkrankh. Pflanzenschutz* **90** : 345-51.
- Singh, M. N., Khurana, S. M. Paul and Nagaich, B. B. 1983b. Transmission of potato virus M by *Myzus persicae* Sulz. *Indian J. Pl. Pathol.* **1** : 163-69.
- Syller, M. J. 1978. Efektywnosc przenoszenia winsow M, SIY ziemniaka przez mszyce brzoskwiowo-ziemiaczana (*Myzus persicae* Sulz.) Z Roslin porazonych kompleksami wirsow M+YIS+Y. *Biuletyn Instytutu Ziemiaka* **22** : 63-76.
- Van Hoof, H. A. 1980. Aphid vectors of potato virus Y^N. *Neth. J. Pl. Pathol.* **86** : 159-62.
- Watson, M. A. and Plumb, R. T. 1972. Transmission of plant-pathogenic viruses by aphids. *Ann. Entomol. Res.* **17** : 425-52.
- Wetter, C. and Volk, J. 1960. Versuche zur vbertvagung der kartoffelviren M-und-S durch *Muzus persicae* (Sulz). *Eur. Potato J.* **3** : 158-63.

STUDIES ON THE TRANSMISSION OF PAPAYA MOSAIC VIRUS BY *RHOPALOSIPHUM MAIDIS* (FITCH.)

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SUMMARY

Detailed investigations were undertaken on the relationship of papaya mosaic virus (PMV) and *Rhopalosiphum maidis* Fitch. The unstarved aphids transmitted the virus to the extent of 20 per cent. Pre-acquisition starvation, however, increased the efficiency of the vector and the maximum infection was obtained after 30 min pre-acquisition starvation. Fifteen minutes feeding was found to be optimum for each of acquisition and transmission of the virus. The infection increased with the increase in number of aphids per test plant and the maximum infection was obtained with five apterous adult per test plant. Winged and non-winged aphids transmitted the virus with the same efficiency. Adults were more efficient vectors than the nymphs. Aphids lost the virus rapidly after inoculating the 1st test plant, indicating a non-persistent type of virus-vector relationship.

Papaya mosaic virus causes heavy losses to the papaya crop in areas where it is being cultivated. It is because of this disease, the cultivation of papaya, a fast growing, early fruiting and a medicinal plant is unorganised and scattered within the State of Punjab. PMV is known to be transmitted by a number of aphid species (Raychaudhuri and Nariani, 1977). Recently, *R. maidis* has been reported to be an efficient vector of PMV for the first time (Cheema and Reddy, 1983). Present investigations were undertaken to study the relationship of PMV and *R. maidis* and the results are presented in this paper.

MATERIAL AND METHODS

Virus-vector relationships were studied under insect-proof cages at the Department of Plant Pathology, Punjab Agricultural University, Ludhiana. The culture of PMV was maintained on healthy plants of papaya cultivar Honey Dew. The non-viruliferous aphids were reared on healthy maize plants of variety Ganga-5. Twenty days old seedlings of papaya (cultivar Honey Dew) were used as the test plants. Unless stated otherwise, five apterae adults were employed on each test seedling. Other methods were same as described by Nagaich and Vashisth (1962).

RESULTS AND DISCUSSION

Acquisition feeding period. The aphids were starved for one h and given an acquisition feeding periods ranging from 1 to 60 min (Fig. 1). One min feeding was enough to acquire the virus. However, the efficiency of the vector was increased with increase in acquisition access. It was highest at an acquisition

feeding period of 15 min after which it began to decline and infection was zero per cent when the acquisition feeding period was 60 min.

Inoculation feeding period. After one h pre-acquisition starvation and 15 min acquisition feeding period the aphids were transferred to test plants and allowed to feed from 1 to 60 min (Fig. 1). The results presented in Fig. 1 showed that

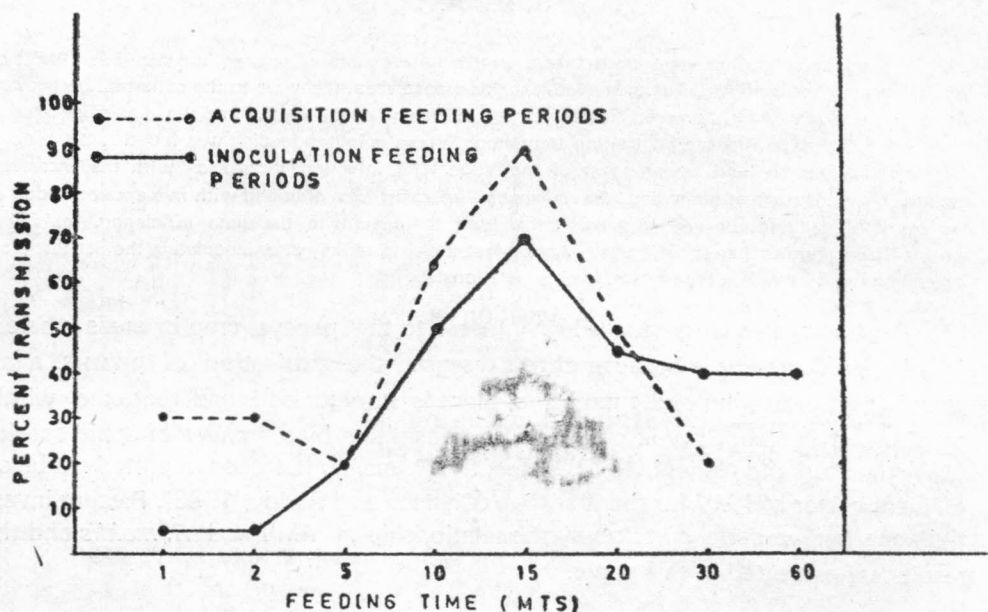


Fig. 1. Effect of different acquisition and inoculation feeding periods on the efficiency of *Rhopalosiphum maidis*.

one min access on test plant was enough to initiate the infection by aphids. However, the percentage of transmission was increased with prolonged transmission period. The maximum infection (70%) was obtained after 15 min of feeding period, on test plants, after which it began to decrease and became almost the same upto 60 min of feeding period.

Effect of pre-acquisition starvation. Non-viruliferous aphids were given varying pre-acquisition starvation periods ranging from 0 to 60 min. The starved aphids were then given acquisition feeding and transmission feeding period for 15 min each. From the results given in Fig. 2, it is obvious that unstarved aphids transmitted the virus to the extent of 20 per cent. However, pre-acquisition

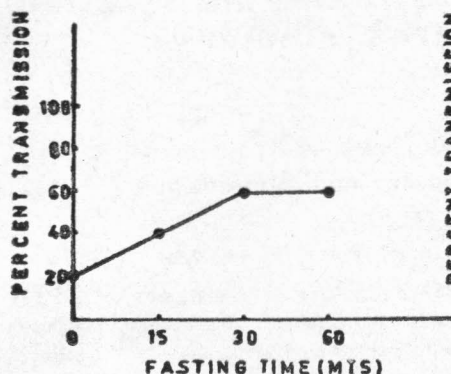


Fig. 2. Effect of pre-acquisition fasting periods on efficiency of *Rhopalosiphum maidis*.

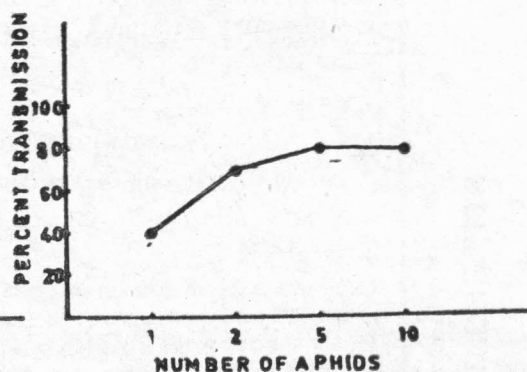


Fig. 3 Relationship between the number of aphids and transmission efficiency.

starvation increased the efficiency of the vector. The highest infection (60%) was obtained with 30 min pre-acquisition starvation and then it became almost constant.

Efficiency of various morphological forms of the vector. Three morphological forms viz., nymphs, alate adults and apterae adults were given pre-acquisition starvation for 30 min, acquisition and inoculation feeding for 15 min each (Fig. 4). The results showed that adults were more efficient vectors than nymphs. The alate and apterae adults transmitted PMV with almost the same efficiency.

Persistence of virus in the vector. After 30 min pre-acquisition starvation and 15 min acquisition, the aphids were individually transferred to a series of five test plants at an interval of 15 min (Table 1). The data presented in Table 1 indicate that out of 10 aphids tested, only four (aphids) could transmit the virus and out of these none could infect more than one plant.

Present investigations have revealed that unstarved aphids transmitted the virus to the extent of 20 per cent. These findings are in conformity with those of Chatterjee and Raychaudhuri (1965) who reported that *R. maidis* was capable of transmitting the mulberry mosaic virus without pre-acquisition fasting. Pre-acquisition starvation increased the per cent infection upto 30 min duration. Longer starvation, however, has no beneficial effects. Similar observations have been reported by Singh (1972) while working with PMV and *Aphis gossypii* as vector.

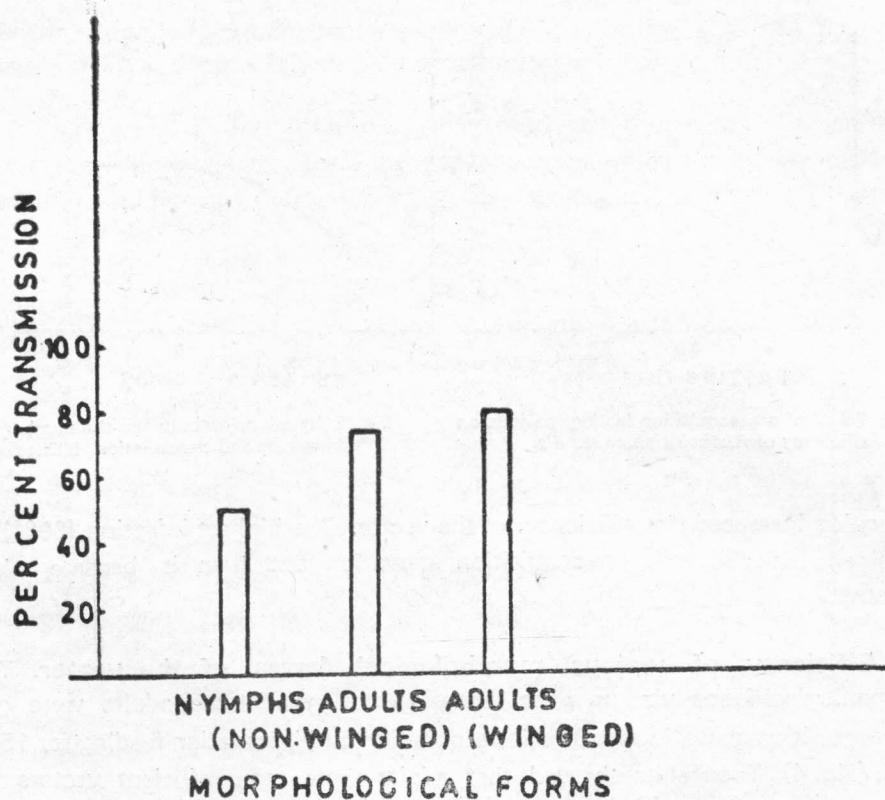


Fig. 4. Effect of different morphological forms on the efficiency of *Rhopalosiphum maidis*.

TABLE 1

Persistence of PMV in the vector (*Rhopalosiphum maidis*)

S. No. of test plants	S. No. of aphids									
	1	2	3	4	5	6	7	8	9	10
1	—	÷	+	—	—	—	+	—	+	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—

The optimum acquisition and inoculation feeding periods were 15 min each. Prolonged feeding period did not improve the efficiency of the vector. Similar results were obtained by Singh (1972) while testing the efficiency of *A. gossypii* in transmitting PMV. Presumably the aphid carries less and less virus after each probe and the possibility of successful infection is progressively reduced (Mathews, 1981).

The present investigations have also indicated that per cent infection increased with increase in the number of aphids and maximum infection was obtained with five aphids per test plant Fig. 3. These findings are in conformity with those of Singh (1972). The adults of *R. maidis* are already known to be more efficient vectors of mulberry mosaic virus (Chatterjee and Raychaudhuri, 1965).

R. maidis lost the virus rapidly after inoculating the first test plant indicating a non-persistent type of virus-vector relationship. Earlier reports indicated that *R. maidis* transmitted eleusine mosaic virus and *A. gossypii* transmitted PMV in non-persistent manner (Raychaudhuri, 1977; Singh, 1972).

REFERENCES

- Chatterjee, S. N. and Raychaudhuri, S. P. 1965. A note on aphid transmission of mosaic disease of mulberry. *Indian Phytopath.* **18** : 319-20.
- Cheema, S. S. and Reddy, R. S. 1983. *Rhopalosiphum maidis* Fitch—A new vector of papaya mosaic virus (PMV). *Curr. Sci.* (Submitted for publication).
- Mathews, R.E.F. 1981. Relationships between Plant Viruses and Invertebrates. In. *Plant Virology*. Academic Press, New York. pp. 501-607.
- Nagaich, B. B. and Vashisth, K. S. 1962. Studies on the transmission of broad bean mosaic virus by *Aphis craccivora* Koch. *Indian J. Ent.* **27** : 188-95.
- Raychaudhuri, S. P. 1977. Eleusine mosaic virus. In. *A Manual of Virus Diseases of Tropical Plants*. Mc Millan Company of India Ltd., Delhi. pp. 158.
- Raychaudhuri, S. P. and Nariani, T. K. 1977. Papaya mosaic. In. *Virus and Mycoplasma Diseases of Plants in India*. Oxford & ICH Publishing Co., New Delhi.
- Singh, A. B. 1972. Studies on the transmission of papaya mosaic virus by *Aphis gossypii* Glover. *Indian J. Ent.* **34** : 240-45.

MANIPULATION OF PLANTING AND HAULMS CUTTING DATES FOR BETTER HEALTH STANDARDS IN SEED POTATO CROPS

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SUMMARY

Field trials to determine the appropriate period for planting and haulms cutting to obtain seed potato crop with tolerable limit of health standards for viral diseases in North-Western plains of India were carried out at Central Potato Research Station, Jalandhar from 1979 to 1983. In first three years of the trial a check set was planted using Breeder's Seed. The crop was planted on three dates at 10 days interval starting from September 30 and haulms were cut on four dates at 10 days interval starting from December 25. Aphid build-up was recorded at 10 days interval from 2nd week of November to last week of January. The produce of check set served as exposed set for planting in subsequent years. The exposed set was planted on October 10 and haulms were cut on December 31 every year. Incidence of viral diseases (mosaics) and yield was recorded.

Aphid build-up beyond 20 aphids per hundred compound leaves was critical in transmission of viruses. Viral diseases were within permissible limit of 1% when the haulms were cut on December 25. Aphid build-up rose approximately three times of the critical limit on January 5 and the incidence of viral diseases also increased two times. Beyond January 5 the aphid build-up and disease incidence increased considerably. The younger plants were more susceptible to mosaic than the older plants. In conclusion the combination of first planting (September 30) and first haulms cutting date (December 25) resulted in lowest incidence of viral diseases. However, since tuber yield is also an important consideration alongwith good health standard, this requirement was satisfied when planting was made between first 10 days of October and the haulms cutting was made up to first week of January.

The low level of disease incidence encountered during the present investigation was not sufficient to cause significant difference in yield in different treatments in the exposed set.

With the development of 'Seed Plot Technique' it has become possible to raise seed potato crops in plains of North-Western India during low aphid period ranging from October to December (Pushkarnath, 1959, 1967; Nagaich *et al.*, 1968; Nagaich, 1975; Vashisth *et al.*, 1981). One of the recommendations of the technique is to cut the haulms during the last week of December when aphid population touches critical level of 20 aphids per 100 compound leaves (Davies, 1934). However, there is no conclusive experimental evidence to show that if the haulms cutting is delayed beyond December, how much increase in incidence of viral diseases will take place. Seed crops in Punjab are planted from last week of September to third week of October. If early haulms cutting (end of December) is recommended, the crop remains immature and considerable yield has to be sacrificed without exactly knowing the impact on seed health standards. In

Punjab, among degenerative diseases, only mosaics are common (Vashisth *et al.*, 1982). Field trials were carried out at Central Potato Research Station, Jalandhar, to determine the increase in incidence of viral diseases when haulms cutting is delayed from last week of December to fourth week of January in relation to varying dates of planting and their effect on yield. The results of four years of trials are presented in this paper.

MATERIAL AND METHODS

Field experiments were planted for four autumn seasons of 1979 to 1982. Split plot design with four replications was used. Main plots consisted of three dates of planting : September 30 (D₁), October 10 (D₂), and October 20 (D₃). Sub plot treatments consisted of four dates of haulms cutting : December 25 (H₁), January 5 (H₂), January 15 (H₃), and January 25 (H₄). The most popular cultivar Kufri Chandramukhi was used. Plot size was 4 x 3 m with row to row 60 cm and plant to plant 20 cm distance. The recommended cultural practices were followed.

There were two sets of the trials viz., check set and exposed set. In first three years of the trials, a check set was planted every year using Breeder's seed. Aphid build-up was recorded in the main plots of the check set at 10 days interval from second week of November up to last week of January. Aphids were counted from 100 compound leaves taken from thirty four plants (Davies, 1934) in each main plot. Individual plants were harvested plot-wise and one tuber per plant was taken. The produce of the check set (one tuber per plant) served as exposed set for planting in years from 1980 to 1982. The exposed sets were planted in the same layout but the planting was done on October 10 and the haulms were cut on December 31 every year. The incidence of viral diseases in different treatments was recorded visually twice during the season and per cent incidence was calculated. Yield was recorded plot-wise in both check and exposed sets.

RESULTS

There was less incidence in the first date of haulms cutting (Dec. 25) which gradually increased with the delay in haulms cutting and was maximum in the last date (Jan. 25) irrespective of the planting dates in all the three years (Fig. 1). The critical level of one per cent viral diseases (standard laid down for Foundation Stage I crops raised from Breeder's seed) was crossed when the haulms cutting was delayed to January 5. Considering the planting dates, the disease incidence was less in case of D₁, increased further in D₂ and was maximum in D₃. Similar trend of

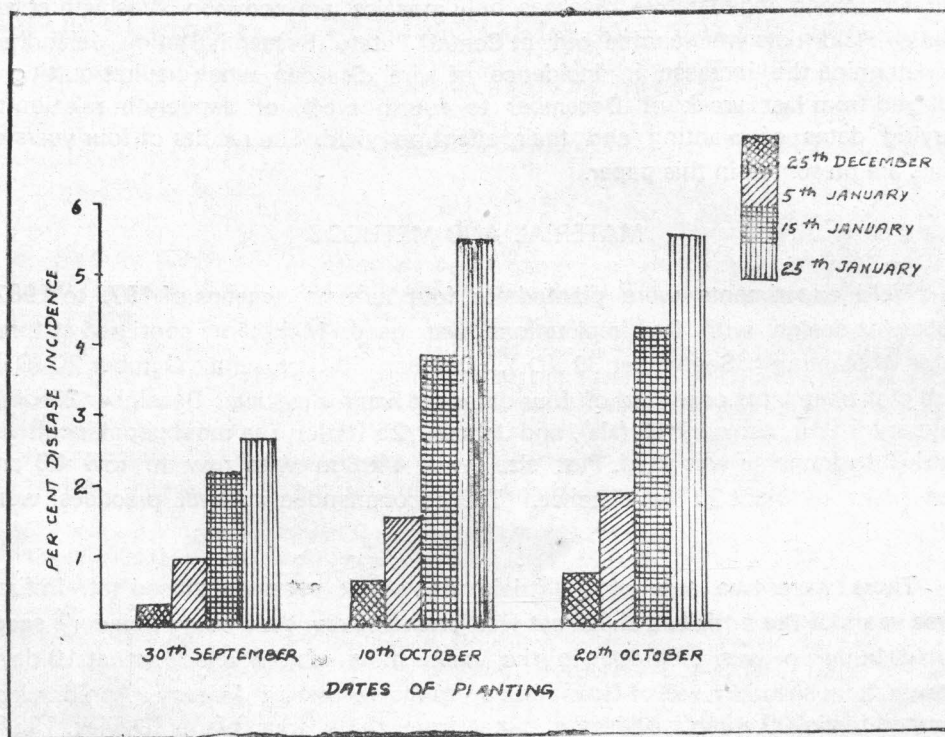


Fig. 1. Average disease incidence during autumn 1980 to 1982.

disease incidence in different treatments is also apparent from the data presented for individual years in Table 1. Although apparent differences of biological significance were observed in different treatments, statistically the interaction between dates of planting and dates of haulms cutting was not significant in all the three years.

The data on aphid population is presented in Table 2 and the data averaged over three years is presented in Fig. 2. The data show that the level of 20 aphids per hundred compound leaves, the critical level adopted for seed crops (Davies, 1934), was crossed on December 25 in case of D₁ and D₂ planted crops and on December 15 in case of D₃ planted crop. There was continuous increase in aphid population such that on January 25 the aphid population in all the treatments had crossed 200 aphids per hundred compound leaves.

Better Health Standards in Seed Potato Crops

TABLE 1
Incidence of mosaic diseases in the exposed set

Treatments*	Average per cent disease incidence		
	1980-81	1981-82	1982-83
D ₁ H ₁	0.26	0.51	0.28
D ₁ H ₂	0.52	1.76	0.79
D ₁ H ₃	1.04	3.32	2.33
D ₁ H ₄	1.31	3.56	3.20
D ₂ H ₁	0.53	0.76	0.78
D ₂ H ₂	0.77	2.27	1.79
D ₂ H ₃	2.83	4.14	4.66
D ₂ H ₄	4.85	5.56	6.20
D ₃ H ₁	0.51	1.02	0.79
D ₃ H ₂	0.78	2.29	2.58
D ₃ H ₃	2.79	4.31	5.72
D ₃ H ₄	5.11	5.88	5.88
C. D. (5			
Dates of planting (D)	N. S.	N. S.	0.15
Dates of haulms cutting (H)	0.07	0.05	0.10
D x H	N. S.	N. S.	N. S.

*Planting dates: D₁= September 30, D₂= October 10, and D₃= October 20

Dates of haulms cutting: H₁=December 25, H₂= January 5, H₃=January 15, and H₄=January 25

TABLE 2
Aphid build-up at different planting dates in check set

Dates of count	Dates of planting								
	September 30			October 10			October 20		
	1979-80	1980-81	1981-82	1979-80	1980-81	1981-82	1979-80	1980-81	1981-82
Nov. 25	0	0	1	0	0	0	0	0	1
Dec. 5	1	0	11	11	0	11	5	0	7
Dec. 15	21	3	28	14	0	18	16	8	51
Dec. 25	40	23	133	32	28	50	39	22	112
Jan. 5	54	62	87	47	70	72	61	45	158
Jan. 15	171	103	158	193	111	159	193	333	202
Jan. 25	200	200	200	200	200	200	200	200	200

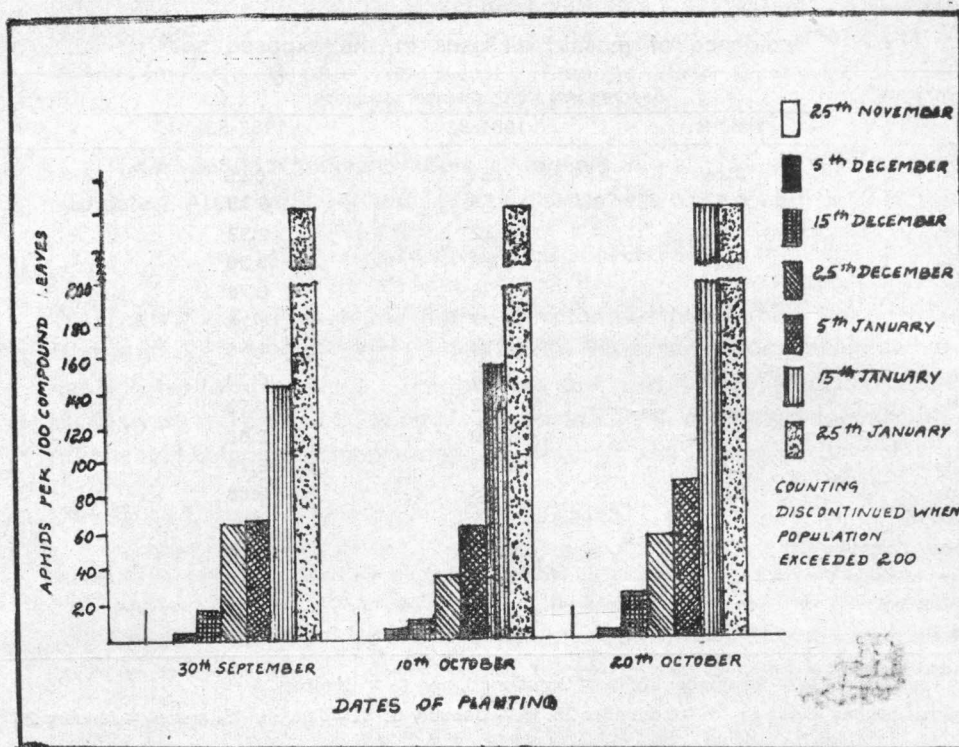


Fig. 2. Average aphid build-up during autumn 1979 to 1981.

Yield data of check sets and exposed sets are presented in Table 3. In check set there was increase in yield as the haulms cutting was delayed in all the three years. There was, however, no consistent trend of yield in the three planting dates. The interaction between dates of planting and haulms cutting was significant in 1979-80 and 1981-82. The yield was the lowest in case of third date of planting and first date of haulms cutting in all the three years. In case of exposed set, there were no significant differences in yield between any of the treatments.

DISCUSSION

In the first haulms cutting date (Dec. 25) when the aphid population was up to 20 aphids, the incidence of viral diseases was lowest and within permissible limit of standards. This was true in case of all the planting dates and so supported the findings of Davies (1934).

TABLE 3
Yield of tubers in check and exposed sets

Treatments*	Yield (q/ha)					
	Check set			Exposed set		
	1979-80	1980-81	1981-82	1980-81	1981-82	1982-83
D ₁ H ₁	237.62	281.67	290.94	334.01	217.87	367.29
D ₁ H ₂	229.28	309.17	316.15	342.99	230.62	368.54
D ₁ H ₃	232.62	325.07	324.90	350.11	261.04	394.00
D ₁ H ₄	256.06	336.67	325.94	333.85	227.92	398.33
D ₂ H ₁	240.88	308.13	272.19	336.72	260.21	395.21
D ₂ H ₂	179.06	332.92	308.37	340.05	248.96	381.04
D ₂ H ₃	277.77	359.59	298.96	337.50	247.92	410.42
D ₂ H ₄	299.90	365.21	321.25	337.24	243.12	386.92
D ₃ H ₁	184.83	246.04	185.83	317.94	258.75	383.33
D ₃ H ₂	258.98	290.84	235.94	328.33	240.71	391.04
D ₃ H ₃	270.21	320.42	298.75	324.07	246.57	387.08
D ₃ H ₄	297.19	334.59	305.11	317.32	257.92	385.62
C. D. (5%)						
Dates of planting (D)	17.92	21.20	41.16	N. S.	N. S.	N. S.
Dates of haulms cutting (H)	8.55	13.17	20.69	N. S.	N. S.	N. S.
D x H	29.07	N. S.	52.31	N. S.	N. S.	N. S.

*Dates of planting : D₁=September 30, D₂=October 10, and D₃=October 20

Dates of haulms cutting : H₁=December 25, H₂=January 5, H₃=January 15, and H₄=January 25

As the haulms cutting was delayed to January 5, the aphid build-up rose approximately three times of the critical limit and the incidence of viral diseases also increased about two times. Beyond January 5, the aphid build-up increased considerably and there was also proportional increase in viral diseases.

The age of the plant appeared important as the disease incidence was consistently less for first date as compared to other two planting dates. Such relation of senescence of plant tissue with disease translocation is known (Beemster, 1972).

The first planting date and first haulms cutting date appeared to be the best combination. However, the haulms cutting could be delayed to first week of January by keeping the incidence of viral diseases at marginal permissible limits when seed crops are raised in healthy surroundings like research-cum-seed farm of Central Potato Research Station, Jalandhar. It is likely that in farmer's fields the process of the build-up of viral diseases may progress at faster rate due to compa-

tively unhealthy surroundings (Verma and Vashisth, Unpublished). The next combination which is suitable appears to be the second planting date (Oct. 10) and first haulms cutting date (Dec. 25). Therefore, planting during first 10 days of October and haulms cutting in last week of December is most desirable.

There was no significant difference in yield in case of exposed set planted on one date. Apparently, the low level of disease was not enough to affect the yield.

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The authors are grateful to the Director, Central Potato Research Institute, Shimla and Officer-in-Charge, Central Potato Research Station, Jalandhar for providing facilities for the work. The authors are also highly thankful to Dr. O. M. Bambawale and Shri B. D. Sharma, Scientists, CPRS, Jalandhar, for valuable suggestions and help in MS preparation.

REFERENCES

- Beemster, A. B. R. 1972. Virus translocation in potato plants and mature-plant resistance. In *Viruses of Potatoes and Seed Production*. (ed. J. A. de-Boks). Centre for Agricultural Publishing and Documentation, Wageningen. pp. 144-51.
- Davies, W. M. 1934. Studies on aphid infecting the potato crop. II. Aphis survey : Its bearing upon the selection of district for seed potato production. *Ann. Appl. Biol.* **21** : 283-99.
- Nagaich, B. B., Pushkarnath, Bharadwaj, V. P., Giri, B. K., Anand, S. R. and Upreti, G. C. 1968. Production of disease-free seed potatoes in the Indo-Gangetic plains. *Indian J. agric. Sci.* **39** : 238-43.
- Nagaich, B. B. 1975. Controlling degenerative diseases in potatoes. In *Advances in Mycology and Plant Pathology*. (eds. Raychaudhuri, Anupam Verma, K. S. Bhargava and B. S. Mehrotra) Professor R. N. Tandon's Birthday Celebration Committee, New Delhi. pp. 249-62.
- Pushkarnath. 1959. Producing healthy seed potatoes in the plains : A new approach. *Indian Potato J.* **1** : 63-72.
- Pushkarnath. 1967. Seed potato production in the sub-tropical plains of India. *Am. Potato J.* **44** : 429-41.
- Vashisth, K. S., Verma, A. K., Chaubey, I. P. and Nagaich, B. B. 1981. Comparative degenerative effect of viral and MLO diseases during autumn and spring seasons on Indian potato varieties in Punjab. *Seed and Farms* **7** : 25-28.
- Vashisth, K. S., Sharma, V. C., Verma, A. K. and Chaubey, I. P. 1982. Status of potato diseases in Punjab : 1972-1979. *Seeds and Farms* **8** : 27-33.

EFFECT OF YELLOW VEIN MOSAIC ON PHYSIOLOGY OF OKRA*

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SUMMARY

The physiological changes in Bhendi due to yellow vein mosaic virus infection were studied. Chlorophyll and carbohydrates levels declined with severity of disease symptoms, while nucleic acids level increased in the diseased leaves as compared to healthy control. The total protein contents declined in diseased tissues while its insoluble fraction increased in diseased tissues as against soluble fraction which lowers in the diseased tissues. The total phosphorus content was low in initial stages while it was almost equal at the later stages followed by an increase in final stages as compared to the control. The fractionation of these indicates the increase in lipid P and nucleic acid P fractions. The mechanism of pathogenesis has been discussed in the light of these results.

MATERIAL AND METHOD

Seeds of var. Pusa Sawani, a susceptible cultivar of *bhendi*, were procured from the Agronomy Department of OUAT, Bhubaneswar and grown in pots containing soil and farmyard manure (1:1) in a net house. Since yellow vein mosaic is a whitefly transmitted disease, 200 plants out of the 400 plants grown in pots under insect proof conditions were kept interspread in an adjacent plot having heavy infestation of the disease. After 24 h. plants were removed and kept in a glasshouse ($28 \pm 2^\circ\text{C}$ 60% R. H. and normal daylength). Controls were shifted directly. The regular observation of the exposed plants showed only 60% infection after 20-25 days. Since the exact time of infection could not be ascertained, leaves from top three nodes showing visible symptoms of virus were collected for analysis. The leaves of these nodes were respectively 25-25 days (first node), 30-35 days (second node,) and 40-45 days (third node).

Sampling was done randomly and the leaves were collected early morning and washed with tap water followed by distilled water. The leaves were then blotted dry and weighed equally for analysis.

The chlorophyll contents were extracted and estimated in 80% acetone as described by Arnon (1949) using 80% acetone as a blank and the results in terms of mg/g fresh weight are calculated.

The carbohydrates were extracted in 80% alcohol and the aliquots obtained were treated with charcoal for absorption. The sugars were estimated as reducing

*Part of Ph. D thesis awarded to senior author.

sugars according to Nelson (1944). However, the total sugars were estimated by the extracts treated with 1N H_2SO_4 (Sulfuric acid) at 49°C for 1 h. The non-reducing sugars were obtained by deducing the reducing sugars from the total sugars.

The nucleic acid and proteins were extracted as described by Schmadt and Thannhauser (1948) as modified for plant material by Broughton (1970). The estimation of the nucleic acids in the extracts was done according to procedures of Meijbaum and Burton, respectively, as detailed by Webb and Levy (1956).

The proteins were, however, estimated by biuret method described by Gornall *et al.* (1949).

RESULTS AND DISCUSSION

There was a loss of 87% of total chlorophyll in the leaves of the first node as against 83 and 76 percent on second and third node (Fig. 1). The loss in the level

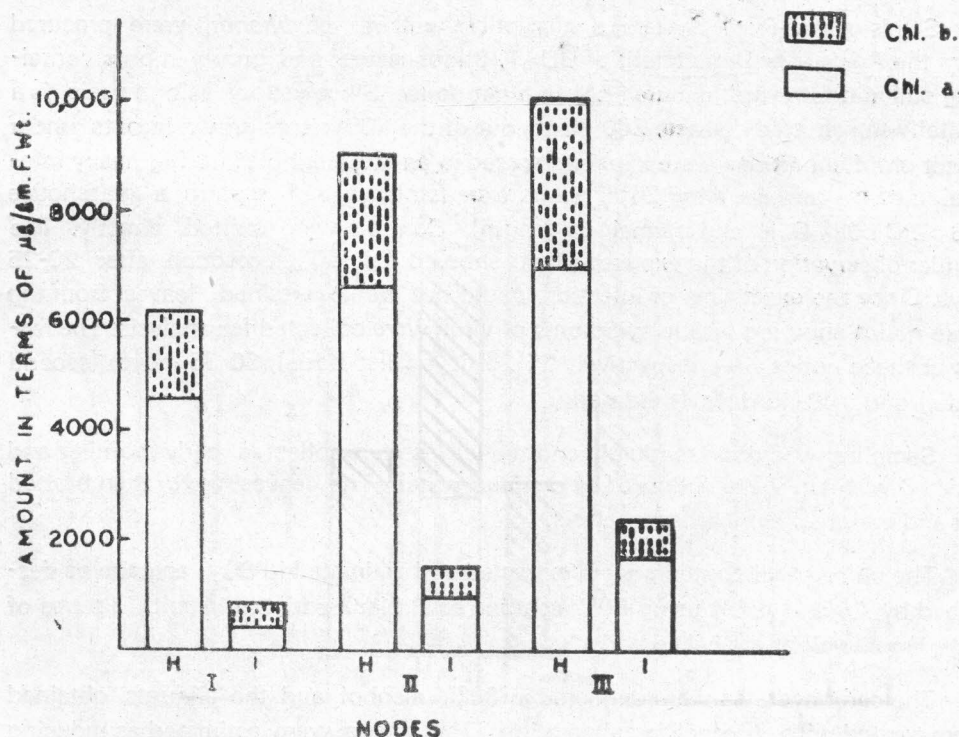


Fig. 1.—Changes in chlorophyll composition of healthy and diseased Okra leaves.

of chlorophyll-b was much higher as compared to chlorophyll-a. The chlorophyll-b contents reduced upto a maximum of 90% at first node and 76% at the third node. The chlorophyll-b remained almost constant at all the nodes and ranged between 76-70 per cent only. The loss in the level of chlorophyll has also been observed in the same host virus combination by Mandahar and Singh (1972). However, Mandahar and Singh (1972) observed a greater loss of chlorophyll-b as against chlorophyll-a. The loss has been observed due to a reduced number of chloroplast following virus invasion and utilization of plastid proteins for the virus synthesis (Bawden, 1956).

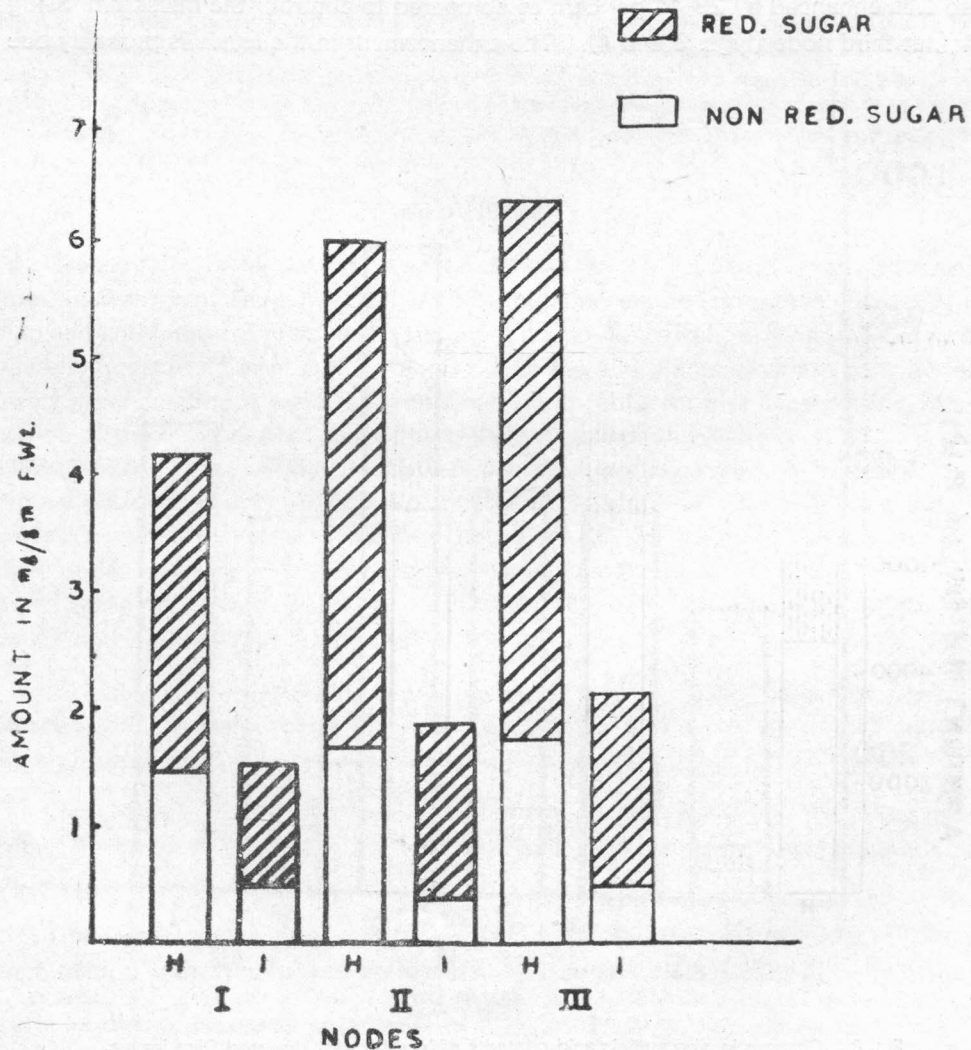


Fig. 2. Changes and sugars composition of healthy and diseased Okra leaves.

The sugar content was reduced by 63-69 per cent in diseased tissues (Fig. 2). The same ranges between 59-65 per cent for reducing sugars and 66-78 per cent for non-reducing sugars. As evident from lower chlorophyll levels, the lower synthesis as per expectation is the cause. Jeyrajan and Ramakrishnan (1968) and Singh and Shrivastava (1974) also found the similar reasons for lowered level of sugars:

The RNA contents were 60% high as compared to control at the second node, while it is 26 and 35 per cent respectively on third and first node. DNA content also was enhanced by 29-44 per cent as compared to control, the maximum being 44% at third node (Figs. 3 and 4). The enhancement in the levels is probably due

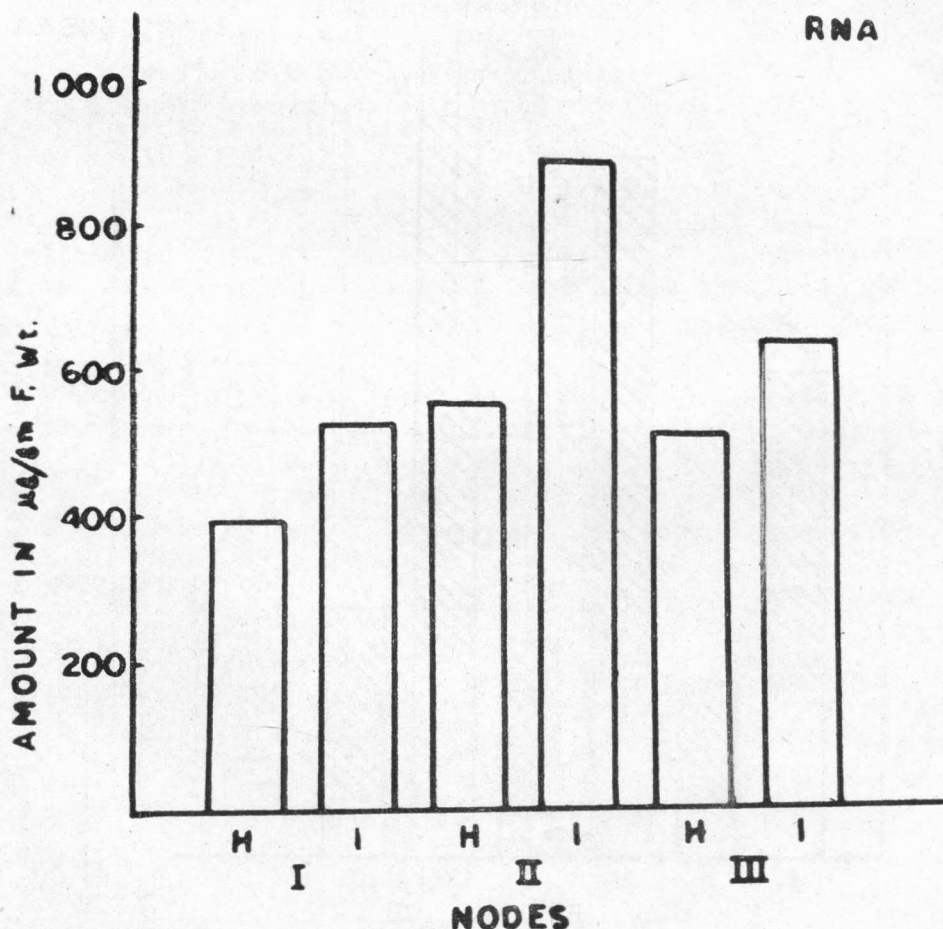


Fig. 3. Changes in ribo nucleic acid contents of healthy and diseased Okra leaves.

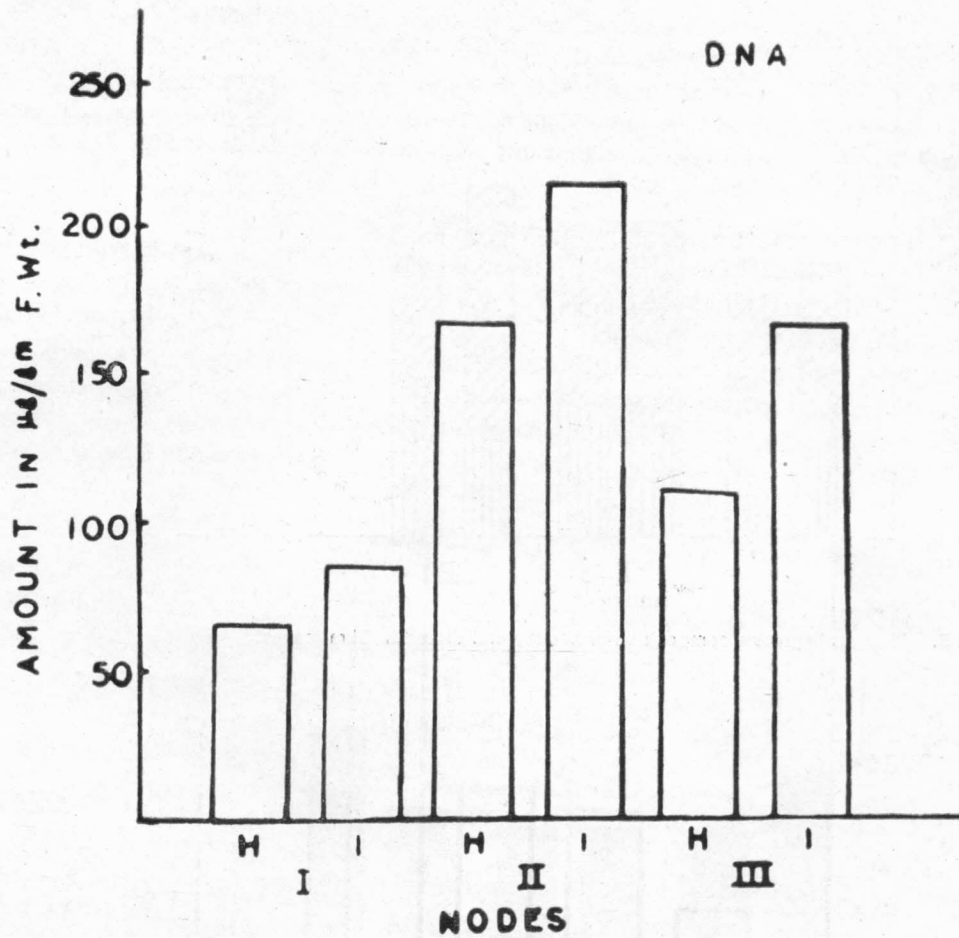


Fig. 4. Changes in deoxyribo nucleic acid contents of healthy and diseased Okra leaves.

to enhanced level of RNA of both the host and virus. The level of the DNA might increase due to lower DNase activity. Similar results were reported by Ahmed *et al.* (1975) and Zafar and Naqui (1977)

Total protein content was slightly less in diseased tissues as compared to control. The insoluble protein contents were high in diseased leaves and ranged between 23-51 per cent, respectively. The soluble protein contents on the contrary are decreased (Fig. 5).

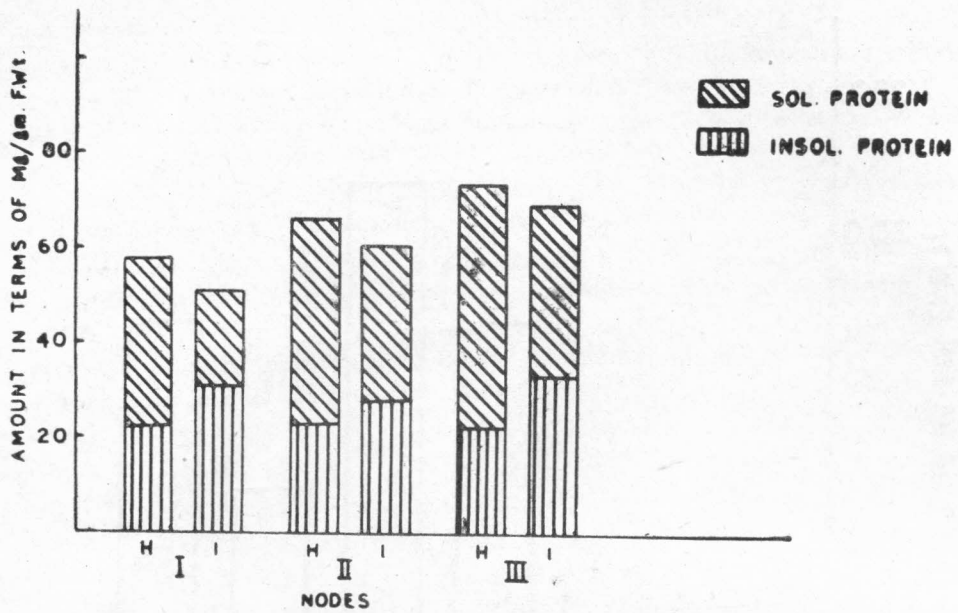


Fig. 5. Changes in protein composition of healthy and diseased Okra leaves.

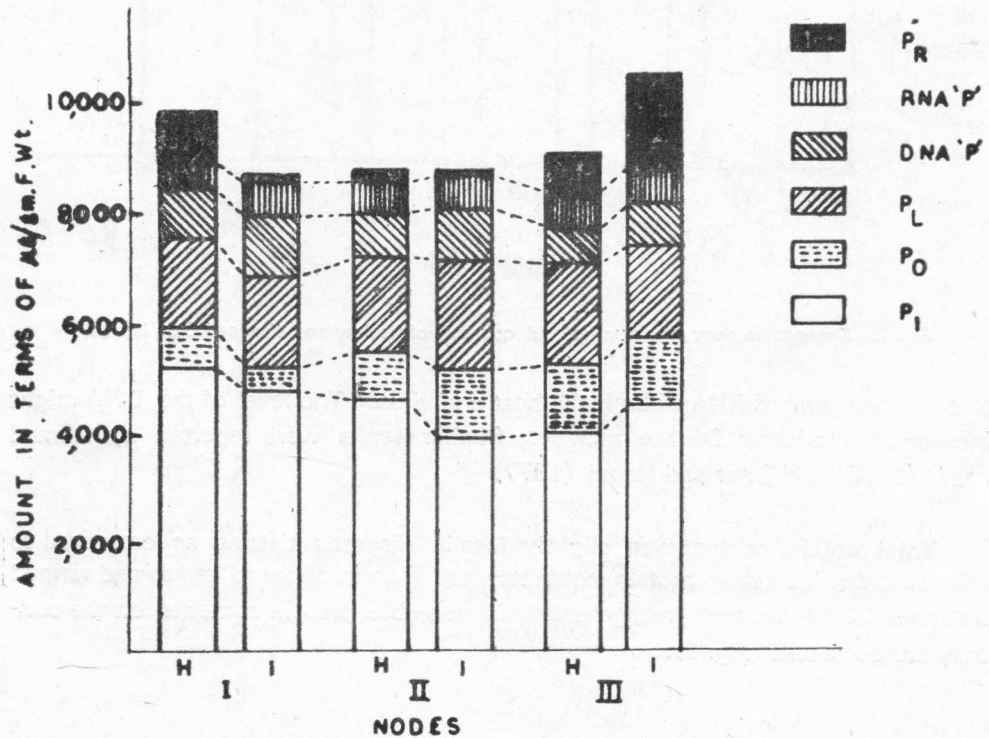


Fig. 6. Changes in phosphorus fractions in healthy and mosaic infected Okra leaves.

Although the total phosphorus content was reduced in the initial stage, it increased in the later stages of infection (Fig. 6). The total acid soluble phosphorus and inorganic phosphorus were increased in comparison to control at third node, while they were lesser than the control at the second and first node. Total organic phosphorus showed an enhancement over the control in the samples of third and second, but a decrease in the same was recorded at first node. Lipid RNA and DNA phosphorus were greater in all samples at all stages. These findings are in agreement with that of Sayavendra and Boninsegua, (1972). Increase in RNA and DNA phosphorus is due to presence of heterogenous RNA and impairment of DNase activities. The lipid phosphorus might be correlated with the enhanced respiration rate. The increase in inorganic phosphorus might be related with the increased absorption/translocation per unit time as reported by Araujocaldas *et al.* (1968). The enhanced level of residual organic phosphorus might belong to virus fraction of viral nucleoprotein.

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REFERENCES

- Ahmed, J. Quaseem, S. M., Ahmed, Awuil, Khalique, Abdul and Wasiuddin. 1975. Biochemical aberrations by yellow vein mosaic virus in *Abelmoschus esculentus* (L.) Moench. var. Pusa Sawani. *Geobios* 2 : 90-91.
- Arnon, K. I. 1949. Copper enzymes in isolated chloroplast. I. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* 24 : 1-15.
- Araujocaldas, Crocone, R., Barioni, O. J., Gusman, A. and Gusman, L. C. 1968. Influentia de virus 'vira cabeca' na absorcao de fosforo em plantas de tomato. *Phyton*. 25 : 29-34.
- Bawden, F. C. 1956. Plant viruses and virus diseases. Chronica Bot. Co. III edn. Waltham Mass. pp. 335.
- Broughton, W. J. 1970. Determination of total nucleic acid in plants. *Anal. Biochem.* 38 : 291-96.
- Gornall, A. G., Bradwill, C. S. and David, M. M. 1949. Determination of serum proteins by means of biuret reagent. *J. Biol. Chem.* 177 : 751.
- Jeyrajan, R. and Ramakrishnan, K. 1968. Studies on the physiology of chilli plants (*Capsicum annum* L.) affected potato virus Y. 1. Effect on metabolism of carbohydrate organic acids phenolics and respiration. *Phytopath. Z.* 63 : 142-52.
- Mandahar, C. L. and Singh, J. S. 1972. Effect on Bhendi yellow vein mosaic virus on it's host. *Acta. Phytopath. Acad. Scient. Hung.* 7 : 187-91.

J. K. JOSHI AND B. PADHI

- Nelson, N. 1944. *J. Biol. Chem.* **153** : 375 (Cf. Methods in Enzymology, Vol. III).
- Sayavendra, G. A. and Boninsegua, J. A. 1972. Phosphorus distribution in tomato bushy stunt virus infected *Lycopersicon esculentum*. *Phytopath. Z.* **75** : 156-62.
- Schmidt, G. and Thonnhäuser, S. J. 1948. Methods of extraction and estimation of nucleic acids. *J. Biol. Chem.* **161** : 63.
- Singh, R. and Srivastava, R. P. 1974. Physiological changes induced by potato virus X on tomato, *Lycopersicon esculentum* Mill. *Indian J. Exptl. Biol.* **12** : 209-11.
- Webb, J. M. and Leby, H. B. 1956. New Developments in the chemical determinations of nucleic acids. *Methods of Biochem. Analysis*. VI. Edited by D. Glick, Interscience N. Y., New York.
- Zafar, A. and Naqvi, Q. A. 1977. Nucleic acid changes in *Petunia* roots as a result of Petunia mottle virus infection. *Curr. Sci.* **46** : 573.

STUDIES ON THE EFFECT OF SOME ALTERNATIVE CULTURAL
METHODS ON THE INCIDENCE OF YELLOW VEIN MOSAIC
VIRUS (YVMV) DISEASE OF OKRA (*Abelmoschus*
esculentus (L.) Moench.)

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SUMMARY

Yellow colour polyethylene mulch significantly delayed the appearance of YVMV disease symptoms in Okra. It took as long as 69 days from the date of sowing for the appearance of first disease symptoms in mulched crop, whereas disease was noticed only 28 days after sowing in non-mulched crop. The extent of disease incidence in mulched crop was 24.3%, whereas it was 58.6% in control.

In an another field experiment, mixed cultivation of pumpkins (*Cucurbita moschata*) with Okra enhanced both the total incidence of YVMV disease and its rate of spread in Okra. The extent of disease observed in mixed crop was 96.43%, whereas in control plot it was only 42.17 per cent.

Yellow vein mosaic virus (YVMV) disease is a serious problem of Okra cultivation. The disease occurs throughout India, wherever this crop is grown and causes an average loss as high as 93.80% to the crop (Sastry and Singh, 1974). Extensive research has been conducted in different parts of the country on different aspects of the disease, particularly on the chemical control. But unfortunately till date no solid recommendation is available for the control of this disease. There are other methods of approach to the problem of the control of plant virus diseases besides chemical control, such as cultural methods. They are not, of course, all applicable to all the diseases, but may be quite effective in certain diseases. Therefore, present studies were undertaken to find out the effect of mulching and mixed cropping on the incidence of YVMV disease of Okra and are reported hereunder.

MATERIAL AND METHODS

YVMV disease of Okra is transmitted by whiteflies. Since the whiteflies are known to be positively phototrophic, especially to yellow colour (Cohen, 1981), therefore, an experiment was conducted under field conditions at Plant Virus Research Field, Kalyani (W. B.) to study the effect of yellow colour polyethylene mulch on the incidence of the disease. A square plot of 20 × 20 m was prepared following

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normal agronomical techniques for Okra cultivation. Yellow colour polyethylene sheetings purchased locally were cut into 40 cm wide and 20 m long strips. These strips were then spread on the prepared land before sowing keeping five cm gap between two strips. Suitable arrangements were made to keep the sheets stretched in the field during the period of observation. The seeds of 'Pusa Sawani' variety were sown in February (16-2-1982) keeping plant to plant and row to row distance of 45×45 cm. No inputs, such as, fertilizers/pesticides were applied. The interculture operations were carried out as usual and the plants were irrigated as and when required.

An another plot of similar size was prepared in same way as control on the side of the experimental plot. No polyethylene mulch was applied in the case. The experimental as well as control plots were checked every 24 h for appearance of disease symptoms. Final observations were taken on 110th day after sowing. The total incidence of disease was recorded in terms of per cent infection.

Whiteflies have got number of susceptible hosts, pumpkins being one among them and is very commonly grown. Therefore, in an another experiment pumpkin (*C. moschata*) was selected to study the effect of mixed cultivation of pumpkins and Okra on the incidence of YVMV disease. A field experiment was conducted at Plant Virus Research Field, Kalyani (W.B.), where an area of 26×30 m was prepared and was divided into five plots of 2×30 m alternating with four plots of 4×30 m size, keeping 2×30 m plots as borders. The seeds of pumpkins were sown in hills forming two rows in 2×30 m plots on 14-9-81. Five seeds were sown in each hill. Thirty days after when pumpkin plants were sufficiently grown, seeds of Okra (Pusa Sawani) were sown during middle of October (14-10-81) in 4×30 m plots at 45×45 cm spacings from plant to plant and in between rows. Both the crops were raised following usual agronomical practices. No fertilizer or pesticide was applied to any of these crops. The plants were however irrigated and weeds were removed as and when necessary. The Okra crop was kept under observation and the data was recorded on the incidence of YVMV disease.

RESULTS AND DISCUSSION

It appeared from the data in Table 1, that the yellow colour polyethylene mulch had significant effect on the incidence of yellow vein mosaic disease of Okra. It was found that mulching significantly delayed the appearance of first disease symptoms. It took as long as 69 days from the date of sowing for the appearance of first disease symptoms in mulched crop, where as disease was noticed only 28 days after sowing in case of control. The total disease incidence on 110th day of observation was found much lower in mulched crop as compared with control. The extent of

Yellow Vein Mosaic Disease of Okra

TABLE 1

Effect of yellow colour polyethylene mulching on the incidence of yellow vein mosaic virus disease of Okra

	Date of sowing	Date of first disease appearance	Time taken to exhibit first symptoms (days)	Total disease incidence on 110th day (per cent infection)
Mulched crop	16-2-82	25-4-82	69	24.30
Control	16-2-82	15-3-82	28	58.60

disease incidence in mulched crop was 24.30% whereas it was 58.60% in control. Thus, mulching not only delayed the appearance of first disease symptoms but also reduced the incidence of YVMV disease. Cohen (1981) also got similar results in case of cucumber vein yellowing and tomato leaf curl diseases with yellow polyethylene mulch. The delay may be due to the colour of the mulch and heat reflected by it. The temperature just above the surface of the polyethylene mulch is higher than the prevalent atmospheric temperature. Thus, viruliferous incoming whiteflies got attracted to yellow colour of the mulch and YVMV disease inoculum carried by them got inactivated by the prevalent high temperature above the surface of the mulch, thereby protecting the crop from infection at early stage, which is very crucial for the incidence and intensity of the disease (Sastry and Singh, 1974).

The data in Table 2 revealed that raising of pumpkins mixed with Okra crop enhanced the spread and incidence of YVMV disease. On the first day of observation (1-11-1981), no disease was observed. On the second day of observation (8-11-1981), only 1.70% disease was found in mixed crop, whereas no disease was observed in control plot. During the third day of observation (30-11-1981), the

TABLE 2

Effect of mixed cultivation of pumpkins and Okra on the incidence of yellow vein mosaic virus disease of Okra

S. No.	Date of observation	Percentage of infection	
		Mixed crop	Control
1.	1-11-1981	0.00	0.00
2.	8-11-1981	1.70	0.00
3.	30-11-1981	89.47	2.04
4.	17-12-1981	91.48	20.40
5.	23-12-1981	96.43	42.17

number of infected plants in mixed crop increased enormously and the field showed 89.47% infection as compared to 2.04% infection in case of control. Thereafter the increase was slow in mixed crop and total infection was found 91.48% on the fourth day of observation (17-12-81), whereas rapid increase in disease incidence was observed in case of control (20.40%).

The total disease incidence observed on fifth day of observation (23-12-81) was found to be 96.43 and 42.17 per cent in case of mixed crop and control, respectively. Thus, mixed cultivation of pumpkins and Okra enhanced both total incidence of YVMV disease and its rate of spread in Okra crop. This may be because, pumpkin is one of the favourite hosts of the whiteflies. Therefore, cultivation of pumpkins with Okra crop has attracted more number of whiteflies than ordinarily would have been attracted by the Okra plants, thus resulting in more inoculum, which ultimately enhanced the rate as well as incidence of YVMV disease. So, pumpkins should not be grown along with Okra.

REFERENCES

- Cohen, S. 1981. Control of whitefly vectors by non-conventional methods. Abstract of papers. Physiology of virus infected plants. Department of Experimental Physiology, Oxford. July 27, 1981.
- Sastry, K. S. M. and Singh, S. J. 1974. Effect of YVMV infection on growth and yield of Okra crop. *Indian Phytopath.* 27 : 294-97.

OCCURRENCE OF A NEW VIRUS LIKE DISEASE OF GRAPEVINE IN HARYANA

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SUMMARY

A virus like disease of grapevine exhibiting vein yellowing, chlorotic mottle and leaf smalling has been observed occurring in Haryana. The axillary buds sprouted early, bearing yellow leaves on newly emerged weak shoots. Internodes were drastically reduced. This gave a bushy appearance to the plant. The flowers of the diseased plants were sterile. Sometimes rudimentary berries were observed. The yield of the diseased plants was partially or completely suppressed. The disease was transmitted through grafting but not by mechanical means. Field reaction of 83 varieties/hybrids of grapevine has been determined.

A new virus like disease of grapevine has been observed for the last seven years in the grapevine orchard of the Department of Horticulture, Haryana Agricultural University, Hisar. During the year 1977, only six plants were infected but currently in the year 1983 total thirty one plants of different varieties were found infected with this virus. The diseased plants were moribund with very little (short berry) or no crop. This communication describes symptomatology, transmission and the occurrence of the disease in the field on different varieties of grapevine in Haryana.

MATERIAL AND METHODS

Cuttings of different grape varieties and seeds of different *Chenopodium* sp. were sown in 15 cm clay pots containing a mixture of soil, sand and farm yard manure. These cuttings/seeds were collected from healthy plants. At random seedling raised from these cuttings/seeds were first ensured to be virus free before being used for mechanical inoculations. Mechanical inoculation was done with forefinger as usual using 400 mesh carborundum powder as abrasive. One g of diseased leaves was crushed with one ml of distilled water.

For grafting, cuttings of grape variety Kishmish were used as rootstocks which is a susceptible host. The scion of two already infected varieties viz., Karachi and J. Salsaic were used for whip grafting. The grafting was done on one year old healthy rootstocks in the month of February before bud burst.

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Observations on varietal reaction on different varieties of grapes were made particularly at the Haryana Agricultural University grapevine orchard. Total 83 varieties were assessed on the basis of number of infected plants per variety. These observations were made at two different times, one in the spring season (March-April) before harvesting and the other in the monsoon season (July-August) after harvesting.

RESULTS AND DISCUSSION

Symptoms. Foliar symptom included chlorotic mottle and small leaves. The chlorosis started as vein yellowing. The new emerging shoots, leaves and flower clusters were yellow. The internode distance was reduced remarkably, whereas the growth of tendrils was restricted and they were curved in shape. All the axillary buds tended to sprout early with chrome yellow leaves. The cane growth was very much arrested thus exhibiting a bushy appearance. The flowers of the diseased plants were found to be sterile and no fruit setting was observed. However, some short berries (without fertilization) were observed in the diseased plants.

Transmission. The virus was not found sap transmissible on the following hosts : *Vitis vinifera* var. Thompson Seedless, var. Perlette, var. Beauty Seedless; *Chenopodium amaranticolor*; *Chenopodium quinoa*; and *Chenopodium murale*.

The virus was transmitted through grafting. The first symptom appeared 20 days after the bud burst as chrome chlorotic leaves. Leaves were slightly cupped in size as compared to the leaves of the healthy plant. The interveinal areas also showed general chlorosis. Diseased plants were restricted in size, had shortened internodes and developed abnormal growth from the normally latent axillary buds.

Varietal reaction. Out of 83 varieties assessed in the field, only 19 varieties viz., Thompson Seedless, Karachi, EC-27824, Gumposia 215, J. Salsaic, Vineland 35081, Trentham Black, Siebel 13047, Madras Local, Black Ladyfinger, Phakindi, Pirovano, Kishmish, Kishmishi, HR-13-3, HR-13-5, HR 13-9, HR-14-3 and Black Mackarack were found susceptible. Rest of the 64 varieties did not show any symptom in the field. Except Thompson Seedless, all the commercial varieties like Anab-e-Shahi, Perlette, Beauty Seedless, Pusa Seedless, Delight and Champion were found free from the disease in the field conditions.

The virus was not related to grapevine yellow mosaic and fan leaf (Dias, 1963) as they were sap transmissible. Grapevine leaf roll virus (Singh *et al.*, 1975)

and grapevine necrosis virus (Vanek, 1973) were symptomatically different. Arabis mosaic, yellow speckle, fleck and enation were also reported on this host (Woodhan *et al.*, 1973). Pop. (1972) observed a new virus of grapevine in Rumania, which was characterized by chlorotic areas along the veins, might show some resemblance to this virus.

REFERENCES

- Dias, H. F. 1963. Host range and properties of grapevine fanleaf and grapevine yellow mosaic viruses. *Ann. appl. Biol* **51** : 85-95.
- Pop, I. 1972. Investigations on viruses and virus diseases of grapevine and virus in Rumania. *Analele. Institutin. de Cercetari. Pentru. Protectia Plantelor*. **8** : 81-87.
- Singh, S. J., Sastry, K. S. M. and Sastry, K. S. 1975. Leaf roll—A new disease of grapevine in India. *Indian J. Mycol. Pl. Pathology* **5** : 205-06.
- Vanek, G. 1973. The incidence of virus diseases of the main grapevine varieties in U. S. S. R. *Polnokospodarstvo*. **19** : 684-92.
- Woodhan, R. C., Taylor, R. H. and Krake, L. R. 1973. New diseases and new data on known diseases. *Rivista di Patologia Vegetale* **4** : 15-77.

EVALUATION OF ROUGH LEMON STRAINS AGAINST CITRUS TRISTEZA VIRUS AND CITRUS EXOCORTIS VIROID

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SUMMARY

Fifteen rough lemon strains collected from India and abroad were tested for their reaction to citrus tristeza virus (CTV) and citrus exocortis viroid (CEV). None of the strains was susceptible to CTV, indicating their possible tolerance. Coorg, Florida A, Florida B, Florida C, Indian Limoneria, Milam, Miri, South Africa-I, South Africa-II and Volkamar were found tolerant to CEV. None of the strains showed tristeza symptoms.

Rough lemon (*Citrus jambhiri* Lush), the most prominent root stock in India is known to have a large number of strains which show a wide variability with respect to their potentialities as root stock as well as tolerance to various citrus diseases.

Three virus and virus-like diseases, namely, citrus tristeza virus (CTV), citrus exocortis viroid (CEV) and citrus greening were possible maladies in citrus decline in the state of Punjab. Out of these CTV and CEV are associated with the decline of different root stock-scion combinations (Chohan and Knorr, 1970). Most of the root stocks being used in citrus industry are either susceptible to CEV or CTV. The rough lemon (*C. jambhiri* Lush) which is the most predominant root stock in Northern India, however, is tolerant to both the maladies (Chohan and Knorr, 1970; Kapur *et al.*, 1974). Since a large number of rough lemon strains are available in the world, it was considered desirable to test their tolerance/susceptibility to CTV and CEV to find a better strain which could also be used as a root stock.

MATERIAL AND METHODS

To test tolerance/susceptibility to citrus tristeza (CTV) and citrus exocortis viroid (CEV), the seeds of fifteen rough lemon strains were collected from within and outside the country and sown in the screen house. One-year old seedlings were bud-inoculated with CTV and CEV separately and simultaneously healthy buds from the test plants were inserted into the inoculated seedlings. The seeds of fifteen rough lemon strains were collected from India and abroad. These were sown in sterilized soil under insect proof screen house at Punjab Agricultural University, Ludhiana, during March, 1977. One-year old uniform seedlings of each strain were used for inoculation tests. Six seedlings of each strain were inoculated

Evaluation of Rough Lemon Strains

with CEV and five with CTV. For CEV, two buds from Etrog citron infected with severe isolate of CEV were inserted into each of the test seedlings by 'T' budding so as to provide sufficient inoculum. A bud from a healthy plant of Etrog citron was also inserted into each seedling on the same day to serve as test budling. For CTV, two buds from Kagzi lime infected with a severe isolate of CTV and the second from the healthy Kagzi lime were inserted into each test seedlings. The infected buds were not allowed to develop, whereas the growth of the test budlings was encouraged by cutting back the rough lemon seedlings. The same number of seedlings was kept as check. The observations were recorded periodically on the inoculated seedlings for the appearance of the visual symptoms. The test seedlings which did not show symptoms after one year of inoculations were re-inoculated. The final observations were recorded two years after the first inoculation. The source of rough lemon strains and per cent infection with each agent are presented in Table 1.

TABLE 1
Evaluation of rough lemon strains against citrus exocortis viroid (CEV)
and citrus tristeza virus (CTV)

Strain	Source	CEV	CTV
		Seedling infected/inoculated	Seedlings infected/inoculated
California	Riverside (U. S. A.)	6/6	0/5
Florida-A	"	0/6	0/5
Florida-B	"	0/6	0/5
Florida-C	"	0/6	0/5
Indian	"	0/6	0/5
Limoneria	"	0/6	0/5
Milam	"	0/6	0/5
Miri	"	1/6	0/5
South Africa-III	"	0/6	0/5
Stow	"	4/6	0/6
South Africa-II	Akola (M.S.)	0/6	0
Australian	Tirupati (A.P)	6/6	0/5
Coorg	"	0/6	0/5
South Africa-I	"	0/6	0/5
Volkmar	"	0/6	0/5

RESULTS AND DISCUSSION

Citrus exocortis viroid. The results presented in Table 1 show that the fifteen rough lemon strains artificially inoculated with CEV, three, namely, Australian, California, and stow were infected. The remaining strains did not show any

visual symptoms even after re-inoculations, indicating their possible tolerance. Earlier workers, however, have reported rough lemon as a tolerant root stock for the viroid (Kapur *et al.*, 1974). Possibly they have tested only standard rough lemon.

Citrus tristeza virus. None of the strains inoculated with CTV showed visual symptoms indicating their possible tolerance to the virus. These findings are in conformity with those of Copper (1975).

It is noteworthy that five rough lemon strains, namely, Milam, Miri, South Africa-I, South Africa-II and Volkamar which have already been reported to be tolerant to greening disease of citrus (Cheema *et al.*, 1982/83) also did not show symptoms for CEV and CTV. These five strains have been planted in the field to evaluate their suitability as future citrus root stocks.

REFERENCES

- Capoor, S. P. 1975. Role of tristeza virus in citrus die-back complex. *Indian J. Hort.* **32** : 1-6.
- Chohan, J. S. and Knorr, L. C. 1970. Diseases : In. *Citrus Decline in India: Causes and Control*. A Joint Publication of Punjab Agricultural University and Ohio State University.
- Cheema, S. S., Kapur, S. P. and Chohan, J. S. 1982/83. Evaluation of rough lemon strains and other root stocks against greening disease of citrus. *Scientia Hort.* **18** : 71-75.
- Kapur, S. P., Dhillon, R. S., Cheema, S. S. and Deol, I. S. 1974. Citrus exocortis in the Punjab. *Punjab Agric. univ. J. Res.* **11** : 376-79.

PURIFICATION, ELECTRON MICROSCOPY AND SEROLOGY OF STRAINS A AND F OF SUGARCANE MOSAIC VIRUS

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SUMMARY

Purification of strains A and F of sugarcane mosaic virus was done by differential centrifugation followed by sucrose density gradient centrifugation. The virus culture was multiplied on *Sorghum bicolor*. For extraction, low molarity phosphate buffer of neutral pH with 0.01 M DIECA was more useful. Virus containing pellet was suspended in 0.05M borate buffer pH 8.5 supplemented with 0.01M EDTA and 0.5M Urea. For further purification and concentration the preparation was dialysed and subjected to sucrose density gradient centrifugation. The length of majority of virus particles ranged between 670-770nm. The titre of antisera raised was 1/2048. Presence of virus was detected in leaves, rind, bud and root of sugarcane by ELISA test. The concentration of virus was maximum in youngest leaves and minimum in root of mature older plant as determined by ELISA.

In an earlier study, strains A and F of sugarcane mosaic virus (SCMV-A and SCMV-F) were identified from India (Bhargava *et al.*, 1972). In the present study attempts were made to purify these two strains of SCMV (Fig. 1) and study their morphology under electron microscope. The purified preparations were used for raising antisera. Earlier attempts have been made to purify SCMV (Damirdagh and Shepherd, 1970; Gillaspie, 1972; Snazelle *et al.*, 1971. Tomic *et al.*, 1974; Chen, 1978). However, none of the methods used by these workers, gave high yield. The titre of the antisera raised from purified preparations was also not high.

MATERIAL AND METHODS

Healthy vigorously growing seedlings of *Sorghum bicolor* were raised in insect proof net house and inoculated with SCMV-A and SCMV-F by mechanical means using carborundrum as an abrasive. Young leaves showing bright symptoms were harvested seven days after the appearance of symptoms and frozen at -18°C for 12-14 h. The entire experiment was done at 4°C. The frozen leaves were thawed and homogenized in 0.067M phosphate buffer, pH 7.0, supplemented with 0.01M sodium diethyldithiocarbamate (DIECA) in the ratio of 1:4 (w/v) and extracted with double layer of cheese cloth. The filtrate was clarified by shaking with equal volume of chloroform for 20 min. Further purification was carried on as given below. The viability and concentration of

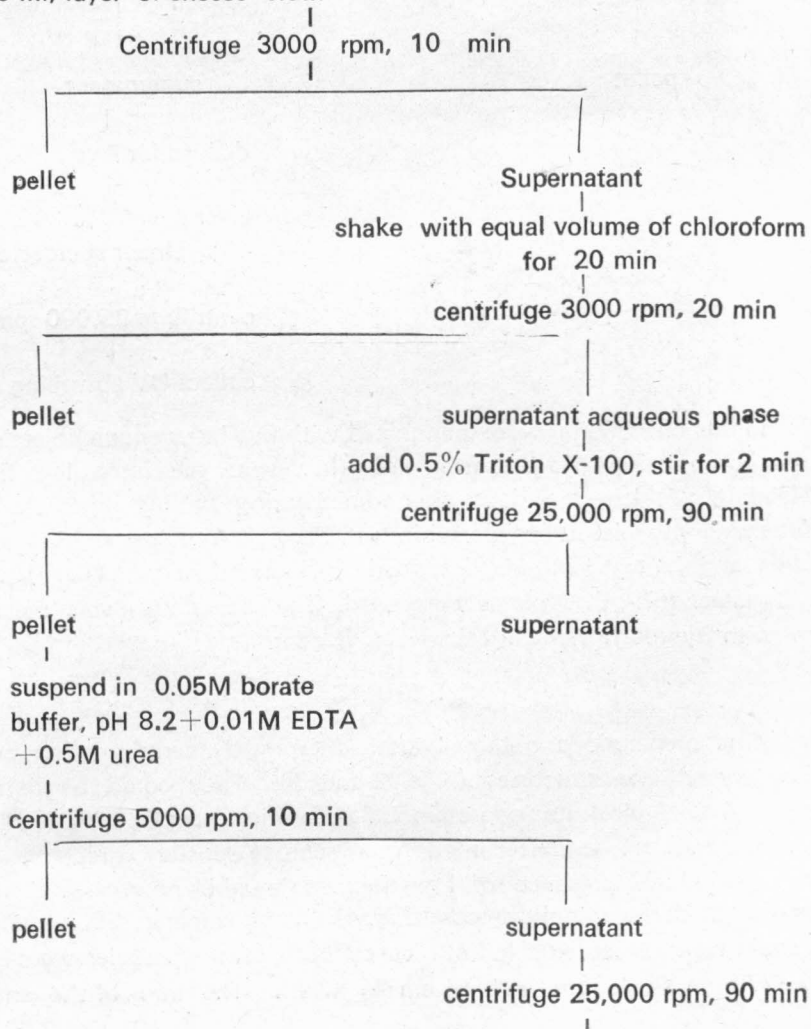


Fig. 1. Mosaic symptoms on sugarcane leaves.

Purification of Sugarcane Mosaic Virus

virus was checked at each step by visual observation under electron microscope and infectivity test on *Sorghum bicolor*. For electron microscopic observations, purified preparation was subjected to negative staining with 2% PTA (phosphotungstic acid), pH 8.0 by mixing droplets in the ratio of 1:1. Preparations, thus, obtained were observed under Hitachi HU-12 electron microscope.

Chill 100 g of leavey at -18°C for 14-16 h, thaw and macerate in 0.067M phosphate buffer, pH 7.0 + 0.01M DIECA and extract through double 400 ml, layer of cheese cloth



RESULTS

82

Purification of Sugarcane Mosaic Virus

SCMV-A and SCMV-F was 1/2048 as determined by microdrop precipitin test. The presence of the virus was detected by ELISA test in the extracts obtained from leaf, rind, bud and root of mosaic affected sugarcane plants. The maximum concentration was in the youngest leaves and the minimum in root of older mature plants.



Fig. 2. Purified preparation of SCMV—A after differential centrifugation. Note large cylindrical crystal like structures. (X 80,000)

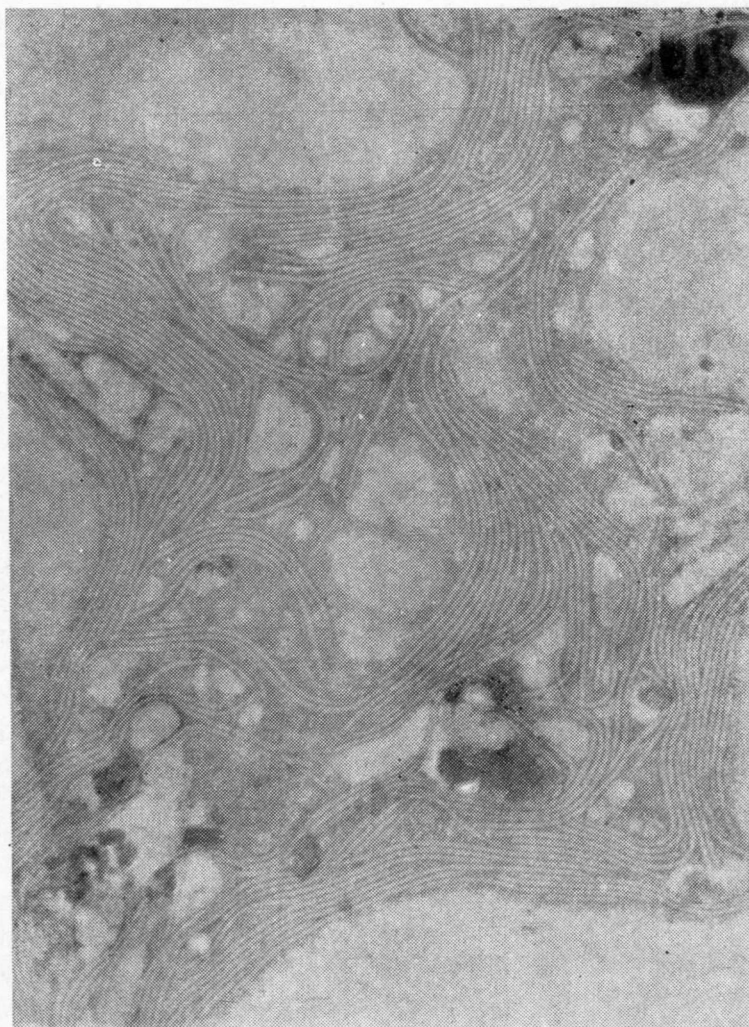


Fig. 3. Purified preparation of SCM-V-F after differential centrifugation. Note cylindrical crystal like structures on extreme left and right. (X 60,000)

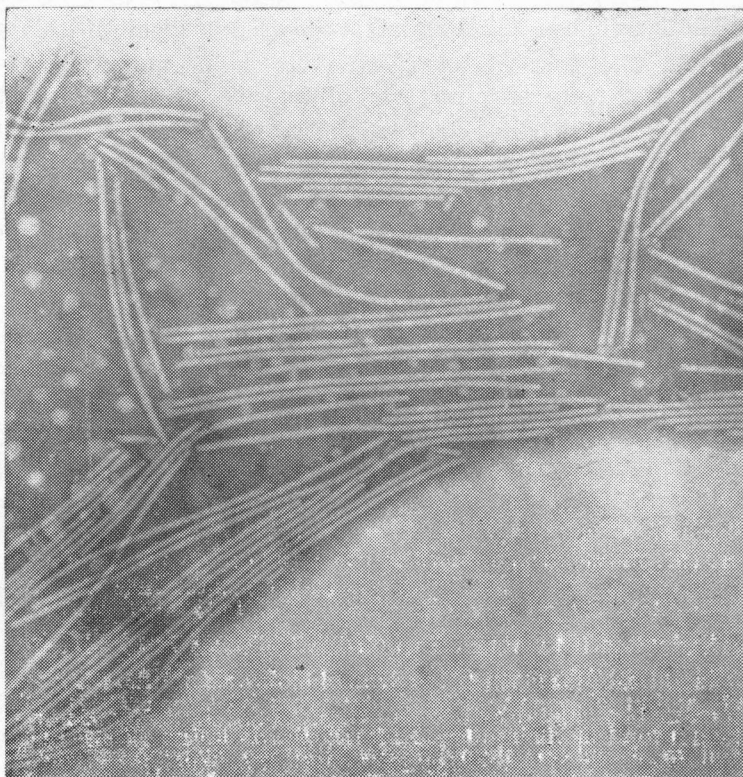


Fig. 4. Purified preparation of SCMV-A after sucrose density gradient centrifugation. (X 60,000)

DISCUSSION

Purification of several potyviruses is difficult and gives only low yield. The problem is usually irreversible aggregation of the virus during the process of purification and its loss in low speed centrifugation. In the present study, freezing and thawing the tissue, use of low molarity buffer with neutral pH and addition of DIECA during extraction gave best results. It appears that one of the factors for loss of infectivity was oxidative degradation and a factor active in freshly extracted juice. Use of DIECA, an antioxidant and freezing and thawing the tissue were useful in overcoming these problems. Change of buffers to sodium citrate and tris-hydrochloride did not give better results. Similarly, use of other stabilising additives such as sodium bisulphite (NaHSO_3), sodium sulphite (Na_2SO_3) and thioglycolic acid were no better. The clarification of extract was much

better when DEAE cellulose (5g/100 ml) was used instead of chloroform but this led to almost total loss of infectivity. The clarification was not very good with chloroform but there was no loss of infectivity. Earlier, Handojo and Noordam (1972) reported poor clarification with chloroform. Damirdagh and Shepherd (1970) used 0.5 M urea and 0.02M Na_2SO_3 while Bond and Pirone (1971) used borate buffer with 0.01M EDTA for resuspending the virus containing pellet. We obtained better result by resuspending the virus containing pellet in 0.05M borate buffer, pH 8.2 supplemented with 0.01M EDTA and 0.5 M urea. The length of the virus particles reported earlier was 686 and 679 nm for SCMV-A and SCMV-F, respectively which was based on observations on only a few particles obtained by dip preparation (Rishi, 1969). The length of majority of virus particles in present study ranged between 670 to 770 nm. It appears that both SCMV-A and SCMV-F are moderately good immunogen. The titre rose to 1/2084 after administering five injections of purified preparations each obtained from 100 g leaves. In earlier report on virus could be determined in the rind of mature cane and old buds in case of SCMV-F and root by inoculation of the extract of these parts on test plant (Rishi, 1969). In the present study presence of virus in these parts of diseased sugarcane plant could be easily detected.

REFERENCES

- Bhargava, K. S., Joshi, R. D. and Rishi, N. 1972. Occurrence of strains A and F of sugarcane mosaic virus in Uttar Pradesh. *Proc. Congr. Int. Soc. Sugarcane Tech.* 14th, 1971, pp. 949-54.
- Bond, W. P. and Pinone, T. P. 1971. Purification and properties of sugarcane mosaic virus strains. *Phytopath.* **71** : 56-65.
- Chen, C. T. 1978. Sugarcane mosaic in Taiwan. I. Purification and electron microscopy of causal agent. Rep. Taiwan Sugar Research Inst. No. 82, 13-18.
- Clark, M. F. and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* **34** : 475-83.
- Damirdagh, I. S. and Shepherd, R. J. 1970. Purification of the tobacco etch and other viruses of potato Y group. *Phytopathology* **60** : 132-42.
- Gillaspie, A. G. Jr. 1972. Sugarcane mosaic virus. Purification. *Proc. Congr. Int. Soc. Sugarcane Tech.* 14th, 1971, pp. 961-70.
- Handojo, H. and Noordam, D. 1972. Purification and serology of sugarcane mosaic virus. *Proc. Congr. Int. Soc. Sugarcane Tech.* 14th, 1971, pp. 973-84.
- Rishi, N. 1969. *Studies on the mosaic disease of sugarcane in Uttar Pradesh*. Ph.D. thesis, University Gorakhpur. pp. 1-142.
- Snazelle, T. E., Bancroft, J. B. and Ulstrup, A. J. 1971. Purification and serology of maize dwarf mosaic and sugarcane mosaic viruses. *Phytopathology* **61** : 1059.
- Tosic, M., Ford, R. E., Moline, H. E. and Mayhew, D. E. 1974. Comparison of techniques for purification of maize dwarf and sugarcane mosaic viruses. *Phytopathology* **64** : 439-42.

RECOVERY OF FOOT-AND-MOUTH DISEASE VIRUS IN BOVINE KIDNEY CELL CULTURE SYSTEM

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SUMMARY

Baby mice is the most common system being employed for recovery of foot-and-mouth disease (FMD) virus from field samples. However, samples where the material is either in insufficient quantity or have been collected in later stages of the infection prove non-viable in mice. For such samples as well as those samples showing irregular mortality pattern in mice, bovine kidney cell culture (BKCC) system was employed for the recovery of the virus. A total of 100 samples were processed in BKCC and of these, 28 (28%) samples produced cytopathic effects and were successfully typed (type O-22, type C-3 and type Asia I-3). BKCC is a conventional method for recovery of FMD virus, however, the study indicated better typability rate when infected cell culture fluid was purified with fluorocarbon and concentrated with polyethyleneglyco.

Foot-and-mouth disease (FMD) is endemic in India and a large number of outbreaks occur every year. The presence of FMD virus in field samples at this laboratory is routinely confirmed by typing of suspected material (tongue, gum, feet epithelium etc.) by direct complement-fixation test or through recovery of the virus from the material in suckling mice and retesting by complement-fixation test. Because of various reasons, sometimes it is not possible to collect the suitable sample at right time as well as in sufficient quantity. Such samples are usually not typed when put to complement-fixation test with antigen prepared from original material. Moreover, these samples when passaged in suckling mice generally turn non-lethal to them. Bovine kidney cell culture (BKCC) system has proved to be a superior method for recovery of FMD virus (Patty *et al.*, 1965; Cottral *et al.*, 1966; Tewari *et al.*, 1980). In the present study, BKCC was used for recovery of FMDV from such samples with a modified technique.

Foot-and-mouth disease samples were either collected by the staff of Regional Centre, Hisar or received from field in the form of tongue/gum/feet epithelium. These samples were either scanty in quantity or have proved non-viable in baby mice on testing in the laboratory. A total of 100 samples (31 of 1981, 13 of 1982 and 56 of 1983) were studied.

The sample was triturated and an approximately 10% suspension was prepared in cell culture medium aseptically. The suspension was centrifuged and the supernate was used for infection of BKCC. At least two to three blind passages were

Short Communication

given before declaring the samples negative and those showing specific cytopathic effects (CPE) were further processed (Figs. 1 and 2).

The cell culture fluid of samples of 1981-82 showing CPE was given directly into baby mice, whereas similar fluid of samples of 1983 showing CPE was first shaken with equal amount of fluorocarbon (Arklone, ICI Mond Division, Cheshire, U. K.) for 30 min and centrifuged at 3000 rpm for same time. They were then concentrated against polyethyleneglycol 6000. Thus, concentrated cell culture fluid was inoculated into baby mice. In both cases, mouse carcass antigen, prepared as per standard method, was used and the virus was typed by micro complement-fixation test (Forman, 1974) with slight modifications.

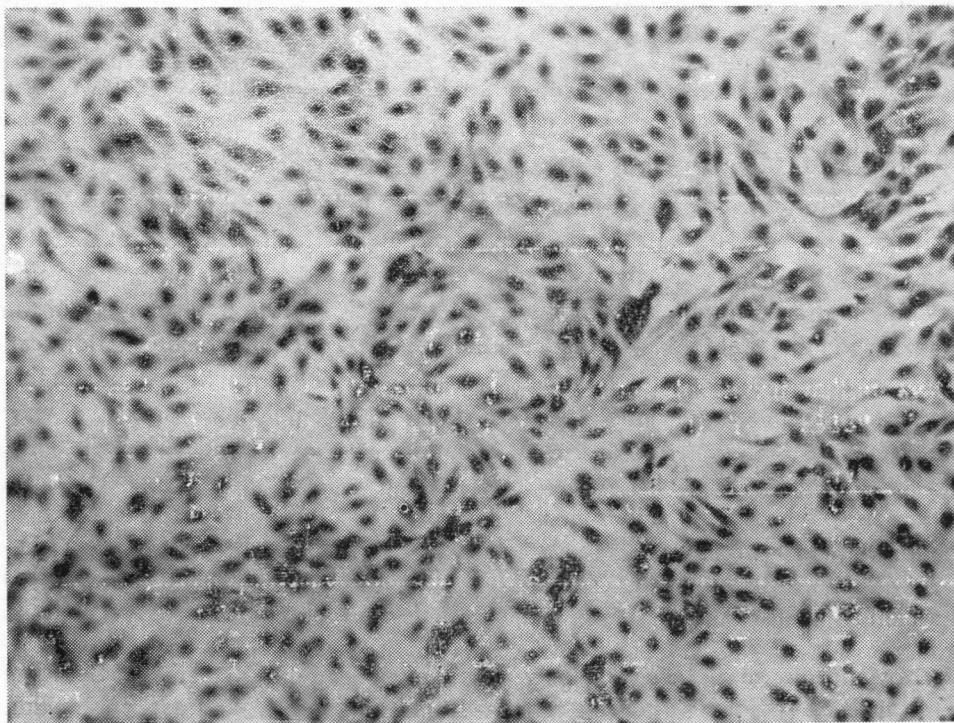


Fig. 1. Bovine kidney cell culture after 168 h of cultivation Giemsa x 100

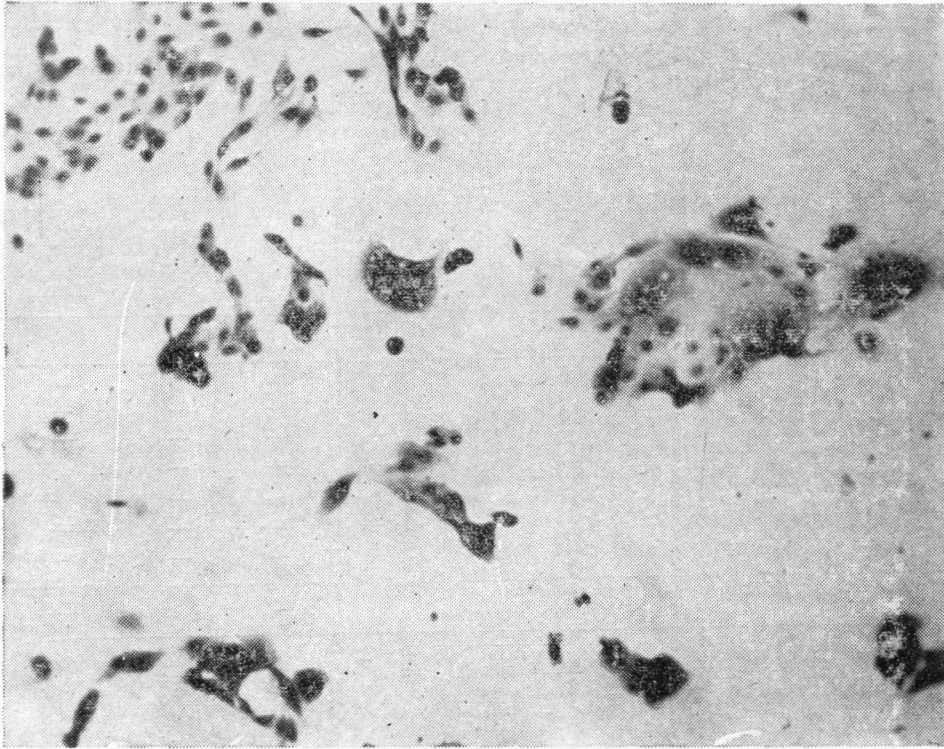


Fig. 1. Bovine kidney cell culture 48 h after infection with type O virus. Giemsa X 100

A total of 100 samples were processed and the virus was recovered from 28 (28%) samples. In 44 samples of 1981-82, which were not given fluorocarbon and polyethyleneglycol treatment, virus could be recovered from 5 (11.3%) samples, however, in case of the remaining 56 samples (all of 1983) which were given such treatment, the virus could be recovered from 23 (41.1%) samples (Tables 1 and 2). It is worthwhile to mention here that recovery of FMD virus from the samples of treated group of 1983 was much higher (41.1%) than untreated (11.3%) samples of the 1981-82. Therefore, the present findings are improvement on the earlier findings of Tewari *et al.*, (1980) who recorded 20% viability/typability. However, these authors did not carry out fluorocarbon-polyethyleneglycol treatment to the samples. It might appear that the treatment of fluorocarbon helps in release of infectious virus particles (Bachrach, 1968) and polyethyleneglycol increases the

number of virus particles by concentration. The present study, while confirming the earlier observations of Tewari *et al.* (1980) about the successful use of BKCC system for recovery of FMD virus, showed that the recovery rate could be further improved with the modified method as reported above.

TABLE 1

Recovery of foot-and-mouth disease virus from field samples in cell culture system
(Not treated with fluorocarbon and polyethyleneglycol). Year 1981-82*

Species	No. of samples processed	No. of samples found viable	Virus types			
			0	A	C	Asia I
Cattle	26	3	1	—	2	—
Buffalo	11	1	1	—	—	—
Sheep	1	—	—	—	—	—
Goat	1	—	—	—	—	—
Pig	2	1	1	—	—	—
Deer	1	—	—	—	—	—
Nilgai/Churi	2	—	—	—	—	—
Total	44	5	3	—	2	—

*Viability percentage 11.3

TABLE 2

Recovery of foot-and-mouth disease virus from field samples in cell culture system
(Treated with fluorocarbon and polyethyleneglycol) Year. 1983*

Species	No. of samples processed	No. of samples found viable	Virus types			
			0	A	C	Asia I
Cattle	32	14	11	—	1	2
Buffalo	22	9	8	—	—	1
Sheep/goat/ pig/others	2	—	—	—	—	—
Total	56	23	19	—	1	3

*Viability percentage 41.1

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Short Communication

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REFERENCES

- Bachrach, H. L. 1968. Foot-and-mouth disease. *Annual Review of Microbiology* **22** : 201-244.
- Cottral, G. E., Patty, R. E., Gailiunas, P. and Scott, F. W. 1966. Relationship of foot-and-mouth disease virus plaque size on cell cultures to infectivity for cattle by intramuscular inoculation. *Archives gesmate Virusforschung* **18** : 276-93.
- Forman, A. J. 1974. A study of foot-and-mouth disease virus strains by complement-fixation. A comparison of tube and microplate test for differentiation of strains. *Journal of Hygiene, Cambridge*. **72** : 407-13.
- Patty, R. E., Cottral, G. E. and Gailiunas, P. 1965. Comparative assay of foot-and-mouth disease virus in cattle, mice and cell culture. *Bulletin Office International Des Epizooties* **63** : 1595-1606.
- Tewari, S. C., Ramakant, Prasad, S., Ahuja, K. L. and Sharma, V. K. 1980. A note on the use of bovine kidney cell culture for recovery of foot-and-mouth disease virus from field samples. *Bulletin Office International Des Epizooties* **92** : 167-69.

EFFECT OF INSECTICIDES ON YELLOW MOSAIC OF MOTH (*VIGNA ACONITIFOLIA* (JACQ) MARECHEL)

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SUMMARY

Yellow mosaic, a most wide spread and destructive disease of **mothbean** (*Vigna aconitifolia* (Jacq.) Marechel.), is transmitted by the whitefly, *Bemisia tabaci* Genn.

Studies were conducted to test the efficacy of five insecticides in reducing the incidence of the disease and in increasing the yield. Rogor (0.02%) sprayed twice at an interval of 15 days, when the plants were 20 days old, proved to be the most effective in reducing the incidence of the disease (9.66%) and in increasing the yield (419.4 kg/ha) significantly as compared to control (48.66% and 111.1 kg/ha, respectively).

A field trial in RBD with three replications in plots of 2×2 m size accommodating four two-meter long rows at 50 cm apart Baleshwar-12, a highly susceptible variety, spread out with a plant to plant distance of 15 cm, was conducted to test the efficacy of five insecticides (Table 1). Insecticides were sprayed twice at an interval of 15 days at recommended doses. Check plots received water spray. First spraying was given with the initiation of symptoms in a few plants. Twenty days after the second spray, per cent disease intensity was recorded in each plot and later grain yield in each plot. The data was subjected to statistical analysis.

Table 1 indicates that the per cent disease intensity in all the treatments was lower than the control (48.66%), lowest for Rogor (9.66%), followed by Dimecron (14.9%), Metasystox (21.94%) and Ekalux (27.22%) and Malathion (36.94%). The yield in all the treatments was higher than the control. Highest yield was recorded with Rogor (419.4 kg/ha) followed by Dimecron (333.3 kg/ha) and Metasystox (286.5 kg/ha).

Control of whitefly transmitted virus diseases by the application of insecticides was reported with varying degrees of success (Varma, 1963; Nour Eldin *et al.*, 1969; Sastry and Singh, 1971; Yassin and Abu Sahib, 1972; Sastry *et al.*, 1974). Reduction in the incidence of yellow mosaic of soybean and also the whitefly population due to carbofuran and aldicarb was also observed by Singh *et al.* (1971). Nene (1972, 1973) observed effective control of the population of *B. tabaci* transmitting yellow mosaic of **mungbean** by Baythion, Malathion and Parathion.

Highest yield (419.4 kg/ha) with Rogor corroborates report of Sastry and Singh (1971) that Rogor, Ekalux, Metasystox, Thimet and Dimecron applied regularly

TABLE 1

Effect of insecticidal treatments on the infection percentage of yellow mosaic of moth

S. No.	Insecticides	Conc. in percentage	Infection percentage/ plot			Average percentage of infection	Average yield ()	Yield/ha (kg)
			R ₁	R ₂	R ₃			
1.	Dimecron 100 Ec	0.03	13.63 (21.64)	18.18 (25.18)	12.94 (21.05)	14.91	200	333.3
2.	Malathion 50 Ec	0.1	29.16 (32.65)	44.44 (41.78)	37.00 (37.46)	36.94	101.66	169.4
3.	Metasystox 50 Ec	0.05	18.75 (25.62)	21.00 (27.27)	26.08 (30.66)	21.94	168.33	280.5
4.	Ekalux 25 Ec	0.075	23.18 (28.73)	26.50 (30.98)	32.00 (34.45)	27.22	120	200.0
5.	Rogor 30 Ec	0.02	11.00 (19.37)	8.00 (16.43)	10.00 (18.43)	9.66	251.66	419.4
6.	Control	—	45.00 (42.13)	46.34 (42.88)	54.65 (47.64)	48.66	66.66	111.1

C. D. at 5% level=4.77; *Values in parentheses are angular values.

Short Communication

from the nursery stage resulted not only in reducing the incidence of tomato leaf curl but also increased the yield.

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REFERENCES

- Broadbent, L. 1957. Insecticidal control of the spread of plant viruses. *Ann. Rev. Entomol.* **2** : 339-59.
- Nene, Y. L. 1972. A survey of viral diseases of pulse crops in U. P. G. B. Pant Univ. of Agric. & Tech. pp. 191.
- Nene, Y. L. 1973. Control of *B. tabaci* Genn.—a vector of several plant viruses. *Indian J. agric. Sci.* **43** : 433-36.
- Nour Eldin, F., Mazyad, H. and Hassan, M. S. 1969. Tomato leaf curl virus disease. *Agri Res. Rev. Cairo* **41** : 49-54.
- Rathore, G. S. 1982. Studies on yellow mosaic of moth (*Vigna aconitifolia* (Jacq.) Marechel.). M. Sc. thesis, Sukhadia University, Udaipur. pp. 57.
- Sastry, K. S. M. and Singh, S. J. 1971. Effect of different insecticides on the control of whitefly (*B. tabaci*) population in tomato crop and the incidence of the tomato leaf curl virus. *Indian J. Hort.* **28** : 304-09.
- Sastry, K. S., Sastry, K. S. M. and Singh, S. J. 1974. Influence of different insecticides on tomato leaf curl virus incidence in the yield. *Pesticides* **1974** : 41-42.
- Singh, H., Sandhu, G. S. and Mani, G. S. 1971. Control of yellow mosaic virus in soybean, *Glycine more* (L.) Merrill. by the use of granular insecticides. *India J. Entomol.* **33** : 272-78.
- Varma, P. M. 1963. Transmission of plant viruses by whiteflies. *Bull. Nat. Inst. Sci., India* **24** : 11-23.
- Yassin, A. M. and Abu Sahib, H. S. 1972. Leaf curl of tomato. *Tech. Bull. Agri. Res. Corporation, Sudan* **3** : 33.

THE DETECTION OF RINDERPEST ANTIGEN BY SINGLE RADIAL HAEMOLYSIS TECHNIQUE

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I. V. R. I., Mukteswar-Kumaon-263 138

SUMMARY

Single radial haemolysis technique was used to detect rinderpest viral antigens in various organs/tissues of the infected animals. In comparison to counter immunoelectrophoresis (CIE) and agar gel precipitation test (AGPT). Single radial haemolysis was found to be more sensitive in detection of rinderpest viral antigens.

The basic principle of single radial haemolysis (SRH) lies on the ability of antigens to lyse erythrocytes which have been sensitized with antiviral antibody in the presence of complement. This technique has been successfully applied for the detection of rinderpest viral antigens in the various visceral organs/tissues of animals experimentally infected with rinderpest virus. The hyperimmune serum was produced in rabbits or in bovines as per technique of Scott and Brown (1961). The lymph nodes collected from rabbits infected with lapinised strain of rinderpest virus were used as positive control; while lymph nodes from healthy rabbits served as negative control. Agar gel precipitation test (AGPT) was performed as per technique described by Joshi *et al.* (1972) and counter immunoelectrophoresis as by Bansal *et al.* (1981). The SRH was conducted as per technique of Russell *et al.* (1975) using chromium chloride in place of potassium periodate for detection of sensitizing erythrocytes as per technique of Clarke *et al.* (1977).

The results are presented in the Table. The results of SRH were obtained in about 26 h after spreading fresh guinea pig serum on slides.

From the Table, it is apparent that this test is more sensitive even for the qualitative estimation of antigen than CIE and with an added advantage that it does not need equipments. Further work with regard to comparison of quantitative estimation with enzyme-linked-immunosorbant-assay and rocket immunoelectrophoresis is in progress.

Short Communication

TABLE

Table showing the average diameter of haemolytic zones by applying SRH from different visceral organs/tissues of infected animals for detection of viral antigen and its correlation with CIE and AGPT

S. No.	Name of the organs/ tissues	Average diameter of haemo- lytic zones (mm)	Percentage positive by SRH	Percentage positive by CIE	Percentage positive by AGPT
1.	Jejunum	4.14	100	100	50
2.	Caecum	4.00	100	75	62.5
3.	Rectum	4.00	88.88	100	90
4.	Spleen	3.90	90.90	90	80
5.	Tongue scrapping	3.88	81.81	63.63	45.45
6.	Gum scrapping	3.88	81.81	54.54	36.36
7.	Caecal tonsil	3.86	85.71	50	50
8.	Lymph nodes	3.73	90.90	90	80
9.	Duodenum	3.71	85.71	66.66	66.66
10.	Tonsil	3.57	100	100	33
11.	Pancreas	3.57	100	66.66	66.66
12.	Abomasum	3.44	100	87.5	87.5
13.	Adrenal glands	3.14	100	66.66	66.66
14.	Haemolymph glands	3.9	85.71	50	50
15.	Colon	2.29	85.71	100	50

Note : (i) Average diameter of haemolytic zones of positive control—4 mm.

(ii) Negative control = No zone of haemolysis.

REFERENCES

- Bansal, R. P., Uppal, P. K., Oberai, M. S. and Joshi, R. C. 1981. Quick diagnosis of rinderpest by detection of antigen by counter immunoelectrophoresis. *Indian J. Anim. Sci.* **51** : 766-69.
- Clarke, Mairin, Janet Boustred, Veterie Seagroatt and Schield, G. C. 1977. The use of single radial haemolysis for rubella antibody studies. *J. Hygiene* **79** : 355-64.
- Joshi, A. C., Dhukla, D. C. and Bansal, R. P. 1972. Rinderpest diagnosis by agar gel double diffusion test. *Indian Vet. J.* **49** : 449-56.
- Russell, S. N., Mc Cahon, D. and Beare, A. S. 1975. A single radial haemolysis technique for the measurement of influenza antibody. *J. Gen. Virol.* **27** : 1-10.
- Scott, G. R. and Brown, R. D. 1961. Rinderpest diagnosis with special reference to agar gel double diffusion test. *Bulletin Epizootic Diseases of Africa* **9** : 83.

OCCURRENCE OF WOUND TUMOR AND WOODY GALL VIRUS DISEASES OF CITRUS IN INDIA

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SUMMARY

During the survey of virus diseases of citrus in Assam, wound tumor disease was observed on Mosambi (*Citrus sinensis* (L.) Osbeck.) and on Rangpur lime (*C. limonia* Osbeck.) and woody gall on Gul-tenga [*C. limon* (L.) Burmf.] and citrus (*C. medica*). Wound tumor disease was graft transmissible. No insect vector was discovered. Woody gall was transmissible by bud and bark grafting to *C. jambhiri*, *C. volkamariana* and *C. sinensis*.

Citrus is an important fruit crop of North East India in about 15,000 ha with annual production of 2,00,000 tonnes. In Assam, 5750 ha are in citrus cultivation with annual production of 50,210 tonnes. The production of citrus of 7.7 ha in India is far below the average in other countries, due to decline of a large population of the budded seedling trees. Several factors are responsible for citrus decline, virus disease plays an important role. Prevalence of wound tumor and woody gall was observed in this investigation.

Seedlings of *C. sinensis*, *C. limonia*, *C. limon*, *C. medica*, *C. jambhiri* and *C. volkamariana* were raised on 10" earthen pots. Sterilized soils filled up in the pots were kept in insect proof house. Nine-month old healthy seedlings, 5 per variety, were taken for grafting bud and bark taken from infected plants. The plants were kept in insect proof house for study. The insect transmission with *Toxoptera citricida*, *Myzua persicae* and *Aphis gossypii* were negative.

In case of wound tumor disease, the base of the trunk is swollen and forms a tumor like structure. Affected plants are dwarfed in internodes shortened and the leaves show interveinal chlorosis. In winter months, affected plants show yellowing of the leaves which subsequently drop off and when new flushes come, the chlorosis becomes evident in all the leaves. The plants produce several twigs close together so as to give bushy appearance. The virus was transmitted from Rangpur lime to Rangpur lime and Mosambi seedlings by bark and bud grafting but not by leaf grafting on 9-month old plants. In Rangpur lime root stock the same symptoms as described earlier was observed when coorg mandarin was used as scion. No insect vector was discovered. Symptoms appeared 80-90 days after grafting as interveinal chlorosis on leaves

and later on tumor appeared on tree base. Young plants die after 2-3 years of infection. In old plants, the disease was not observed.

Symptoms of woody gall were evident as small knots on trunks, branches, at joints between leaf petiole and the stem, and on bark as small swellings which enlarge to form galls of various sizes. These galls are covered by greyish roughened bark and at times they may coalesce to form an out growth with cauliflower like surface. Knots of woody gall adhere tightly to the woods. The woody galls were transmitted on *C. limon*, *C. jambhiri*, *C. volkamariana* and *C. sinensis*.

The indexing on rough lemon seedlings initially indicated development of vein enation symptoms (Wallace, 1968). From India Mali *et al.* (1975) first reported its presence. This not presents the occurrence of wound tumor disease on Rangpur lime in Assam as the first report in India and that of woody gall disease on *C. limon* and *C. medica* as the second report from India.

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REFERENCES

- Mali, V. R., Chaudhuri, K. G. and Rane, S. D. 1975. Vein enation and woody gall virus disease of citrus. *FAO Plant Prot. Bull.* 23 : 190-91.
- Wallace, J. M. 1968. Vein enation and woody gall. In: Chilled, (ed. J.P.L.) Indexing for 15 virus diseases of citrus. pp. 44-49. Washington D. C., 4-5, *Deptt. of Agriculture Hand Book* No. 333.

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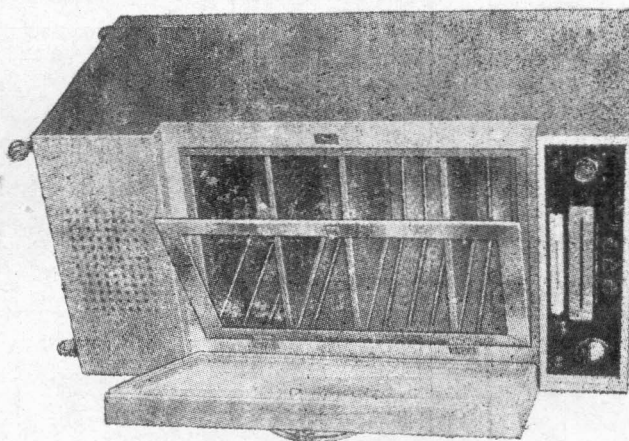
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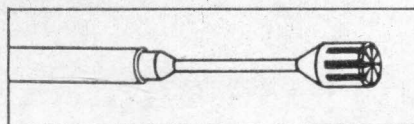
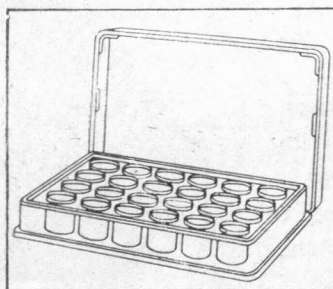
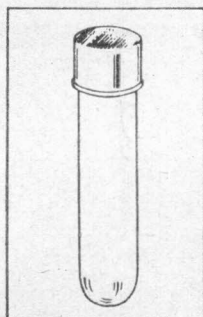
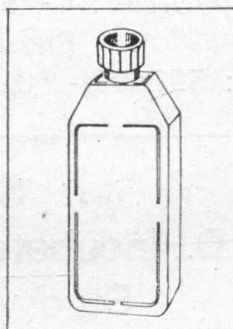
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