

**SOYBEAN MOSAIC VIRUS (SMV): METHODS FOR
DETECTION AND MECHANISM OF TRANSMISSION
IN SOYBEAN (*Glycine max* L. MERRIL)**

Thesis

Submitted to the

**Govind Ballabh Pant University of Agriculture & Technology,
PANTNAGAR-263 145 (U.S. Nagar), Uttarakhand, INDIA**



By

Vikram Singh

***IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF***

Doctor of Philosophy
(PLANT PATHOLOGY)

JULY, 2010

Acknowledgements

“Emotions can not be expressed in words because then they are transformed into mere formality. My gratitude is much more than what I am able to express here”.

I thank almighty for carrying me when I could not walk and helping me safe under his company of love, care and endless blessings without which this tedious and wearisome task could not be accomplished.

At the outset of this epistle, I consider myself fortunate and greatly privileged to have worked under the supervision and guidance of Dr. (Mrs.) Karuna Vishunavat, Professor, Department of Plant Pathology, and Chairperson of my Advisory committee. Words are inadequate to express my sincere and deepest feelings of gratitude originating from the innermost core of my heart for his benevolent guidance, meticulous supervision, whole hearted encouragement, critical appreciation in execution of my work and for all the trust her had in my ability.

With stupendous ecstasy and profundity of complacency, I pronounce utmost of gratitude to members of my Advisory Committee, Dr. R.P. Awasthi, Professor, Dr. V.S. Pundhir, Professor, Department of Plant Pathology and Dr. S.N. Tiwari, Professor, Department of Entomology. Besides their relentless efforts, sagacious guidance and faultless planning, I am grateful to them for their salutary advice, kind co-operation and congenial discussion on an array of topics.

I am gratefully indebted to Dr. J. Kumar, Head and Director CAFT, Department of Plant Pathology, all the teachers and staff members of Department of Plant Pathology for their valuable help and cooperation.

My inexplicable gratitude goes to Hon'ble Vice-Chancellor; Dean, College of Agriculture; Dean, Post Graduate Studies; Dean Students Welfare; Director, Experiment Station; Director, Mushroom Research and Training Center and University Librarian, other University authorities for providing necessary facilities for this investigation.

My sincere thanks are goes to Praveen Kumar sir, Farha, Santosh, and Jairam, (field attendant) of seed pathology lab for their all time support and cooperation during the course of present study.

Best feelings can never be expressed in words, and I find it hard to describe in words the love, blessings, selfless help, support as elder brother and amenable company at Pantnagar of my senior Dr. Bipin Chandra deserve for special thanks.

My sincere thanks goes to Dr. G. P. Gangwar for his kind cooperation for providing me his Laptop for thesis writing.

The author acknowledges moral support of Dr. Anjani Khulbe, Abhishek Pandey, Pradeep Singh, Ranjeet Kumar, Sanjay Chaudhary Sir, Nisha Singh, and Anchal Singh and their family members time to time when I feel lonely.

I also extend heartfelt thanks to my respected seniors Doctor's, C.S. Patni, V. S. Mehra sir, K.K. sir, Rashmi Tiwari mam, Amit sir, Mayank Pandey sir, Rikesh sir, D.J. sir, S. Mehta sir, Himanshu sir, Sanjay sir, Ashutosh Mishra sir, Arvind sir, and Hemant Pandey sir,

beloved juniors especially Bhupendra, Anoop Mishra, Manoj Yadav, Priyanka, Ghatak, Santosh, Himanshu, Amit, Vinod, Danish, Mohd. Ansar and Upreti for the help and understanding they endowed me during the long and strenuous course of study.

As author it was always cheerful to have loving and joyous company of Dr. C.P. Singh, Bhanu Bhaiya, Vipin Kumar Misra, Dr. Ramwant Gupta, Bhuvan Da. Jitendra sir, Rakesh Nainwal, Vipin, Bhaskar, Shailesh Pandey, Saurabh, P.P., Balwant, Kamal, Smita Puri, Usha Suyal, Geetanjali, Dinesh Pathak, Sheetanshu, Vikram Singh, Rishendra, Dr. Dinesh Tiwari, Dr. R.K. Chaurasia and Dr. Akhilesh Pandey, the whole course of degree programme. I highly acknowledge them for providing the warm company, love & care, encouragement during the degree programme. Blissful moments shared with them will remain cherishable.

It is difficult to express the gratitude I owe to my nearest friends especially Ashis, Manoj, Umesh, Naveen, Sanjay, Kaushal, Pankaj, Abhishek, Dr P.C. Singh, Virendra, Kirti, Prashant, and Vijya for their constant help and moral support.

Alas! Before this manuscript reach to my father Sri Baleshwar Singh was no more in this world. He was a constant source of inspiration of my life. He always inspired me to acquire a great achievements in my life and he want to see me as a plant doctor. Although his death has caused a grievous loss for me and my family but he as always will be the torch bearer in the darkest path of life and guiding me to achieve great success at every step in life. Oh! God please keeps his soul in space.

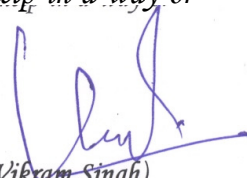
Where the emotions are involved, words cease to express. My vocabulary fails to accentuate my profound reverence and sincere regards to my amma Suneeta Singh and younger brother Mr. Rahul Singh (Lalu) for their ceaseless inspiration, moral and spiritual support, profound love and unending encouragement, guiding me to achieve success at every step in life.

Words fail to express my profound sense of respect, indebtedness and sincere feeling for my beloved Tauji, Sri Rameshwar Singh and Mausaji's especially Sri A.K. Singh and Sri D.K. Singh and Mausiji's Smt. Suman Singh, and Smt. Subhashini Singh, Mama's Sri Radheshyam Singh, Sri Ghanshyam Singh and Sri Ramesh Singh for their constant support, inspirations, love blessings and sacrifice throughout my academic career.

I am obliged to university for awarding me university fellowship during the course of study at G.B. Pant University of Agriculture and Technology.

Thanks are also due to Bhanu Bhaiya and Ravindra Prasad, for typing the manuscript with utmost care.

I apologize for the faux pass of the person who have extended the help in a way or other and deserved such thanks.


(Vikram Singh)
Author

Pantnagar
July, 2010

Dr. (Mrs.) K. Vishunavat
Professor



Department of Plant Pathology
College of Agriculture
G.B. Pant Univ. of Agric. &
Tech., Pantnagar - 263 145,
Distt.- U.S. Nagar,
Uttarakhand, INDIA

CERTIFICATE

This is to certify that the thesis entitled **“Soybean Mosaic Virus (SMV): Methods for detection and mechanism of transmission in Soybean (*Glycine max* L. Merrill)”** submitted in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** with major in **PLANT PATHOLOGY** and minor in **ENTOMOLOGY** of the College of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona-fide* research carried out by **Mr. Vikram Singh, Id. No. 34018**, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

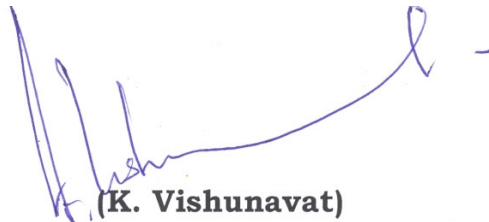
The assistance and help received during the course of this investigation have been duly acknowledged.

Pantnagar
July, 2010

(K. Vishunavat)
Chairperson
Advisory Committee

CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Mr. Vikram Singh, Id. No. 34018**, a candidate for the degree of **DOCTOR OF PHILOSOPHY** with major in **PLANT PATHOLOGY** and minor in **ENTOMOLOGY**, agree that the thesis entitled **“Soybean Mosaic Virus (SMV): Methods for detection and mechanism of transmission in Soybean (*Glycine max* L. Merrill)”** may be submitted in partial fulfilment of the requirements for the degree.



(K. Vishunavat)
Chairperson
Advisory Committee



(R.P. Awasthi)
Member



(V.S. Pundhir)
Member



(S.N. Tiwari)
Member



Ex-Officio Member
Head of the Department

CONTENTS

S. No.	CHAPTERS	PAGE
1.	<i>INTRODUCTION</i>	
2.	<i>REVIEW OF LITERATURE</i>	
3.	<i>MATERIALS AND METHODS</i>	
4.	<i>EXPERIMENTAL RESULTS</i>	
5.	<i>DISCUSSION</i>	
6.	<i>SUMMARY</i>	
	<i>LITERATURE CITED</i>	
	<i>APPENDICES</i>	
	<i>VITA</i>	
	<i>ABSTRACT</i>	

Introduction

Soybean, *Glycine max* (L.) Merrill a native of Eastern Asia, is an ancient oil crop with hundreds of food, feed and industrial uses. It is known as the “golden bean” of the 20th Century. Soybean is being grown since last century under various names as Bhat, Bhatman, Bhatmas, Ramkulthi, Bhut, Kalitur, Telikulth and Garryakaly (**Singh and Saxena, 1979**). Due to very poor cookability and digestibility on account of inherent presence of trypsin inhibitor, it can not be utilized as a pulse and is widely used for soybean oil. It is now the second largest oilseed in India after groundnut. It grows in varied agro-climatic conditions. It has emerged as one of the important commercial crop in many countries. Due to its worldwide popularity, the international trade of soybean is spread globally. Several countries such as Japan, China, Indonesia, Philippines, and European countries are importing soybean to supplement their domestic requirement for human consumption and cattle feed. Soybean possesses a very high nutritional value. It contains about 20 per cent oil and 40 per cent high quality protein (as against 7.0 per cent in rice, 12 per cent in wheat, 10 per cent in maize and 20-25 per cent in other pulses). Soybean protein is rich in valuable amino acid lysine (5%) in which most of the cereals are deficient. In addition, it contains a good amount of minerals, salts and vitamins (thiamine and riboflavin).

A large number of Indian and western dishes such as bread, chapati, milk, sweets, pastries etc., can be prepared with soybean. Wheat flour fortified with soybean flour makes good quality and more nutritious chapati. Soybean oil is used for manufacturing vanaspati ghee and several other industrial products. Soybean is used for making high protein food for children. It is widely used in the industrial production of different antibiotics. Soybean builds up the soil fertility by fixing large amounts of atmospheric nitrogen through the root nodules, and also through leaf fall on the ground at maturity. It can be used as fodder; forage can be made into hay, silage etc. Its forage and cake are excellent nutritive foods for livestock and poultry. Soybean being the richest, cheapest and easiest source of best quality proteins and fats and having a vast multiplicity of uses as food and industrial products is sometimes called a wonder crop. It is also an excellent source of unsaturated oil.

Recently, in India, the importance of soybean has been recognized because newer products of soybean are becoming more and more popular in formulating new, low cost, nutritionally balanced, high protein food and beverages for human consumption. Soybean mixed with other cereals is capable of increasing Protein Efficiency Ratio (PER), a parameter of protein quality. According to **Mehta (1987)** the PER of wheat and rice is increased from 1.53 to 2.57 and 2.10 to 2.77 respectively when mixed with soybean.

Most of the soybean varieties contain an average of about 20 per cent vegetable oil. Forty percent of world supply of edible vegetable oil comes from soybean alone and 80 per cent of which is used in margarine, salad oil and cooking oil (**Sinclair, 1984**). For those Asians, who do not drink animal milk, the soybean quickly became indispensable source. The beans, soaked in water to yield a white liquid, were known affectionately as 'The cow of China'. In 206 BC in Han Dynasty of China, soybeans were first fermented to make *douchi*, the predecessor of soy sauce and *miso* (what is called *douche* by the Chinese and *hamanatto* by the Japanese). It is also called 'salted black beans' as the soybean turn black during fermentation. SoyafLOUR, powder, or curd were fermented to make *miso* (Soy paste), *shoyu* (soy sauce), *doufu* (soy curd), *natto* (soy cheese), and *tempeh* (a soybean cake invented in Indonasia), as well as *yuba*, *kinako*, *hamanatto* and *kochu chang*. Steamed green beans, roasted soy nuts, and soybean sprouts were also favored and were highly nutritious (**Warshall and Imhoff, 1999**).

Although a native of China, United State is the major producer of soybean and ranks first in its production. Brazil, Argentina and China rank second, third and fourth position, respectively in terms of production. India occupies fifth place. Soya is now a global staple food and about 220.35 million tonnes of beans are produced, mainly in

the United States (33%), Brazil (28%), Argentina (21%) and China (6%) and the rest (12%) is produced in India, Paraguay, Canada and others. Presently, the world's total area under soybean cultivation is in 91.29 million hectares mainly in United States (31%), Brazil (26%) and Argentina (20%). The rest 33 per cent is contributed by China (10%), India (10%) and Paraguay (3%) **(Anonymous, 2008)**.

In the recent past, soybean cultivation has increased manifold as compared to any other oilseed crop in India and stands next only to groundnut, though commercial production of soybean began only in 1971-72. Soybean production is mainly confined to Madhya Pradesh (also known as soybean bowl of India), Maharashtra, Rajasthan, Andhra Pradesh, Karnataka, Uttar Pradesh and Chhattisgarh. The soybean crop is grown mainly in the states of Madhya Pradesh, Maharashtra and Rajasthan. About 53 per cent of the cropped area under this crop falls in Madhya Pradesh. However, the yield of soybean crop in Madhya Pradesh 2001-02 was as low as 838 kg per hectare whereas it was 1,254 kg and 1,091 kg per hectare in Maharashtra and Rajasthan, respectively. Soybean acreage and production in the country was increasing till the late 1990s and lost its momentum in the past few years. The area under the crop steadily increased from 22.5 lakh hectares in 1989-90 to 60.02 lakh hectares in 2001-02 but thereafter the area fell sharply in 2002- 03 due to poor weather conditions. Again, the area under the crop increased in successive

years after 2002-03 and it increase upto 88.50 lakh hectares in 2007-08. For the last six years, soybean has been witnessing growth in terms of acreage and it now stands at 88.50 lakh ha. In 1989-90, the production of soybean seeds was mere 18.05 lakh tonnes, which increased to 54.0 lakh tonnes in 2001-02 and then decreased to 43 lakh tonnes in 2002-03. It is only in 2003-04 that production recovered with record of 69.32 lakh tonnes of soybean seeds, thereafter the production fell to 61.26 in 2004-05. However, production rose in consecutive years after 2004-05 and now (2007-2008) it has significantly increased to 94.73 lakh tonnes, an all time record. Similarly, the yield per hectare declined from 1,074 kg per hectare in 2003-2004 to 927 kg per hectare in 2006-07. The yield rose sharply in 2007-08 to 1070 kg per hectare just short of the all-time record yield of 1074 kg per hectare in 2003-04 **(Anonymous, 2008)**.

Although, there are several bottlenecks in the successful cultivation of soybean in India, diseases are considered as one of the major constraints in its cultivation. In recent years increase in acreage, introduction of high yield potential varieties and wider adaptability has necessitated a considerable change in agronomic and cultural practices to suit the growth pattern of these varieties. This has resulted in a sudden and drastic alteration of the plant microenvironment favoring the development of more and newer disease with higher incidence.

Over 100 diseases caused by different pathogens viruses, fungi, bacteria, nematode and other deficiency disorders are known to affect soybean, about 10 diseases are viral diseases which are of economic importance. Among virus diseases like green mosaic, yellow mosaic, bud blight, spotted wilt and rhychosia mosaic appear in epidemic form year after year causing colossal losses. However, green mosaic is seed borne and cause substantial yield losses.

A survey of soybean *kharif* crop grown in Uttarakhand state, crop research centre (CRC) farm revealed the presence of soybean mosaic disease in several varieties and germplasm lines. Chief symptoms of the disease were mosaic, mottling, distortion of leaf lamina, necrosis of leaves and stunting of affected plants. Symptoms begin with transient vein clearing in the upper trifoliate leaves, followed by mosaic. As the disease progresses, the margins of the affected leaves curl downwards and dark green areas along the veins develop into raised puffs or puckering, or into a more general leaf curling and rugosity. The seedling grown from infected seed shows mottling and downward curling in plants. Infected plants are usually stunted because of shortening of the petioles and internodes, particularly when infected while still young or when infected from the seed. Some cultivars develop progressive necrosis of the petioles and stems, bud necrosis, defoliation and plant death with some strains **(Cho *et al.*, 1977; Hunst and Tolin, 1982).**

The pods are reduced in number and size, some are malformed, glabrous or seedless, and the yield may be considerably reduced. The

virus can reduce the size of the seed (**Demski and Jellum, 1975; Hepperly et al., 1979**).

Spread of SMV is by several aphid species (mostly transient alatae), but only a few species are important vectors of the virus (**Ruesink and Irwin, 1986**).

SMV is considered to be one of the most significant soybean viruses recurring worldwide (**Bos, 1972**).

In India, this disease is on increase and appears almost every year. So far, there is little information on different aspects of disease including its seedborne nature and characterization of SMV on soybean.

In view of seriousness of the disease as well as the virus in India, following investigations were undertaken:

1. Maintenance of the soybean mosaic virus culture.
2. Study of symptomatology, host range and transmission and stability of soybean mosaic virus.
3. Purification, electron microscopy and serology of the virus.
4. Study the location of seed inoculum of SMV in different flower and seed parts of soybean.
5. To find out the more attractive colour and shape of trap for monitoring aerial population of aphid.
6. Study the yield losses in soybean due to the soybean mosaic virus.
7. Screening of different germplasms /cultivars lines of soybean both under field and glass house conditions for source of resistance.

Review
of
Literature

2.1 Distribution of Disease

Soybean is presently grown throughout the world and over the recent years has emerged as a hunger fighter in many tropical and subtropical areas of the world. However, disease have become major constraints in enhancing the production of soybean.

A mosaic disease of soybean was first reported by **Clinton (1916)** from Connecticut under the name chlorosis and crinkling, but **Gardner and Kendrick (1921)** at Indiana, USA were first to established the viral nature of disease. Soybean mosaic virus has been reported from various countries by different workers as shown in Table 1.

2.2 Symptomatology

Symptoms in sap-inoculated soybean seedlings begin with transient vein clearing in the upper trifoliate leaves, followed by mosaic. As the disease progresses, the margins of the affected leaves curl downwards and dark green areas along the veins develop into raised puffs or puckering, or into a more general leaf curling and rugosity. The primary leaves of plants grown from infected seed may show mottling and downward curling. Infected plants are usually stunted because of shortening of the petioles and internodes,

Table 1 : Soybean mosaic virus reported on soybean from different countries

Country	Workers	Year
Australia	Anonymous	1948
Brazil	Porto and Hagedon	1973
Bulgaria	Vui-Yui	1961
Canada	Dickson	1924
China	Yu	1939
Czechoslovakia	Baudys	1931
England	Klessner	1954
France	De Guerpel	1942
Germany	Heinze and Kohler	1940
Ghana	Dilghton and Tinsleey	1958
India	Nariani and Pingaley	1960
Italy	Grancini	1941
Jamaica	Martyn	1942
Japan	Murata	1925
Kenya	Anonymous	1972
Malaya	Heath	1958
Morocco	Rieuf	1960
Netherlands	Quantz	1961
New Zealand	Close <i>et al.</i>	1970
Philippines	Reyes	1957
Poland	Garbowski and Juraszkowna	1933
Portugal	De Vasconcelos	1964
Rhodesia	Hopkins	1932
Rumania	Sovulescu	1935
Sweden	Linhell	1939
Taiwan	Han	1959
Tanzania	Anonymous	1962
Uganda	Hansford	1934
U.S.S.R.	Bilyk	1965
Venezuela	Mullar	1941
Yugoslavia	Nikolic and Stakic	1964

particularly when infected while still young or when infected from the seed. Some cultivars develop progressive necrosis of the petioles and stems, bud necrosis, defoliation and plant death with some strains **(Cho *et al.*, 1977; Hunst and Tolin, 1982)**.

The pods are reduced in number and size, some are malformed, glabrous or seedless, and the yield may be considerably reduced. The virus can reduce the size of the seed **(Demski and Jellum, 1975; Hepperly *et al.*, 1979)**.

Particularly in the case of co-infection with BPMV **(Ross, 1968)**, seed germination **(Suteri, 1981)**, seedling vigor **(Kwon and Oh, 1983)** change its chemical composition **(El-Amrety *et al.*, 1985b)**, diminish seed viability **(El-Amrety *et al.*, 1985a)**, and thus the quality of the seed.

There is continuing confusion as to the cause of seed-coat mottling often associated with SMV infection and often considerably increased by it **(Kennedy and Cooper, 1966, 1967)**. However, it may also be caused/increased by other virus infections (Soybean dwarf virus) and by environmental and genetic factors **(Irwin and Schultz, 1981; Bajwa and Pacumbaba, 1996)**. It is not correlated with seed transmission of the virus **(Ross, 1970; Iwai *et al.*, 1985)** and cannot be used as a parameter of infection by SMV and of seed transmission of the virus.

SMV is thought to promote the metabolism of nitrogen compounds and their content in the seed coat of inoculated plants,

and is suggested to be helpful as an indicator of resistance to seed-coat mottling (**Zheng *et al.*, 1998**). Environmental factors causing or influencing seed-coat mottling are location and season and temperature (**Ross, 1968, 1970**).

Ross (1969b) observed that extensive seed-coat mottling developed in inoculated plants exposed to 20°C in growth chambers during flowering, while no mottling occurred in plants exposed to 30°C.

Another factor is time of infection during plant development; incidence of mottling was higher in plants with early infection but it has also been reported to be more distinct when plants are inoculated during flowering than when inoculated earlier (**Bryant *et al.*, 1982; Nakano *et al.*, 1988; Tu, 1992**). Genetic factors are host cultivar (**Wilcox and Laviolette, 1968**), and virus strains (**Ross, 1968**).

Iwai *et al.*, (1985) reported that when two Japanese strains of the virus were inoculated onto the same cultivar under greenhouse conditions, more seed-coat mottling was induced by the strain that caused the least amount of seed infection.

Tu (1977) observed that of mottled and non-mottled seed coats showed higher accumulations of anthocyanin, chlorophyll a, chlorophyll b and ferritin in mottled coats.

Expression of symptoms depends upon the viral strains, the susceptibility of cultivar, the time of infection and temperature. In case of certain cultivars, no symptoms could be observed and the

virus could not be recovered from them where as in some other cultivars, necrotic lesion on inoculated leaves, systemic mottling, systemic necrosis or general necrosis could be observed (**Ross, 1969; Porto and Hagedorn, 1973**).

The mosaic affected plants set fewer pods which are flattened, less pubescent and acutely curved than pods on healthy plants (**Gardener and Kendrick, 1921; Conover, 1948; Nariani and Pingaley, 1960; Quiniones and Dunleavy, 1970**).

Porto and Hagedorn (1974) described stunting of plants and premature leaf abscission as common symptoms on *Phaseolus lathyroides* inoculated with SMV.

Tu (1992) reported that when soybean is inoculated with SMV at different growth stages, the most severe symptoms and the greatest reduction in plant growth and seed yield occurs at early infection than that late infection in each of the cultivars .They established that the temperature giving greatest symptoms severity was 20°C.

Alemeida (1995) observed that strain Gx and G5 induced the highest rates of mottling in all the cultivars tested .He also established that the leaf area (LA) and dry weight of tissues (DWT) differ significantly between healthy and infected cultivars of Santa Rosa and Bragg .It was lower in infected cultivars.

Liao et al. (1995) observed when crosses are made between a parent resistant to SMV and a susceptible parent, then if F1 only

symptom less plants and those with and those with top necrosis occurred. But in F2, an additional wrinkling or mosaic symptoms were observed. The segregation ratio was 3R:1S indicating the top necrosis in soybean is a symptom conditioned by either a single dominant gene or two recessive complimentary genes.

Guo *et al.* (1997) established that in leaf tissue culture of soybean the frequency of bud differentiation for cultures inoculated with strain Sa of SMV lower than uninoculated.

2.3 Electron microscopy

The morphology of SMV in crude sap and as well as in purified virus preparation has been studied by various workers. The virus particles are elongated flaxious type.

Table 2: Morphology of SMV

Virus Examined	Particle Size (nm)	References
Crude sap	750×12-13 nm	Brandes and Wetter, 1959
Crude sap	748×12-13	Quantz, 1961
Partially purified preparation	650-725 ×15-18	Galvez, 1963
Crude sap	650-700 × 13-15	Liu, 1963
Crude sap	750 ± ×--	Bilyk and Legunkova, 1966
Purified preparation	740 ±×--	Rose, 1967
Crude sap	680-750 × 12.5-13	Murayama and Han, 1971
Purified preparation	675-750 × --	Soong and Milbrath, 1980
Purified preparation	580-660 × --	Anjos <i>et al.</i> , 1985
Purified preparation	740-760	Naik and Murthy, 1992
Purified preparation	740 × 13	EI. Ahdal, 1996
--- Not seen/Reported		

2.4 Effect of buffers and varying pH on the infectivity of soybean

Galvez (1963) reported that the virus was inactivated below pH 4.0 and above pH 9.0, and it was most stable at pH 6.0 of 0.3 M phosphate buffer.

Soong and Milbrath (1973) reported that 0.05 M Sodium Phosphate buffer at pH 7.0 gave best result on local lesion production in bean varieties 'Top crop' by SMV.

2.5 Physical properties of the virus in crude sap

In the past, several workers tried to identify the SMV by studying its physical properties. The information on the Thermal Inactivation Point (TIP), Dilution End Point (DEP) and Longevity *in vitro* (LIV) of the virus in crude sap as studied by various workers is given in Table 3.

Nariani and Pingaley (1960) showed that the virus was viable on desiccation of the mosaic affected leaves for seven days at room temperature (25°C-30°C).

Galvez (1963) reported that the longevity of the virus was increased to four month when clarified crude sap from infected plants was kept frozen.

The virus remains infective in expressed plant sap for 2-5 days. It usually has a dilution end point of 0.001, and a thermal inactivation point of 55-70°C. Virus particles are usually flexuous

filaments, about 750 nm long and 15-18 nm wide (**Bos, 1972; Demski and Kuhn, 1989**).

Table 3: Physical properties of SMV

TIP	DEP	LIV	References
58	10^{-4}	3	Pierce, 1955
61	-	3-4	Heinze and Kohler, 1940
64-66	-	4-5	Conover, 1948
64-66	-	4-5	Ling, 1951
50-60	-	-	Brandes and Wetter, 1959
60-62	10^{-3} - 5×10^{-3}	3-4	Nariani and Pingley, 1960
62	10^{-3} - 10^{-5}	1	Quantz, 1961
62	10^{-3} - 10^{-5}	4	Galvez, 1963
55-60	-	3-4	Koshimizu and Iizuka, 1963
65	10^{-4}	4	Nikolic and Stakic, 1964
70	10^{-4}	4-5	Quiniones and Dunleavy, 1970
60	10^{-5}	4-5	Murayama and Han, 1971
54-56	10^{-3} - 10^{-4}	2	Lucas and Hill, 1980
55-60	10^{-2} - 10^{-3}	2-3	Anjos <i>et al.</i> , 1985
55-60	$5 \cdot 10^{-3}$	-	Naik and Murthy, 1992

They have helical symmetry with a pitch of 3.4 nm and contain 5.3% single-stranded nucleic acid (RNA) with a MW of 3, 250, 000 (**Hill and Benner, 1980b**).

The protein coat contains about 2166 subunits of one type of protein with a MW of ca 28, 300 (**Hill and Benner, 1980a**) or ca 32, 150 or 33, 075 according to virus strain (**Soong and Milbrath, 1980**).

The genome of two strains of the virus is 9588 nucleotides long, and a large open reading frame encodes for a large precursor polyprotein that is proteolytically cleft into nine mature proteins including the viral coat protein (**Jayaram *et al.*, 1992**).

2.6 Presence of virus in different parts of infected soybean plant

Iizuka (1973) reported that the virus is pollen transmitted and present in immature seed coats and embryos of infected seeds. it was also demonstrated that embryos and cotyledons of infected seeds can transmit the virus whereas seed coat can not (**Porto and Hegedon, 1973**). **Bowers and Goodman (1979)** detected SMV in flowers, green pods immature seeds, testa, cotyledons and embryos. **Anjos *et al.* (1985)** reported the presence of SMV in leaves, stems and pods but not in the roots during all stages of growth using serological tests. **Iwai *et al.* (1985)** reported that both mild and severe strains of SMV were detected in seed coat and embryo, but only mild strain was detected in the cotyledons using ELISA. **Nicolaescus (1987)** developed a simple method of detection of SMV by individuals testing of embryos from seed. **Bawja and Pacumbaba (1996)** reported that SMV was detected in individual embryo of both mottle and nonmottled soybean seed. It was not detected in individual stained and non stained seed coats and in endosperms of either type of seeds. However, virus was detected in individual hypocotyls in post

germinated seeds. **Dong et al. (1997)** suggested that the principle route of soybean embryo infection was by invasion of ovules.

2.7 Host range studies

2.7.1 Natural hosts

SMV has a narrow natural host range and a high degree of specificity to its main host, soybean. Cultivated soybean is the major natural host of the virus. Natural infection of common bean *Phaseolus vulgaris*, in which the virus is also seed transmissible (**Castano and Morales, 1983**), may also occur. It has increased in Brazil (**Costa et al., 1978**) and been demonstrated in New York State, USA, from *P. vulgaris* plants with symptoms closely resembling those of Bean common mosaic virus (**Provvidenti et al., 1982**). *Vicia faba* in China (**Xu et al., 1986a**) *Lupinus albus* in South Africa (**Vroon et al., 1988; Pietersen and Garnett, 1992**), *Centrosema macrocarpum* in Colombia (**Morales et al., 1990**). Natural infection was also reported from *Cassia occidentalis* in India (**Singh and Gupta, 1996**), and from the non-legumes *Chrysanthemum frutescens* in Italy (**Bellardi et al., 1993**) and *Passiflora* spp. in Colombia (**Benscher et al., 1996**). *Strophostyles helvola* in Arkansas, USA (**Kline et al., 1997**).

2.7.2 Artificial Hosts

Conover (1948) recovered the virus from symptomless inoculated leaves of certain varieties of garden beans.

For many years, soybean was considered to be the only systemic host of soybean mosaic virus (SMV) (**Pierce, 1955**). The virus can be transmitted mechanically to a limited number of plant species, mainly Fabaceae. Systemic infection was induced in the following: *Cassia occidentalis*, *Crotalaria spectabilis*, *Dolichos falcatus*, *Lablab purpureus*, *Hippocrepis multisiliquosa*, *Indigofera hirsuta*, *Tetragonolobus purpureus*, *Lupinus albus*, *L. angustifolius*, *Macroptilium (Phaseolus) lathyroides*, *Phaseolus nigricans*, *P. speciosus*, *P. vulgaris*, *Scorpiurus sulcatus*, *Sesbania exaltata*, *Trigonella caerulea*, *T. foenum-graecum* and *Vigna unguiculata*. The non-legumes *Chenopodium album* and *C. quinoa* react with local lesions (**Quantz, 1961 and Galvez, 1963**).

In general, the virus is poorly infectious to *P. vulgaris*; many cultivars react hypersensitively with necrotic local lesions (also on detached leaves) similar to Bean common mosaic virus (**Quantz, 1961**), but such cultivars may also react with systemic necrosis (**Costa et al., 1978**).

The symptoms of SMV in experimental hosts are similar to those of other potyviruses, and natural infection in such species may often have been mistaken for infection by other viruses. Symptomless infection in several species, including the weed *Phaseolus speciosus* (**Galvez, 1963**), suggests that such species may be a natural source of infection.

Walters (1963) observed that several legumes viz: *Canvalia ensiformis*, *Cyamophsis tetragonoloba*, *Glycine Ussuriensis*, *Lespedeza*

stipulocea, *Lespedeza striata*, *Phaseolus lunatus* and *Stizolobium deeringiarum* and systematic hosts of the virus, whereas, *Vigna sinensis* produce local lesions.

Some cultivars of *Phaseolus vulgaris*, produced local lesion whereas, other cultivars may be systemically infected.

Cassia occidentalis and *Sesbania exaltata* were established as host of soybean mosaic virus by **Galvez (1963)**. *Lupinus albus* and *Phaseolus lathyroids* were confirmed as other hosts (**Walters, 1963 and Galvez, 1963**). Certain varieties of cowpea, bean and the weed *Phaseolus* species were found to be symptomless carriers. *Phaseolus vulgaris* was reported as symptomless carriers (**Galvez, 1963**). **De Vasconcelos (1964)** reported *Mucuna deeringiaa*, *Mucuna utilis* and *Vicia narbonensis* as hosts.

Local lesions were obtained in *Cyamopsis tetragonoloba*, *Lablab purpureus* and *Macrotyloma uniflorum* (**Ross, 1967; Dunleavy et al., 1970; Quiniones and Dunleavy, 1970**).

Murayama and Han (1971) reported that when *Chenopodium amaranticolor* and *Chenopodium album* were inoculated with SMV, local lesions were formed on the inoculated leaves. **Porto and Hagedorn (1973)** reported *Galactia sp.* and *Glycine wightii* as new systemic hosts of soybean mosaic virus. Potentially important species, such as *Macroptilium lathyroides* a possible forage plant in Brazil (**Porto and Hagedorn, 1974**), indicates the potential significance of

the virus for cultivated plant species other than soybean. Nearly 20 systemic hosts, 11 local lesion host and 6 symptomless carriers of the virus have been reported (**Anonymous, 1975**). Pathogenicity of the virus on a number of cultivated plant species, such as *P. vulgaris* (**Costa et al., 1978**). **Anjos et al. (1985)** reported *Calopogonium spp* as new systemic hosts of the virus. They also observed that Brazilian isolates of SMV are restricted to seven species of leguminosae and *Chenopodium quinoa*. Symptomless local infections were obtained in *Nicotiana benthamiana* and *N. clevelandii* (**Vroon et al., 1988**).

Naik and Murthy (1992) reported *Dolichos lablab* and *Dolichos biflorus* as two new leguminous plants of hosts of soybean mosaic.

Singh and Gupta (1996) reported the natural occurrence of soybean mosaic virus of coffee senna (*Cassia occidentalis*).

2.8 Seed transmission

Gardner and Kendrick (1921) and **Kendrick and Gardner (1924)** reported transmission of SMV through seeds and observed that they led to reduction in yield to an extent of 30-75% in various countries. Up to 40% plants raised from infected seeds were found infected (**Heinze and Kohler, 1940**). Seed transmission of SMV ranging from 2.7 to 76.9 % have been reported by various workers (**Conover, 1948; Johnson and Chamberlain, 1953; Nariani and Pingaley, 1960; Quantz, 1961; Walters, 1963; Koshimizu and**

Iizuka, 1963; Ross, 1963; Kennedy and Cooper, 1967; Van Nieberk and Lombard, 1967; Ross, 1968; Dunleavy *et al.*, 1970 Murayama and Han, 1971; Iizuka, 1973 Porto and Hagedorn 1973, 1975a; Bowers and Goodman, 1979; Goodman *et al.*, 1979; Goodman and Oard, 1980; Lu *et al.*, 1981; Chen *et al.*, 1981; Suteri, 1981 and Anjos *et al.*, 1985).

Effects of combined infection with Bean pod mottle virus on seed transmission of SMV are conflicting; one report suggested that it was increased (**Quiniones *et al.*, 1971**) and another that it was decreased (**Ross, 1969**).

SMV antigen can be detected in the seed testa serologically, the particles are no longer infectious (**Porto and Hagedorn, 1975b; Tu, 1977; Bowers and Goodman, 1979**) also revealed that infectious SMV was recovered from embryos and cotyledons, but not the testae of mature seeds. It was, however, found in the taste of immature seeds (**Porto and Hagedorn, 1975a; Bowers and Goodman, 1979; Tu, 1992**), but non-infectious antigen persisted in testae of mature seeds as shown by serology (**Bowers and Goodman, 1979; Iwai *et al.*, 1985**).

The pathogen was found extensively in the flowers and pods of inoculated plants (**Bowers and Goodman, 1979**) and was transmitted to seeds through both pollen and ovules (**Iizuka, 1973**).

Seed transmission was demonstrated in a field environment by plants developing the disease when grown from infected seeds while

protected from insect-borne inoculums by insect-proof cages. The lack of an over wintering host in soybean production areas of the northern USA further suggested the seed to be the primary inoculums source for the pathogen **(Hill et al., 1980)**. **Tu (1989)** also demonstrated seed to be the primary inoculums source for the pathogen transmission rates as high as 75.6%.

Transmission of SMV can occur from both mottled and non-mottled seeds, although the rate tended to be higher for mottled seeds **(Hill et al., 1980; Laguna et al., 1987)**, but others found no significant relationship between seed transmission rate and extent of mottling **(Kim et al., 1996)**.

Some one hundred eleven viruses may infect soybean out of which 35 have been encountered on soybean in nature and several of them may occur in mixed infections **(Irwin and Schultz, 1981; Demski and Kuhn, 1989)**.

Zhang et al. (1988) found that the disease can be effectively controlled by using seeds at low or zero transmission rate removal of disease seedling and suitable sowing dates. In plants infected with SMV .resistance to seed coat mottling was positively correlated with soluble protein content in the seed coat and that this feature could be used as biochemical index to identify resistance to seed coat mottling.

Blottenberg and Irwin (1992) observed that mixed cropping and soybean with sorghum could limit seed mottling induced by SMV.

Tu (1992) tested different cultivars. Early infection produced more severe disease symptoms, higher virus titre, greater reduction in plant growth and seed yield and higher per cent of mottled seeds and seed transmission than did late infection. **Almeida et al. (1995)** observed that seed transmission of SMV was different for the different strains Gx and G1 had higher transmission rather than strain G5, rate of mottled seeds ranged from 22.6 to 38.4 % in cultivar Bossier and 65.7 to 89.9 % in cultivar Santa Rosa. The latter shows highest rate of seed transmission. **Almeida (1995)** identified different strains of SMV from Brazil as their influence on seed mottling was observed. They reported that relative virus content measured by indirect- ELISA was highest in plants, kept at lower temperature regime.

There is a higher percentage of seed transmission when infection of SMV occurs at or before flowering compared to infection after flowering (**Ren et al., 1997**).

Usually little seed transmission occurs when plant infection is after flowering. Seed-coat mottling, though often enhanced by infection by SMV, is not directly caused by the virus and there is no correlation between the extent of seed-coat mottling and the rate of seed transmission of the virus

Symptoms on seed appear pigments spread in regular streaks across the seed coat resulting in conditions which have been commonly referred to as “Mottling”. It was first described by the

Woodworth and Cole (1924). Black and brown streaks were formed on yellow to green color seed coats.

When the soybean plant was inoculated with the virus, it leads to with mottled seed coat. (**Koshimizu and Iizuka, 1963; Cooper and Kennedy, 1966; Van Nierkerk and Lombard, 1967; Kennedy, and Copper, 1967; Wilcox and Laviolette, 1968; Ross, 1963, 1968; Murayama and Han, 1971 and Lu *et al.*, 1981).**

The percentages of seed coat mottling in eight soybean lines evaluated at harvest in inoculated plants with both bean pod mottle virus (BPMV), and soybean mosaic virus (SMV), during growing season, inside an insect-proof cage in the field, indicate that plants infected with BPMV and SMV, alone or in combination, produced seed coat mottling but no mottled seed (**Hobbs *et al.*, 2003).**

According to **Patil and Byadgi (2005)** the soybean mosaic virus (SMV) was readily sap transmissible to test plants (soybean cv. JS-335) and produced systemic disease symptoms.

Domier *et al.* (2007) also reported seed-borne infection as the primary sources of inoculum for SMV.

2.10 Insect Transmission

Soybean mosaic virus is transmitted efficiently by various species of aphids in a non persistent manner .SMV was known to be transmitted by several species of aphids like *Dorsalis (Aphis) fragulae*,

D. rhamni, *D. fabae*, *Microsiphum solanifoli*, *M.pisi*, *Myzus oronatus*, *Neomyzus circumflexus*. *Myzus persicae* and *Aulacorhynchus pseudosolani* (Heinze and Kohler, 1940; Conover, 1948; Baldacci, 1949; and Ling, 1951).

Nariani and Pingaley (1960) reported *Aphis craccivora*, *Myzus persicae*, *Lipaphis erysimi* and *Aphis gossypii* as vectors of soybean mosaic virus. *Megoura viciae*, *Dactynotus sonchi*, *Microsiphum rosae* and *Aphis craccivora* were reported to be the vector of SMV by De Vansconcelos (1964). *Myzus persicae* was reported as an aphid vector of soybean mosaic virus by Galvez, 1963; Murayama and Han, 1971 and Anjos *et al.* 1985. Transmissibility of isolates of the pathogen has been shown to differ between vector species (Lucas and Hill, 1980). Global transfer of the virus and initial introduction in a certain region prior to epidemic development may be in alien commercial seed or germplasm (Cho and Goodman, 1979; Irwin and Schultz, 1981).

Halbert *et al.* (1981) reported *Aphis craccivora*, *Microsiphum euphorbiae*, *Myzus persicae*, *Rhopalosiphum maidis* and *Rhopalosiphum padi* while, Chen *et al.* (1981) reported *Myzus persicae*, *Aphis gossypii*, *Aphis glycines*, *Aphis craccivora* and *Rhopalosiphum pseudobrassicae* as vector of virus.

Halbert and Irwin (1981) have investigated the use of ground cover manipulation to manipulate the spread of soybean mosaic virus.

SMV is transmitted non-specifically by aphids in the non-persistent stylet-borne manner. Soybean has not been reported to be colonized by aphids to any extent. In central Illinois, USA, more than 60 aphid species are transient in soybean fields each year and large numbers of aphids are reported to alight and briefly probe soybean plants (**Halbert et al., 1981**).

Myzus persicae optimally transmitted SMV after acquisition probes of 30-60 seconds; transmission was lower after acquisition probes of 15 seconds and access times of 15 minutes or more (**Schultz et al., 1983**). **Cho et al. (1984)** reported *Myzus persicae*, *Aphis spiraecola* (*A. citricola*), *Aphis glycine*, *A. gossypii*, *Lipaphis erysimi*, *Macrosiphum euphorbiae* and *Aulacorthum solani* as vectors of soybean mosaic virus.

The potential for virus spread by aphids is determined by the availability of the aphids, the time they are present during crop development, their population density, activity and vector efficiency. SMV is transmitted non-persistently in the field by a large number of aphid species and extensive studies have been made on the epidemiology of the disease in relation to aphid flights (**Schultz et al., 1985**).

Spread of SMV is by several aphid species (mostly transient alatae), but only a few species are important vectors of the virus (**Ruesink and Irwin, 1986**).

Zhang et al. (1988) established the factors for epidemics of SMV and integrated measures for its control. They calculated that increased rates of seed transmission was positively correlated with the epidemics and that the disease could be effectively controlled by using seed of low or zero transmission rates and by suitable sowing dates to prevent infestation of alate vectors during flowering of crops.

Li and Pu (1991) reported that's epidemics of SMV were closely related to the immigration time of aphid vectors .When immigration occurred before flowering, the SMV incidence was high but when immigration occurred after flowering the incidence was low.

Naik and Murthy (1992) reported that SMV was transmitted by two species of aphids (*Aphis craccivora* and *Myzus persicae*).

Quimio and Calilung (1993) found that out of eighteen aphid species found in soybean field, seven were responsible for transmission. These are *Aphis glycine*, *A. gossypii*, *T. nigribdominalis*, *A. craccivora*, *Melanaphis sacchari*, *R. maidis* and *S. spiraeicola*.

SMV may also affect the population density and behavior of vector aphids. *Rhopalosiphum maidis* was found to leave SMV-infected soybean plants sooner after probing than healthy plants, which increases the probability of successfully transmitting the virus to other plants (**Fereres et al., 1999**). For SMV such influx from even farther away cannot be excluded (**Irwin, 1999**).

SMV infection may also adversely affect the biology of aphid predators such as *Orius insidiosus* that facultatively feeds directly on soybean. Via the predator, SMV may increase transmission of the virus by aphids (**Armer *et al.*, 1999**).

It was established by **Ren *et al.* (2000)** that increase in pubescence density reduced the incidence level of SMV infection. Extra dense pubescence provided resistance to SMV by both reducing the maximum incidence of infection and delaying the time at which maximum SMV spread occurs.

Kim *et al.* (2000) studied the seasonal occurrence of aphids and reported that there are two peaks in aphid population (late June and mid August). They suggested that virus spread was strongly correlated with increased colonization of aphids.

Madden *et al.* (2000) have developed an approach to predict the yield loss of crops in relation to infection by systemic disease. They have applied this model to SMV and analyzed data on soybean yield loss in relation to infection by SMV.

Wang and Ghabrial (2002) found that the soybean aphid (*Aphis glycines*) was a poor vector (0.83% transmission) when the aphids were allowed overnight acquisition feed on Soybean mosaic virus (SMV)-infected soybean leaves. However, *A. glycines* was shown to be a very efficient vector (34.72% transmission) when individual aphids were allowed a 1-min acquisition probe on the same infected leaves used for the feeding treatment.

Burrows et al. (2005) found that the soybean aphid, *Aphis glycines*, causes yield loss and transmits soybean mosaic virus (SMV) in soybean (*Glycine max*). *A. glycines* transmitted the potyviruses Bean yellow mosaic virus (BYMV) and Soybean mosaic virus from soybean to soybean more efficiently than *Myzus persicae* (**Wang et al., 2006**).

2.11 Purification

Soybean mosaic virus was partially purified by **Galvez (1963)** by using a combination of rate and equilibrium zonal centrifugation. **Ross (1967)** purified the virus produced antiserum. The procedure involved grinding the tissue in 0.5 M sodium citrate buffer, precipitation of normal plant proteins with n-butanol, two differential centrifugation cycles and density gradient centrifugation, pelleted virus was suspended in 0.1 M Borate buffer. By using **Steere (1956)** method with slight modification, SMV was purified by **Quinions and Dunleavy (1970)**. **Han and Murayama (1971)** purified the virus by using a modification of Ross's method. Later **Soong and Milbrath (1980)** **Anjos et al. (1985)** and **Pin kevich and Krylov (1997)** have purified the virus by using different methods. **Gough and Shukla (1981)** developed a method for purification of SMV. **Vance and Beachy (1984)** purified SMV using Cscl (cesium chloride) centrifugation. **El-Ahdal (1996)** developed a modified steeres method for purification of SMV using chloroform butanol fallowed by PEG precipitation and ultracentrifugation.

2.12 Serology

Quantz (1961) reported that SMV is serologically related to bean common mosaic virus and bean yellow mosaic virus. **Quinions and Dunleavy (1970)** reported that antisera produced against SMV-M and SMV-NC isolates had zero titre after absorption with their homologous antigens. Antiserum titre was 250 after absorption with their heterologous antigens. The homologous titre was 2048.

Murayama and Han (1971) reported that the virus reacted positively with antiserum of SMV isolated SV-15 in interface precipitin test. Antisera with low titres of 1:64 and 1:128 were obtained by virus precipitation at IEP (**Gerdeichuk and Sokolova, 1972**).

ELISA techniques has been used for the detection of SMV by (**Lister, 1978; Chen *et al.*, 1982; Moore *et al.*, 1982; and Diaco *et al.*, 1985**). **Chen *et al.*, 1982** have used polysterene beads, while **Diaco *et al.* (1985)** have used monoantibodies based biotin-avidin in ELISA for the detection of SMV.

Ma *et al.* (1982) purified SMV from infected leaf tissue by a procedure involving precipitation with PEG (mol. wt. 6000), differential centrifugation and sucrose density gradient centrifugation. The virus was detected by ELISA at a titer of 1:2208 in seed and 1:1536 in leaf sap, compared with the sensitivity of the double diffusion test of only 1:28.

Properties of SMV and its isolated protein have been identified by **Hill *et al.* (1984)**. These workers have demonstrated the

translation of SMV, RNA *in vitro* in wheat germplasm and reticulocyte cell free translation system and have identified viral encoded proteins as an approach to determine the translation strategy in vitro.

According to **Anjos *et al.* (1985)** the antiserum has a homologous titre of 1:1024 in micro precipitation tests.

Khetarpal *et al.* (1992) using DAS-ELISA concluded that both mottled and unmottled seed could carry SMV and the frequency of detection even in unmottled seeds was very high. However, the seedling infection (transmission) was detected in seeds that had infected embryo.

Almeida (1995) identified different strain of SMV from Brazil and their influence on seed mottling. He reported that the relating virus content measured by Indirect-ELISA was higher in plants kept at lowest temperature regime. **Almeida *et al.* (1995)** used Indirect – ELISA to confirm the occurrence of a new strain of SMV, designated G5 in Brazil.

Dong *et al.* (1997) used Indirect-ELISA to detect the percentage of infected embryos of F1 hybrids in four crosses.

Jayram *et al.* (1998) raised a monoclonal antibody against a synthetic decapeptide containing the DAG tripeptide (Aspartate- Alanine- Glycine). This reacted with isolates of SMV, as well as several other potyvirus. **Jain *et al.* (2000)** established by DAC-ELISA that Ps TV is serologically related to SMV.

According to **Sun *et al.* (2008)** Soybean Mosaic Virus (SMV), could be detected using double antibody sandwich immunosorbent assay (DAS-ELISA) in soybean cultivars.

2.13 Monitoring of aphid population

Mohanti and Basu (1991) used golden yellow water pan traps for monitoring aerial population of white flies.

Fereres *et al.* (1999) found that *Myzus persicae* (Sulzer) and *Rhopalosiphum maidis* (Fitch) expressed no preference for landing on leaves infected with soybean mosaic virus (SMV) or on chlorophyll-deficient soybean leaves. Furthermore, neither aphid species showed odor-mediated attraction to leaf extracts of SMV-infected leaves. Also, *R. maidis* remained longer on uninfected than on SMV-infected soybean plants before taking flight, although *M. persicae* exhibited no preference.

According to **Hodgson, *et al.* (2005)** Soybean aphid, *Aphis glycines matsumura*, can be an economically important pest in soybean, and evaluated the use of non attractive pan traps to estimate soybean aphid densities in commercial soybean field.

2.14 Effect of soybean mosaic virus on growth and yield components of soybean

Infected plants and their organs are usually smaller in size and weight; plants are often stunted. Reduction in fresh weight of 60-day-old seedlings ranged from 35-73% depending on the virus strain **(Tu, 1989)**. In 10 soybean cv. plant height was reduced by 4.0-16.9%,

number of pods per plant by 8.4-33.4%, pod length by 10-25.6%, number of seeds per pod by 6.4-15.2%, and total seed weight per plant by 6.6-17.8% (**Haque et al., 1993**).

Mohammad et al. (2003) reported that soybean mosaic virus (isolates S1 and P1) caused significant reduction in plant height, number of pods per plant, pod length, number of grains per pod and 100-grain weight in 8 mechanically-inoculated soybean cultivars.

2.15 Screening for Resistance

Ross (1969) reported a few cultivars resistant to SMV .Reaction of thirty soybean lines against SMV were studied by **Sundaresh et al., (1984), Suteri (1986)** screened about 24 cultivars against mild and severe strains of SMV .They have reported resistance in few cultivars.

Akhtar et al. (1992) screened twelve varieties for resistance to SMV .Four varieties (Crow ford, Cico, Zane and 80-B-4007) were found resistant to the virus.

Zhung et al. (1996) made a study of resistance in perennial wild soybean against SMV. Three species (*G. canescens*, *G. clandestine* and *G. tomentella*) showed high levels of resistance to SMV.

Four varieties of soybean mosaic potyvirus resistant soybeans were crossed and F1, F2 and BC, progenies were evaluated. They were found to be highly resistant germplasm, suitable for breeding SMV resistant varieties (**Luan and Luan, 1997**).

Ren et al. (2000) studied six isolines of cultivar Clark and concluded that increased correlated positively with resistance against SMV.

Hayes and Maroof (2000) developed four markers closely linked to RSV1 (Gene) responsible for resistance against SMV by AFLP approach.

Some 348 soybean accessions were screened for resistance to soybean mosaic virus (SMV) by inoculation with SMV3, a strongly virulent strain from northeast China. The results showed that 113 accessions were highly resistant, 113 were moderately resistant and 122 were susceptible (**Zheng et al., 2000**).

A new strain (SMV 95-1) of soybean mosaic virus (SMV) was found infecting resistant cultivars of soybeans (*Glycine max*), causing dwarfing and systemic necrosis. In a screening test conducted with strains SMV 95-1 and SMV 95-2, the cultivars Embrapa 60, Embrapa 61, Embrapa 62, Embrapa 66, Embrapa 133, Embrapa 134, Embrapa 135 and Embrapa 136 were found resistant to both strains (**Almeida et al., 2001**).

Zheng et al. (2003) found that soybean line 'ICGR95-5383', (*Glycine max* (L.) Merr.) a newly released germplasm from China, is resistant (R) to soybean mosaic virus (SMV). In order to investigate the inheritance of SMV resistance ICGR95-5383 was crossed to the susceptible (S) cultivars 'HB 1', 'Tiefeng21', 'Amsoy', and 'Williams'.

Yu et al. (2005) Soybean mosaic virus (SMV) is a prevalent virus that infects soybean [*Glycine max* (L.) Merr.] crops world-wide. The Rsv3 gene of soybean confers resistance to three (G5-G7) of the most virulent strains of 7 defined SW strains, which were grouped based on virus-reaction phenotypes of a spectrum of soybean cultivars mainly carrying Psv1 alleles.

Shi et al. (2008) reported Soybean mosaic virus (SMV) to be highly destructive viral disease. Three loci with 12 alleles conferring SMV resistance have been identified in soybean [*Glycine max* (L.) Merr.

Materials

and

Methods

The details of materials used and procedures followed to carry out the present investigation on the “Soybean Mosaic Virus (SMV): Methods for detection and mechanism of transmission in Soybean (*Glycine max* L. Merrill)” are described below.

3.1 Experimental Site

Pantnagar falls in the *tarai* belt, adjoining the foot hills of Shivalik range of Himalayas with an altitude of 343.84 m above mean sea level. It is located at 29°N latitude and 79.3°E longitude. The climate of Pantnagar is humid subtropical. The field trial was conducted in plot number C/6 of the N.E. Borlaug, Crop Research Centre of the G. B. Pant University of Agriculture and Technology, Pantnagar during *kharif* of 2007 and 2008, while glass house experiments were carried out in glasshouses of Department of Plant Pathology. Experiments related to ELISA, virus purification and Electron Microscopy were conducted at Plant Quarantine Division, National Bureau of Plant Genetic Resources (NBPGR), New Delhi and other experimental study of SMV was conducted at Pantnagar Centre for Plant Genetic Resources (PCPGR), Laboratory, G.B. Pant University of Agriculture & Technology, Pantnagar.

3.2 Experimental Materials

3.2.1 Soil

Fallow soil collected from Crop Research Centre (CRC) and homogenized with farm yard manure and sand in 4:1:1 ratio was used for raising the plants in pots under glass house conditions.

3.2.2 Pots

For growing on test, seedlings were raised in six inches plastic pots.

3.2.3 Seeds

Different cultivars /germplasm lines of soybean grown at CRC farms was obtained from the All India Coordinated Research Project" on soybean. Soybean variety P K-416 was used as a test variety through out the studies.

3.2.4 Fungicidal Seed Treatment

Prior to planting, Thiram containing 75 % active ingredient was used @ 2.5 gm/kg seed as dry seed treatment.

3.2.5 Insecticide Application

Carbofuron 3 G was used as soil application and metasystox (@ 0.1 %) as spray to keep the glass house free from insects which might enter accidentally.

3.2.6 Cages

Insect proof cages made up of nylon mosquito net were used for raising seedlings both under glass house and field conditions.

3.3 Isolation and maintenance of virus culture

Soybean crop cultivars grown in the fields of virus (SMV) were brought to the laboratory. The crop was surveyed on different places of Uttarakhand and infected leaves of PK-416 plant, showing symptoms of SMV, were washed with tap water and dried in the folds of blotting paper. Later, the leaves were macerated in a sterile pestle and mortar by adding equal amount (w/v) of distilled water and filtered through a double layer of muslin cloth. The filtrate was used for mechanical inoculations. The inoculations were done by rubbing the forefinger dipped in standard extract on two week old seedlings of soybean cv. PK-416 raised in 6" pots containing well manure soil. Carborundum (600 mesh) was used as an abrasive. After inoculations the test plants were immediately rinsed with tap water. Single local lesion isolations were made from PK-416. The virus culture thus obtained was maintained on *Phaseolus vulgaris* L., cv. Chitra during winter months and on *Glycine max* (L.) Merrill cvs. Bragg and PK-748 and *Vigna sinensis* L. var. Pusa Phalguni during summer months. This culture was used throughout the course of investigations. Periodical electron microscopic examinations were made from the virus culture.

3.4 Symptomatology and host range

Various plant species belonging to different families viz., Amaranthaceae, Chenopodiaceae, Cruciferae, Cucurbitaceae, Leguminosae, Malvaceae and Solanaceae were tested for their susceptibility to the virus by mechanical inoculation. The test plants were raised in 6" pots containing well manure soil. Two week old seedlings were inoculated with the standard extract of the virus. The test plants were kept under observation for 45 days. Symptoms were recorded at various intervals depending on temperature fluctuations prevailing during summer and winter months. Back inoculation tests were done on soybean cv. PK-416 from various test plant species showing no visible symptoms.

3.5 Transmission

3.5.1 Mechanical transmission

For all the mechanical inoculations standard extract of the virus prepared in 0.1 M phosphate buffer pH 6.5 was used, unless otherwise mentioned. Carborundum (600 mesh) was used as abrasive. Reciprocal mechanical cross inoculation with the virus using standard extract were done among two systemic hosts on soybean.

3.5.2 Insect transmission

The following two experiments were conducted in the insect transmission studies.

1. To establish different aphid species as vectors of the virus, and
2. To determine the relative efficacy of the five species of aphids viz., *A. gossypii* Glov., *Lipaphis erysimi* Kalt, *Myzus persicae*, *Aphis fabae* and *Aphis craccivora* Sulz.

The aphid species viz., *A. gossypii*, and *M. persicae* from *Capsicum annuum* L, *L. erysimi*, from *Brassica campestris* var. PT-30 and *Aphis fabae* and *Aphis craccivora* were collected from healthy plant species of *P. vulgaris* var. Chitra, respectively.

The aphid species were identified by the entomologist and each aphid culture was raised from a single adult aphid on a suitable host separately in the insectary. For establishing different species of aphids as vectors of the virus, "tapping" aphid inoculation technique (**Dhingra and Chenulu, 1981**) was followed. A minimum of 15 to 25 aphids were used with pre-acquisition fasting of 2 hours, acquisition feeding of 1 to 2 minutes and inoculation access period of 24 hours in each case per test plant inoculation. For the determination of relative efficacy of four aphid species, conventional method of brush inoculation was used (**Noordam, 1973**). A fixed number of 15 aphids of same age were used with pre-requisition fasting of 2 hours, acquisition feeding of 5 minute and inoculation access period of 12 hours in each case per test plant inoculation. Soybean cv. PK- 416 was used as test plant.

3.5.3 Seed transmission

Seed transmission of the virus was determined in *Glycine max*. The test plants were inoculated mechanically with the virus. In *G. max*

healthy and diseased seeds of ten important soybean cultivars were collected separately in 2006. In *G. max*, 200 seeds were sown in 6"pots containing well manure soil @ 10 seed per pot kept in under insect proof conditions. The plants thus raised were observed for 45 days. The pots were observed upto 30 days for seed germination. In all the cases percentage of seed emergence and diseased plants was recorded. The experiment was performed in two year trial 2007-08 and 2008-09 cropping season, respectively.

3.6 Stability of the virus in crude sap

For the determination of properties viz., Thermal Inactivation - Point (TIP), Longevity in vitro (LIV) were followed. Dilution End Point (DEP) and the methods described by **Hill (1984)**.

3.6.1 Determination of TIP (Thermal inactivation point)

The TIP was determined by heating 2 ml aliquots of standard extract of the virus for 10 minutes at temperature ranging from 50° to 70°C with 5°C interval each. The heated sap was immediately cooled in running tap water. Mechanical inoculations were done starting with sample exposed to highest temperature.

3.6.2 Determination of DEP (Dilution end point)

For determination of DEP, dilutions of 10⁻¹ to 10⁻⁵ were made with crude sap extracted from virus infected plants using 0.1M phosphate buffer pH 6.5. Mechanical inoculations were done for each dilution starting with the sample having highest dilution.

3.6.3 Determination of LIV (Longevity *in vitro*)

For determination of LIV, the standard extract of the virus was prepared and divided into two lots, one kept at room temperature (25-30°C) and the other at refrigerator temperature (5-7°C). Each lot was further divided into eight aliquots each containing 2 ml of standard extract. Aliquots of standard extract were inoculated at one day interval in both the cases. Diseased leaves in case of soybean were harvested and cut into small pieces. These pieces were wrapped in muslin cloth and then desiccated 5-7°C using anhydrous calcium chloride. After complete desiccation the material was distributed into ten lots each containing 1 g of desiccated leaf pieces. These were then packed in butter paper and kept under desiccated conditions in an air tight conical flask at 5-7°C. The tests for virus infectivity were done at three day interval using standard extract of one lot at a time. The young two week old seedlings of soybean cv. PK- 416 were used as test plants and suitable controls were kept in each case.

3.7 Purification

A modification of **Ross (1967)** method was developed and standardized for purification of the virus. The method is depicted through a flow diagram (**Figure 1**). For the purpose of purification; only the young leaf showing severe symptoms from inoculated plants of soybean PK-416 was used. The leaves were harvested 4-5 weeks after inoculation. The virus infectivity was tested at the various stages of purification.

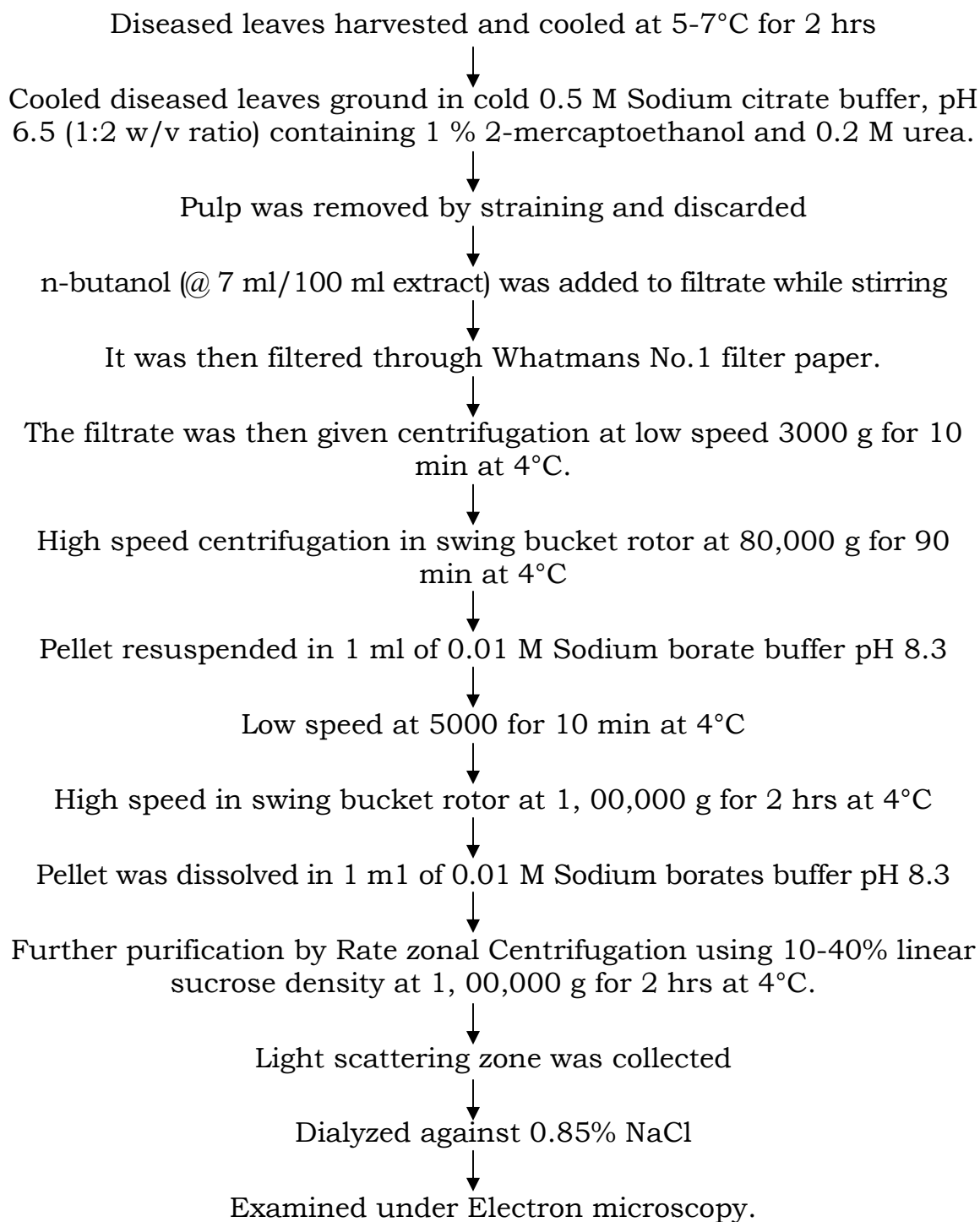


Figure 1 : Flow diagram showing various steps in purification of SMV

3.8 Electron microscopy

For electron microscopy, virus particles in clarified sap of diseased leaves and in purified preparations were used. For tests with diseased leaves, identical leaf pieces were punched with a cork borer. Sap was extracted in five drops of 0.1 M phosphate buffer pH 6.5, with the help of a mini drill. The extract was centrifuged at 7000 rpm for 10 minutes. About 10 μ l drop of the clarified sap was put on copper grid coated earlier with formvar and carbon. After 30 seconds, excess fluid was sucked off with filter paper. Staining was done by rinsing with 8-10 drops of 2% aqueous Uranyl Acetate (UA) and excess stain was drained off. The grid was dried and observed under EOL-100-CX-II electron microscope. For examining purified virus preparation, 10 μ l of suitably diluted preparations were placed on the grids and incubated for 30 seconds. Staining was done with 2% UA. Excess stain was sucked off with filter paper and grids were examined under EOL-100-CX-II electron microscope.

3.9 Spectrophotometric studies

Ultraviolet absorption spectrum of purified virus preparation was determined using 0.01 M sodium borate buffer, pH 8.3 as reference blank in Bausch and Lomb UV spectrophotometer with 1 cm path length. Ultraviolet absorption values at different wavelengths from 220 to 300 nm with 10 nm interval were determined and ratio of A₂₆₀/A₂₈₀ was calculated.

3.10 Enzyme Linked Immunosorbent Assay (ELISA)

Basic ELISA method described by **Clark and Adams (1977)** was followed. Composition of buffers and reagents used are given below:

1. Saturated neutral ammonium sulphate solution

Dissolved 26 g of ammonium sulphate in 40 ml of dissolved water, adjusted the pH to 7.0 with 10 N NaOH and made upto 50 ml with distilled water.

2. Phosphate buffer saline (PHS) pH 7.4

NaCl	8.0 g
KH ₂ PO ₄	0.2 g
Na ₂ HPO ₄ .12H ₂ O	2.9 g
KCl	0.2 g
Distilled water	1000 ml

3. Coating buffer (pH 9.2)

Na ₂ CO ₃	1.59g
NaHCO ₃	2.93g
NaN ₃	0.28g
Distilled water to	1000 ml

4. Washing buffer

IX PBS	100ml
Tween 20	0.5ml

5. Antigen extraction buffer

PBS-T containing 0.2%, ovalbumin and 2% soluble polyvinylpyrrolidone (MW 44,000).

6. Blocking solution

1 % Bovine serum albumin.

7. Conjugate buffer

Same as antigen extraction buffer,

8. Substrate buffer (pH 9.8)

Diethanolamine	9.7 ml
Distilled water	80 ml
NaN ₃	0.02g

Adjusted the pH to 9.8 with concentrated hydrochloric acid and volume made to 100 ml with distilled water.

3.10.1 Preparation of gamma globulin

SMV polyclonal antibody was purchased catalog No. SRA 33300 with format ELISA reagent set from Agdia chemicals as SMV infected plant sample provided and all procedure was done manually. Procedure given by **Hill (1984)** was followed for the preparation of gamma globulin. One ml of antiserum was mixed with 9 ml of distilled water to which 10 ml of neutral saturated ammonium sulphate solution was added, mixed thoroughly and kept for 60 minute at room temperature. The precipitate formed was separated by low speed centrifugation at 5000 g for 10 minutes. The precipitate (IgG) was dissolved in 2 ml of half strength phosphate buffer saline (PBS), pH 7.4 and dialysed against 500 ml of half strength phosphate buffer saline (PBS) at 4°C. A total of three such dialyses were given in 24 hours. The optical density of gamma globulin

(IgG) were measured at 280 nm using half strength PBS as reference blank in Bausch and Lomb UV spectrophotometer. The strength of IgG was adjusted approximately to 1.4 (1 mg/ml) OD. The IgG were divided into half ml aliquots and stored at -18°C in siliconized vials after adding 0.1 % sodium azide.

3.10.2 Preparation of enzyme conjugate

The one step gluteraldehyde method was used for the preparation of antibody alkaline phosphatase conjugate. 0.2 ml (equivalent to 2 mg or 5000 units) of enzyme solution was added to 2 ml gamma globulin (2 mg). Later, freshly diluted 2.5% gluteraldehyde solution was added to make final concentration of 0.05%. This was incubated for 4 hours at room temperature during which time a very faint yellowish brown colour developed. After conjugation, gluteraldehyde was removed by dialysis against 500 ml PBS with. Three changes at 4°C. After dialysis, bovine-serum albumin (BSA) was added to the conjugate to a concentration of 5 mg/ ml and stored at 4°C.

3.10.3 Direct Antigen Coated ELISA (DAC-ELISA) test procedure

DAC-ELISA was done on polystyrene plate (Corning) using the protocol of **Clark and Bar Joseph (1984)**.

1. The plates were coated with 200 µl of crude antigen samples and incubated for 1 h at 37°C. Crude antigen preparation included grinding tissue in coating buffer containing 2%

polyvinyl pyrrolidone (PVP) (MW 44,000) at 1:10 (w / v) ratio. The homogenate was incubated at room temperature for 1 h or overnight at 4°C before centrifuging at 5000 g for 10 min. Pellets were discarded and supernatant obtained was used for coating directly on wells of micro titre plate.

2. The plates were washed and blocking solution was added (200 µl per well) and incubated for 1 h at 37°C.
3. After washing the plate, diluted unfractionated antisera and the plates were incubated at 37°C for 2 h. Each antiserum was diluted at 1:1000 using antibody buffer.
4. The plates were washed and enzyme conjugate (antirabbit IgG alkaline phosphatase; Sigma Chemical Co.) was added (200 µl per well) at 1:2000 dilution. The plates were incubated at 37°C for 2 h.
5. After final washing 200 µl of substrate (p-nitrophenyl phosphate, 0.6 mg/ml) was added and allowed for colour development at room temperature.
6. Intensity of colour was read at 405 nm using a microplate reader.

3.11 Location of seedborne inoculum of soybean mosaic virus in soybean seed

3.11.1 Association of SMV with different flower and seed parts

The study was carried out using both the indicator inoculation test and serological method i.e. enzyme linked immunosorbent assay (ELISA) as described below.

3.11.1.1 Indicator inoculation test

The presence of soybean mosaic virus associated with different parts of flower and seed was studied by preparing the extracts of different parts for assays on soybean plants under glass house conditions. Various steps involved in this method are described below.

3.11.1.2 Raising of plants

Insect proof glass house was used for raising soybean plants. The glass house was sprayed with systemic insecticides routinely at an interval of 10-15 days to keep it free from insects which might enter accidentally. All experimental test plants were raised in a soil: farmyard manure: sand mixture (4:1:1) in plastic pots of 6 inch. The seeds were treated with thiram (@ 0.25 %) before planting. After sowing pots were transferred to insect proof nylon cages and confined there till the experiment was over. The plants were watered regularly to maintain moisture for growth.

3.11.1.3 Preparation of virus inoculum

3.11.1.4 Disinfection

Disinfection of glasswares, mortar, pestle etc. was done by rinsing them with 10 % trisodium phosphate solution and with tap water. After inoculation with infective sap, hands were washed with trisodium phosphate solution or soap. This was followed by washing with tap water. This measure was followed in all the experiments where sap was used as a medium of inoculation.

3.11.1.5 Preparation of potassium phosphate buffer

Potassium phosphate buffer (0.1 M) solution of pH 7.6 was prepared by adding 1 part of 0.1 M $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ solution to 9 parts of 0.1 M KH_2PO_4 solution. Above two solutions were mixed together and shaken thoroughly.

3.11.1.6 Recording the pH

The pH of the phosphate buffer solution was adjusted to 7.6 by using pH meter with glass electrodes.

3.10.1.7 Preparation of extract

(a) Flower parts

Fifty unopened flower buds were collected from systemically soybean mosaic virus infected plants of variety PK-416. These flower buds were separated in to five parts i.e. epicalyx, calyx, corolla, androecium and gynoecium with the help of forceps, needle and blade. The forceps, needle and blade were thoroughly disinfected with 10 % solution of trisodium phosphate (Na_3PO_4) followed by rinsing in soap water and finally in tap water after each handling of flower parts. These parts were separately washed in running tap water for 20 min., then in sterilized water and were kept frozen for 15 min. to facilitate virus extraction. Later each component was separately grounded in a mortar with a pestle in 0.1 M phosphate buffer (pH 7.6). After a thorough grinding, the extract was stained through cheese cloth and separately kept in a test tube. These extracts were used for inoculations.

(b) Seed parts

Fifty seeds obtained from systemically soybean mosaic virus (SMV) infected plants of PK-416 were first soaked in tap water. These were then soaked in distilled water for 24 hours, after which each seed was separated into three parts i.e. seed coat, cotyledon and embryo with the help of needle, forceps and blade. These parts were separately washed in distilled water to avoid contamination before extraction. The extracts of the three parts were prepared in the same way as the extracts of flower. The extracts from different seed parts were then used for inoculations.

3.11.1.8 Inoculation process

Inoculation was carried out by gently rubbing 600 mesh carborundum dusted leaves with the help of fore fingers dipped in the inoculum. Primary leaves (cotyledonary leaves) before the emergence of true leaves were inoculated. The inoculated leaves were immediately rinsed with gentle stream of tap water with the help of plastic wash bottle. The leaves on the control plants were inoculated with only potassium phosphate buffer.

3.11.1.9 Recording observations

The inoculated plants were observed daily and those showing typical leaf crinkling were recorded.

3.11.2 Serological test using ELISA technique

The presence of seedborne inoculum of soybean mosaic virus in different categories of abnormal seeds such as small shriveled, large

shriveled, oversized, undersized, off coloured seeds and mottled seed, in various flower parts viz. epicalyx, calyx, corolla, androecium, gynoecium and seed parts as seed coat, cotyledons and embryo was also confirmed by DAC- ELISA method discussed in section no 3.10 to 3.10.3 of material and methods.

3.12 Monitoring aerial populations of aphid (*Myzus persicae*) vector

For monitoring the aphid populations traps of different colour and shaped were used.

3.12.1 Coloured sticky traps

During the months of July–December, 2007 and 2008 three different coloured sticky traps were used for monitoring aerial populations of aphids. The traps consisted of transparent pieces of plastic plates (20× 15 cm²) **Plate 1**. The traps were painted with bright golden yellow, pale yellow and brown yellow synthetic camel paints. To make surface, sticky hyaline coloured vaseline was used. These traps (three plates of each colour) were tied up with bamboo stand at 75 cm from ground level in vertical position in soybean field. Daily counts of trapped aphid were recorded. After counting, the traps were cleaned by a cotton pad and the plates were coated again with thin layer of Vaseline. Population counts of aphid every week on different coloured traps were plotted on graph to compare the effectiveness of these three coloured traps.



Bright golden yellow coloured plastic plate trap



Brown yellow coloured plastic plate trap



Pale yellow coloured plastic plate trap

Plate 1: Coloured plastic plate traps

3.12.2 Shape of the traps

Two different shaped traps were used to choose the best one for monitoring aerial population of aphid. These traps were wrapped with bright golden yellow cellophane paper were of the following shapes.

3.12.2.1 Cylindrical traps

The thin bright golden yellow cellophane paper wrapped around a cylinder with the help of fevicol. The cellophane paper was changed at monthly interval due to the fed up of the colour which decrease the counts of aphid. The traps measuring 339.4 cm² kept at a height of 75 cm above the ground level with the help of bamboo sticks.

3.12.2.2 Lamp shade type traps

Traps consisted of bright golden yellow cellophane paper wrapped around a soft aluminium wire with the help of fevicol measuring 440 cm². The traps were hooked with bamboo stake at height of 75 cm above ground level **Plate 2**.

Three replications of each kind of trap kept in the soybean field. The surfaces of the traps were coated with a thin layer of hyaline vaseline. After counting the numbers of trapped aphid traps were cleaned with cotton pad every day. The observations were made for two consecutive years in 2007 and 2008. For uniformity, the population of trapped aphid was counted in 300 cm² area of each type of traps. Fortnight average of aphid populations were plotted on graph and the efficiency of various traps were evaluated.



Bright golden yellow coloured lamp shape trap



Bright golden yellow coloured cylindrical shape trap



Collection of insects from trap

Plate 2: Different shape type of traps

3.12.3 Seasonal variations in the aerial populations of aphid

Average population of aphid trapped every fortnights during 2007 and 2008 was plotted on graph and metrological data [average maximum temperature (°C), Average minimum temperature (°C), average relative humidity (%) and total rainfall (mm)] were superimposed to ascertain the effect of weather conditions on aerial populations of aphid.

3.13 Yield losses due to seedborne inoculum of soybean mosaic virus (SMV)

3.13.1 Effect of plant age at infection with SMV on yield contributing factors and yield of soybean

To study the effect of age of soybean plants at the time of infection with SMV and its effect on yield contributing factors and yield of soybean.

Seeds for experiment on the effect of plant age at the time of infection on subsequent seed transmission of SMV were obtained in the following ways.

Soybean plants of variety PK-416 were raised in 6 inch plastic pots containing soil: farmyard manure: sand mixture (4:1:1 ratio) under glass house conditions (20-35 °C with diffuse light). These plants were then sap inoculated by rubbing 600 mesh carborundum powder dusted primary leaves, by fore fingers dipped in sap prepared from soybean mosaic virus infected leaves in

0.1 M potassium phosphate buffer (pH 7.4) at 15, 25, 35, 45, and 65 days after planting (DAP). The inoculated leaves were immediately rinsed with tap water with the help of plastic wash bottles. Check plants were inoculated with only phosphate buffer. A minimum of 3 replications were used in each treatment.

Observations on the number of plants infected, symptoms at different stages of plant growth, number of pods per plant, pod length, number of seeds per pod, number of seeds per plant, 100 seed weight and yield per plant were recorded in each treatment. The per cent reduction in yield was calculated by following formula (**Nene, 1973**).

$$Q = \frac{A - B}{A} \times 100$$

Q = per cent yield reduction

A = average yield of healthy plants

B = average yield of diseased plant

3.14 Screening for disease resistance

Different cultivars /germplasm lines of soybean were grown CRC farms. They were screened for the presence of SMV. The cultivars/germplasm were obtained from the All India Coordinated Research Project" on soybean. The natural incidence of SMV in the different cultivars was observed during *kharif* of 2007-08 and subsequently in *kharif* of 2008- 2009. The evaluation was done by recording incidence of the disease and the reaction of the cultivar

lines to the virus infection at 15 day interval till maturity of the crop. Disease severity was recorded by using a 0-5 point scale, which had six categories (**Table 4**).

Table 4: Disease rating scale for soybean mosaic virus disease

Disease rating scale	Description
0	Escape in nature/no infection, no visible symptoms, plants apparently healthy, Immune (I)
1	0.1-20.0 % plant infection, very mild mosaic (mild mosaic on few leaves/plants) a few necrotic/chlorotic spots, average mosaic mottling upto 10% of leaf lamina, no stunting of infected plant, Resistant (R).
2	20.1-40.0 % plant infection, moderate mosaic (mosaic on many leaves/plant and vein clearing) average mosaic mottling upto 30% of leaf lamina, average necrosis upto 10% and very little stunting, Tolerant (T).
3	40.1-60.0 % plant infection, severe mosaic (severe mosaic and mild mottling) average mosaic mottling up to 50 %, stunting of plants, Susceptible (S).
4	60.1 -80.0 % plant infection, severe mosaic (severe mosaic and severe mottling) average mosaic mottling up to 70 %, stunting of plants, Moderately Susceptible (MS).
5	80.1-100.0 % plant infection, average mosaic mottling up to 90 %, acute stunting of plants, severe mosaic plus severe mottling plus necrosis occasionally death of plant ,Highly Susceptible (HS).

For screening in the field condition, 2 rows of infected cultivar of soybean PK-416 were planted after each germplasm line. The disease incidence was taken at random cultivars/germplasm line reaction to the virus infection.

Some of the economically important cultivars /germplasm which showed 0 and 1 reaction under field conditions were selected and artificially tested with the virus under glass house conditions for determining sources of resistance.

Cultivars/lines falling under 0 and 1 categories can be used in breeding programmes. Category 2 can be used 0 and 1 cultivars/germplasm are not available.

For all the experiments a two week old plant species were used. Suitable controls were kept in each case. The test plants were raised in 6" pots containing well manure soil in an insect proof glass house at temperature ranging from 25 -35 °C. The test plants were sprayed with metasystox (@ 0.1 %) as spray to keep the glass house at different interval for free from insects which might enter accidentally.

Experimental Results

4.1 Isolation and maintenance of virus culture

The soybean mosaic virus was isolated by sap inoculation on a systemic host, soybean cv. PK- 416 from the diseased leaves grown at N.E. Borlaug, Crop Research Centre G.B.P.U.A. & T., and Pantnagar. Further, mechanical inoculations of this isolate were done on local lesion hosts viz., *Chenopodium amaranticolor* and *Phaseolus vulgaris*. The virus produced distinct dark brown to black, chlorotic local lesions, of 1-2 mm size on *Chenopodium amaranticolor* and dark green patches alternating with light green patches with dishaped leaves and puckered leaves on *Phaseolus vulgaris*. The virus was successfully isolated from a single local lesion produced on *Chenopodium amaranticolor* and from leaves sap in *Phaseolus vulgaris*. Presence of the virus in a local lesion from *Chenopodium amaranticolor* and from leaves sap in *Phaseolus vulgaris* were examined under electron microscope. The pure virus culture, thus obtained was maintained: on *Phaseolus vulgaris* L., cv. Chitra during winter months and on *Glycine max* (L.) Merrill cvs. Bragg and PK-748, and *Vigna sinensis* L. var. Pusa Phalguni during summer months.

4.2 Symptomatology

First symptoms appeared after 15 days of inoculation on the young leaves as dark green mosaic mottling. Later the plant developed systemic symptoms. The leaf margins were wavy and showed curling. The leaves were distinctly puckering along the veins and showed

general necrosis along the margins. The leaves showed reduction in size and were misshaped. The affected plants were severely stunted due to shortened internodes. The affected plants produced fewer pods which were small and flattened. Most of the pods remained unfilled and the rest of the pods contained 2-3 seeds per pod. Seeds were of normal size and mottling was observed only at the hilum region.

4.3 Host range studies

The host range of soybean mosaic potyvirus was studied in 24 members of 7 different families. These included commonly used test plants and agronomically important plant species. The plants were tested for their reaction by mechanical inoculation with SMV. The reactions are given in **Table: 5**.

The results show that eight out of 24 species tested showed susceptibility to SMV. Systemic symptoms were observed on *Glycine max* L., *Phaseolus vulgaris* L., *Phaseolus lathyroides* L., and *Vigna sinensis* L. Local lesions were obtained on inoculation in *Cyamopsis tetragonoloba* (L.) Taub, *Chenopodium album* Red, *C. amaranticolor* Costa and Reyn. While *Dolichos lablab* L. was found to be symptomless carriers of SMV.

4.4 Transmission

4.4.1 Reciprocal mechanical transmission

Reciprocal cross inoculation tests made on *G. max* and *P. vulgaris* for multiplication of virus culture required for purification purpose showed that the virus was reciprocally sap transmissible among these two systemic host species.

Table 5: Reaction of different plant species to mechanical inoculation with SMV

Sr. No.	Plant Species	No. of plants infected/ inoculated	Per-cent plant infection
A	Leguminosae		
1.	² <i>Dolichos lablab</i> L.	4/10	40%
2.	<i>Glycine max</i> L.		
	PK-416	14/20	70%
	Bragg	11/20	55%
	PS-1241	9/20	45%
3.	<i>Phaseolus vulgaris</i> L. var. Chitra	6/10	60%
4.	<i>Phaseolus lathyroides</i> L.	5/10	50%
5.	¹ <i>Cyamopsis tetragonoloba</i> (L.) Taub var. Pusa Naubahar	10/10	100%
6.	<i>Vigna mungo</i> (L.) Hepper var. Pant U-35	0/10	Nil
7.	<i>Vigna radiata</i> (L.) Wilczek var. Pant M-3	0/10	Nil
8.	<i>Pisum sativum</i> L. var. Pant P -5	0/10	Nil
9.	<i>Vigna sinensis</i> L. var. Pusa Phalguni	8/10	80%
10.	<i>Arachis hypogea</i> L. var. Kaushal	0/10	Nil
11.	<i>Cajanus cajan</i> (L.) Milsp var. Amar	0/10	Nil
12.	<i>Cicer arietinum</i> L. var. Pant G-186	0/10	Nil
B.	Amaranthaceae		
13.	<i>Amaranthus spinosus</i> L.	0/05	Nil
C.	Chenopodiaceae		
14.	<i>Chenopodium album</i> Red	1/5	20%
15.	¹ <i>C. amaranticolor</i> Costa and Reyn.	5/5	100%
D.	Solanaceae		
16.	<i>Nicotiana tabacum</i> L. var. NP-15	0/05	Nil
17.	<i>Solanum melongena</i> L. var. Pant Samrat	0/10	Nil
18.	<i>Lycopersicon esculentum</i> L. var. Pant Bahar	0/10	Nil
19.	<i>Solanum nigrum</i> L.	0/05	Nil
20.	<i>Capsicum annuum</i> L. var. Pant C 1	0/05	Nil
E.	Cucurbitaceae		Nil
21.	<i>Cucurbita melo</i> L.	0/05	Nil
22.	<i>Cucurbita pepo</i> L.	0/05	Nil
F.	Cruciferae		Nil
23.	<i>Brassica campestris</i> L., var. Varuna	0/10	Nil
G.	Malvaceae		Nil
24.	<i>Abelmoschus esculentus</i> (L.) Moench	0/05	Nil

1=Local lesion host; 2=Symptomless carrier

4.4.2 Insect Transmission

The different aphid species viz., *Aphis fabae* and *Aphis craccivora* were raised on *P. vulgaris* var. Chitra, *Aphis gossypii* and *Myzus persicae* were raised on *Capsicum annuum* L., and *Lipaphis erysimi*, was raised on *B. campestris* L., var. sarson Prain., respectively were successfully raised in the insectaries.

The results of transmission of SMV by different aphid species are given in **Table 6**. The results shows that 5 species of aphid *Aphis fabae*, *Aphis craccivora*, *Aphis gossypii*, *Myzus persicae* and *Lipaphis erysimi* were all capable of transmitting the SMV. However, maximum per cent transmission of 85% was by *Myzus persicae* while minimum transmission of 15% was by *Aphis fabae*.

Table 6 : Transmission of SMV by different species of aphids

Aphid Species	No. of aphid inoculated/ plant	Total number of plant inoculated	Total number of plant infected	Per cent Transmission
<i>Aphis fabae</i>	15	20	3	15
<i>Aphis craccivora</i>	15	20	13	65
<i>Aphis gossypii</i>	15	20	12	48
<i>Myzus persicae</i>	15	20	17	85
<i>Lipaphis erysimi</i>	15	20	6	30

4.4.2.1 The relative efficacy of different aphid species in transmitting SMV

The results of the efficacy of different aphid species in transmitting the virus are given in **Table 7**. It is clear from **Table 7 and Figure 2** that *M. persicae* transmitted SMV with maximum efficacy of 78.33 per cent followed by *A. craccivora* with 61.66 per cent. The least efficiency in transmitting the virus was shown by *Aphis fabae* at 23.33 per cent. *A. gossypii*, and *Lipaphis erysimi* both showed intermediate range of efficacy at 51.66 and 26.66 per cent, respectively.

Table 7: Determination of relative efficacy of different aphid species in transmission of SMV

Aphid species	Per-cent Transmission			Mean
	Trial I	Trial II	Trial III	
<i>Aphis fabae</i>	20	25	25	23.33
<i>Aphis craccivora</i>	65	70	50	61.66
<i>Aphis gossypii</i>	50	55	50	51.66
<i>Myzus persicae</i>	85	70	80	78.33
<i>Lipaphis erysimi</i>	35	25	20	26.66

Test Plant : Soybean
Aphid /Plant : 15
Pre –acquisition fasting period : 2 hrs
Acquisition feeding period : 5 min.
Inoculation feeding period : 12 hr
20 plants each were tested in 3 replicates.

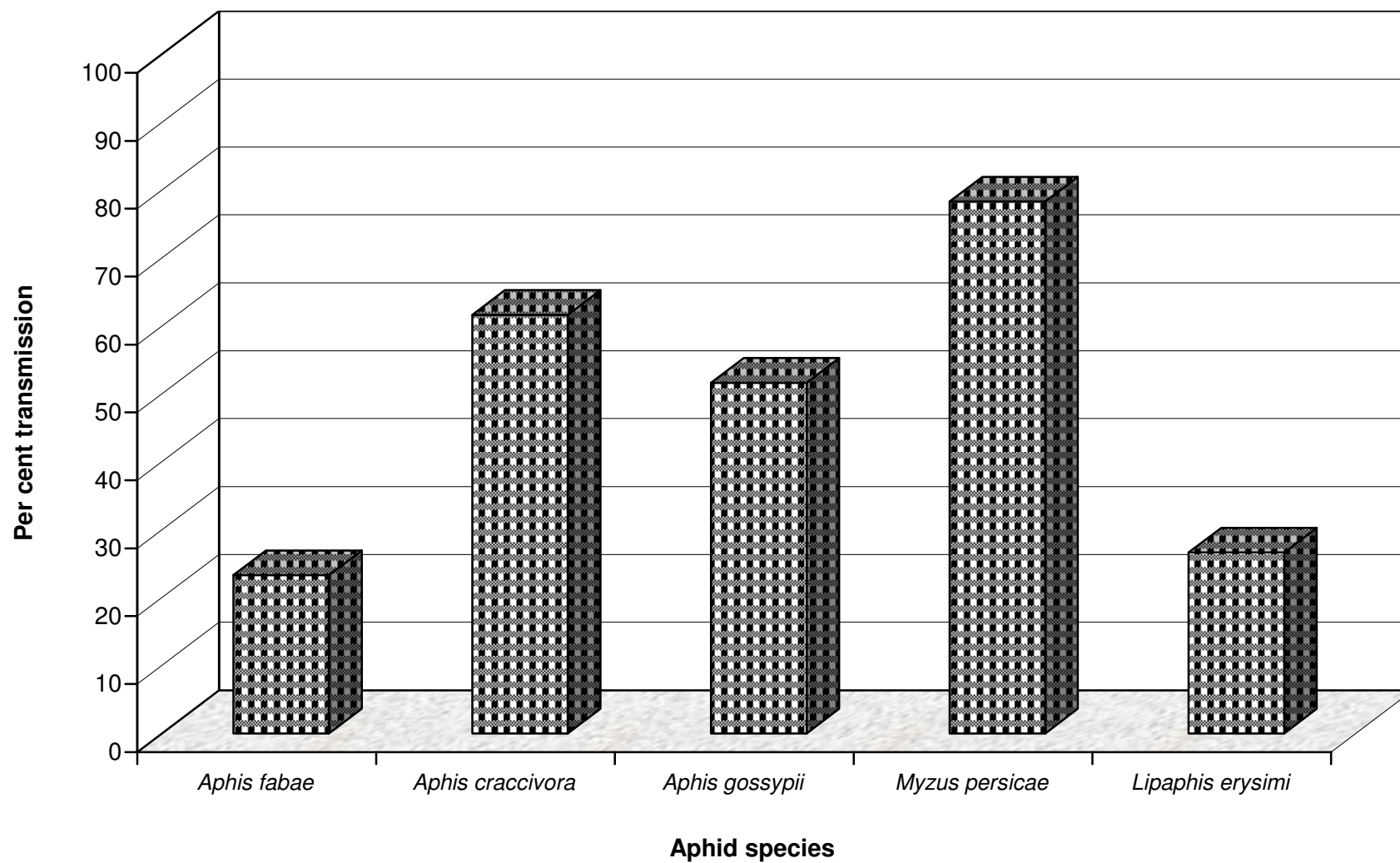


Fig. 2: Determination of relative efficacy of different aphid species in transmission of SMV

4.4.3 Seed transmission

The results of seed transmission of the virus in different cultivars of soybean for *kharif* season 2007-08 were given in **Table 8 and Fig.3.**

The result shows that the virus was transmitted through seed in all the ten cultivars of soybean tested. The per cent seed transmission ranged from 7.54 to 14.72 in different cultivars. The per cent seed germination ranged from 74.0 to 85.5 in different cultivars and in healthy 92.5 per cent was recorded. The maximum seed borne virus transmission was 14.72 per cent and 74 per cent germination was found in cultivar PK-416. While seed borne virus transmission were recorded 13.37, 12.58, and 12.14 percent recorded in PK-308, PK-327 and Shilajeet, respectively. The minimum seed borne virus transmission was 7.54 per cent and with 88.5 per cent seed germination were recorded in cultivar Alnkar.

During the *kharif* season 2008-09 the same 10 cultivars were tested for the seed transmission of SMV in soybean. The results of seed transmission were given in **Table 9 and Fig. 4.**

The data from table revealed that the per cent seed transmission of SMV ranged from 9.67 to 17.82 in different cultivars. Reduction in seed germination in various cultivars depending upon infection severity and seed viability.

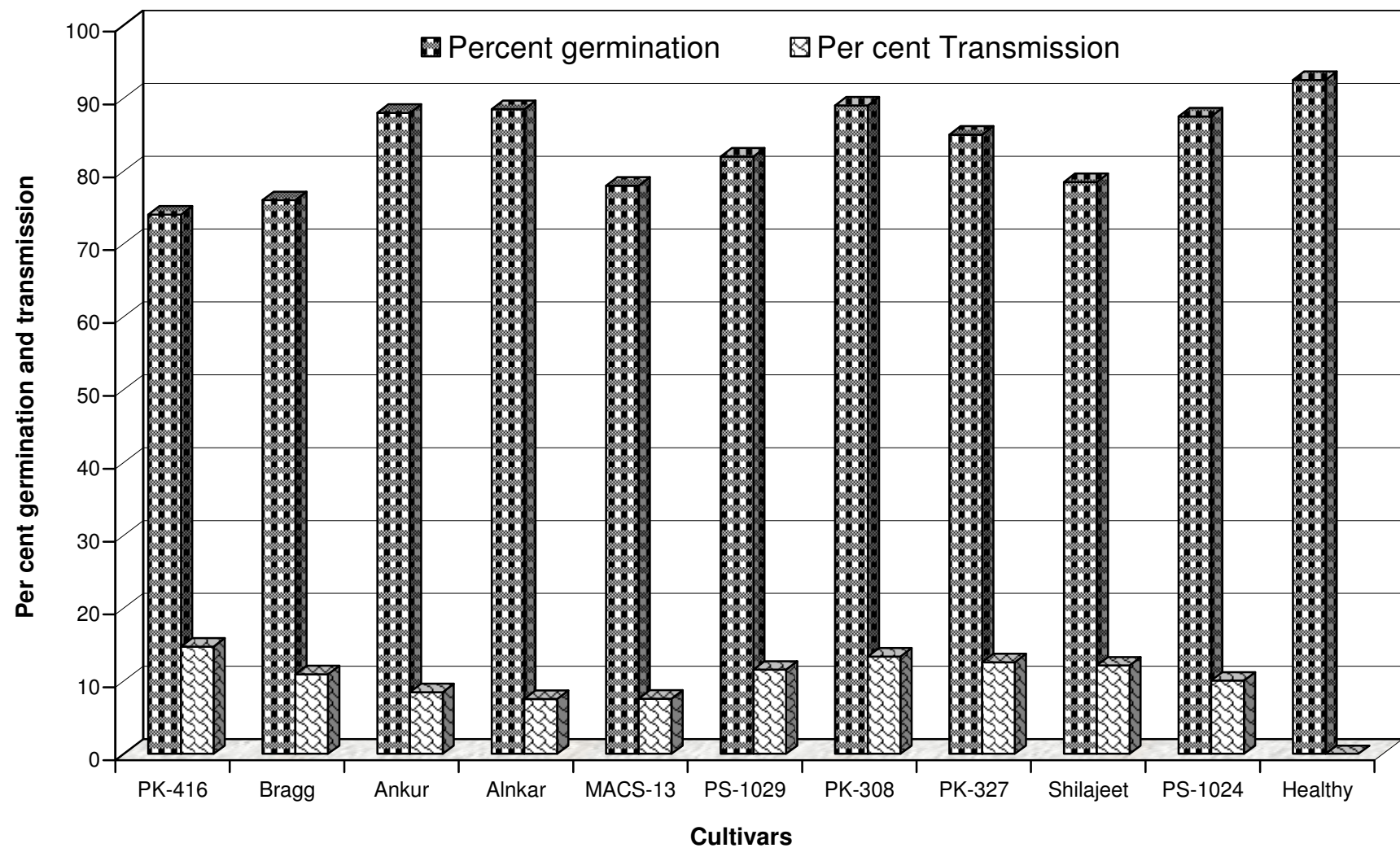


Fig.3: Seed transmission of SMV in different cultivars of soybean in year 2007-08

Table 8 : Seed transmission of SMV in different cultivars of soybean in year 2007-08

Cultivars	No. of seeds germinated	Percent germination	No. of seedlings		Per cent Transmission
			Healthy	Diseased	
PK-416	148	74	129	19	14.72
Bragg	152	76	137	15	10.94
Ankur	176	88	165	14	8.48
Alnkar	171	88.5	159	12	7.54
MACS-13	156	78	145	11	7.58
PS-1029	164	82	147	17	11.56
PK-308	178	89	157	21	13.37
PK-327	170	85	151	19	12.58
Shilajeet	157	78.5	140	17	12.14
PS-1024	175	87.5	159	16	10.06
Healthy	185	92.5	185	Nil	Nil

* Total seeds sown = 200

Table 9: Seed transmission of SMV in different cultivars of soybean in year 2008-09

Cultivars	No. of seeds germinated	Percent germination	No. of seedlings		Per cent Transmission
			Healthy	Diseased	
PK-416	152	76.0	129	23	17.82
Bragg	147	73.5	126	21	16.66
Ankur	173	86.5	156	17	10.89
Alnkar	170	85.0	155	15	9.67
MACS-13	158	79.0	142	16	11.26
PS-1029	160	80.0	141	19	13.47
PK-308	175	87.5	155	20	12.90
PK-327	166	83.0	151	19	12.58
Shilajeet	153	76.5	136	17	12.50
PS-1024	174	87.0	153	21	13.72
Healthy	188	94	188	Nil	Nil

* Total seeds sown = 200

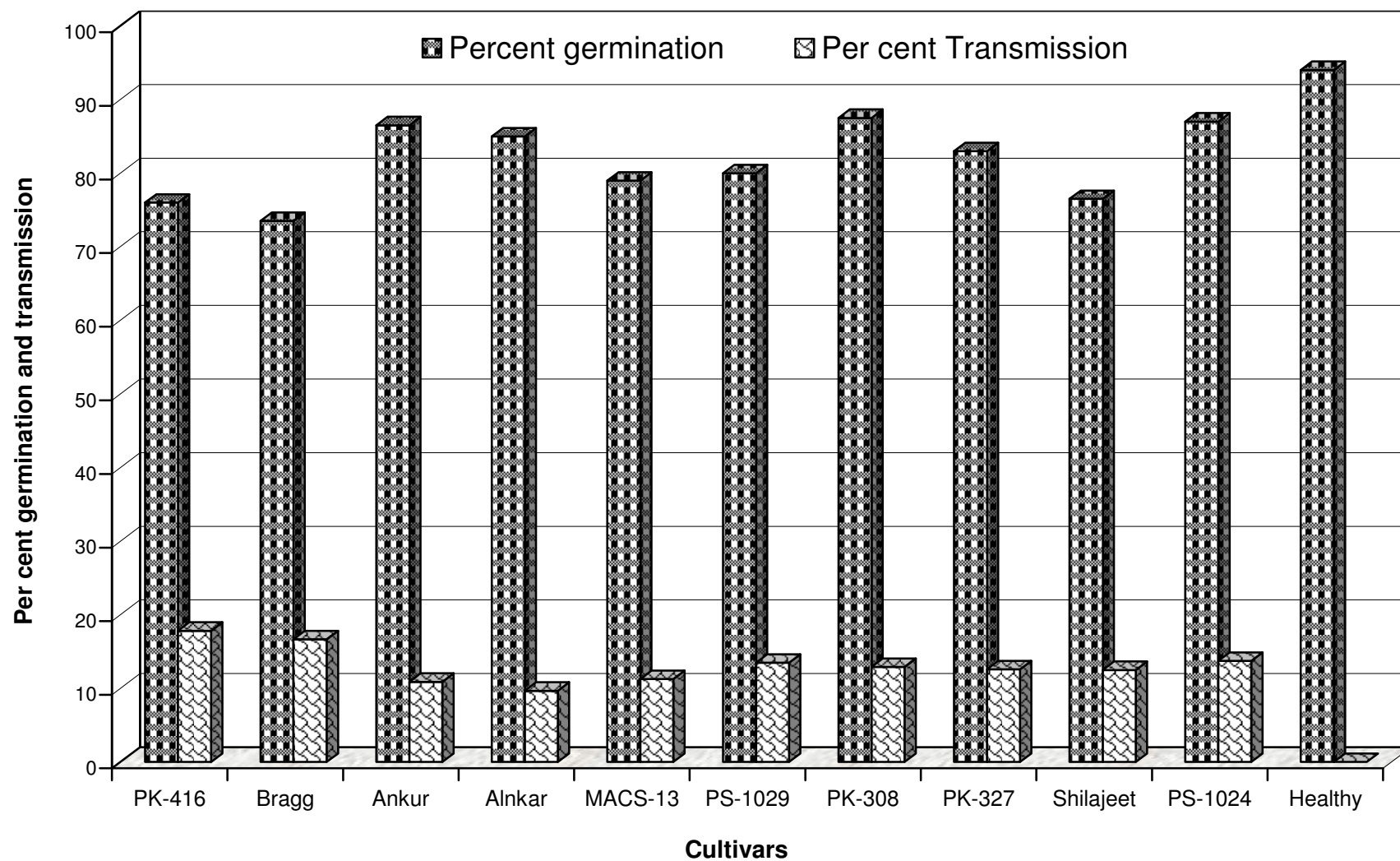


Fig.4: Seed transmission of SMV in different cultivars of soybean in year 2008-09

4.4.4 Stability of the virus in crude sap

Studies on stability of the virus in crude sap consisted of determination of TIP (Thermal inactivation point), DEP (Dilution end point) and LIV (Longevity *in vitro*).

4.4.4.1 Determination of TIP (Thermal inactivation point)

The results of determination of TIP were given in Table 10.

Table 10: Determination of TIP of SMV

Exposure Temperature(°C)	Percent Plant infection
50	80
55	70
60	50
65	Nil
70	Nil
Control	80

* Data is based on 20 plant inoculation

4.4.4.2 Determination of DEP (Dilution end point)

The results of determination of DEP were given in **Table 11**.

Table: 11 Determination of DEP of SMV

Dilution	Percent plant infection
10 ⁻¹	80
10 ⁻²	70
10 ⁻³	50
10 ⁻⁴	20
10 ⁻⁵	Nil
Control	80

* Data is based on 20 plant inoculation

4.4.4.3 Determination of LIV (Longevity *in vitro*) of SMV

The results of determination of LIV were given in **Table.12**

Table 12: Determination of LIV of SMV

Period of storage (days)	Per cent plant infection		
	Sap storage at room temperature (25-35°C)	Sap storage in refrigerator (5-7°C)	Leaves kept at refrigerator temperature (5-7°C)
Immediately	90	80	80
1	70	80	80
2	70	70	80
3	40	70	80
4	20	60	70
5	Nil	60	70
6	Nil	40	60
7	-	10	60
8	-	Nil	40
9	-	Nil	20
12	-	-	10
15	-	-	Nil
18	-	-	Nil
21	-	-	-

* Data is based on 20 plant inoculation

‘-’= not tested

The results given in **Tables 10-12** show that the virus had a TIP between 60-65°C, DEP between 10^{-4} to 10^{-5} and LIV between 4-5 days at room temperature, 7 days at 5-7°C and 15 days at in leaves stored at 5-7°C.

4.5 Purification

The virus was purified after several unsuccessful trials by a modification of **Ross (1967)** method as given in section 3.7 of Material

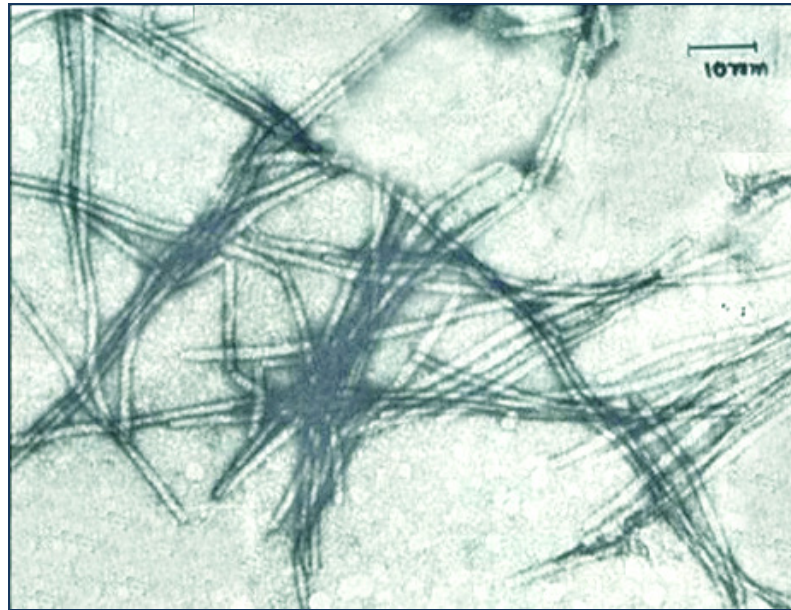
and Methods. The virus was purified from soybean leaves. The use of 0.2 M urea (use of 0.5 M or more urea resulted in the breakage of particles) reduced aggregation of particles. However, loss in particles due to aggregation was still noticed in low speed clarification steps. When the time was increased (>10 min) or when the speed was increased (> 3000 g) there was further loss in particles along with the pellet containing hosts debris. In sucrose density gradient centrifugation virus particles formed a diffused light scattering zone normally between 4 - 5.5 ml from the top of the 12.5 ml gradient tube. In most of the cases, the pellet formed at the bottom of the gradient tube had some aggregated virus particulars along with other host debris.

4.6 Electron microscopy

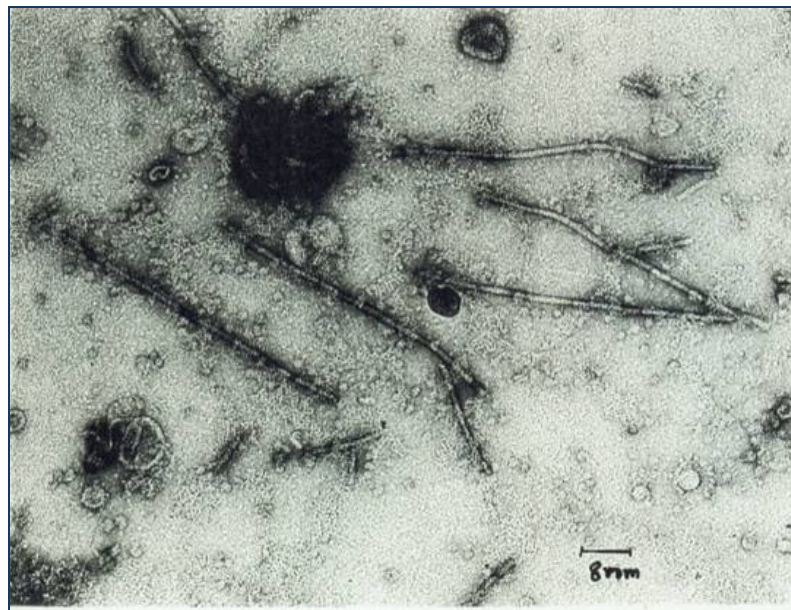
Electron microscopy of negatively stained purified virus of SMV revealed the presence of typical flexous particles (**Plate 3 A and B**). Similar flexous particles were observed in extracts of virus infected leaves, but in a smaller concentration. Long flexous particles of size 680-725 x 12-13 nm were seen in crude sap preparations of virus infected tissue of local lesion hosts *Cyamopsis tetragonoloba*. The average size of the particles was observed to be 680 x 12.5 nm.

4.7 UV spectrophotometry

The UV absorption pattern of the purified virus preparation is given in the **Table 13 and Figure 5**.



(A)



(B)

Plate 3: Electron micrograph of purified preparation (A) and Leaf dip preparation (B) of SMV from soybean

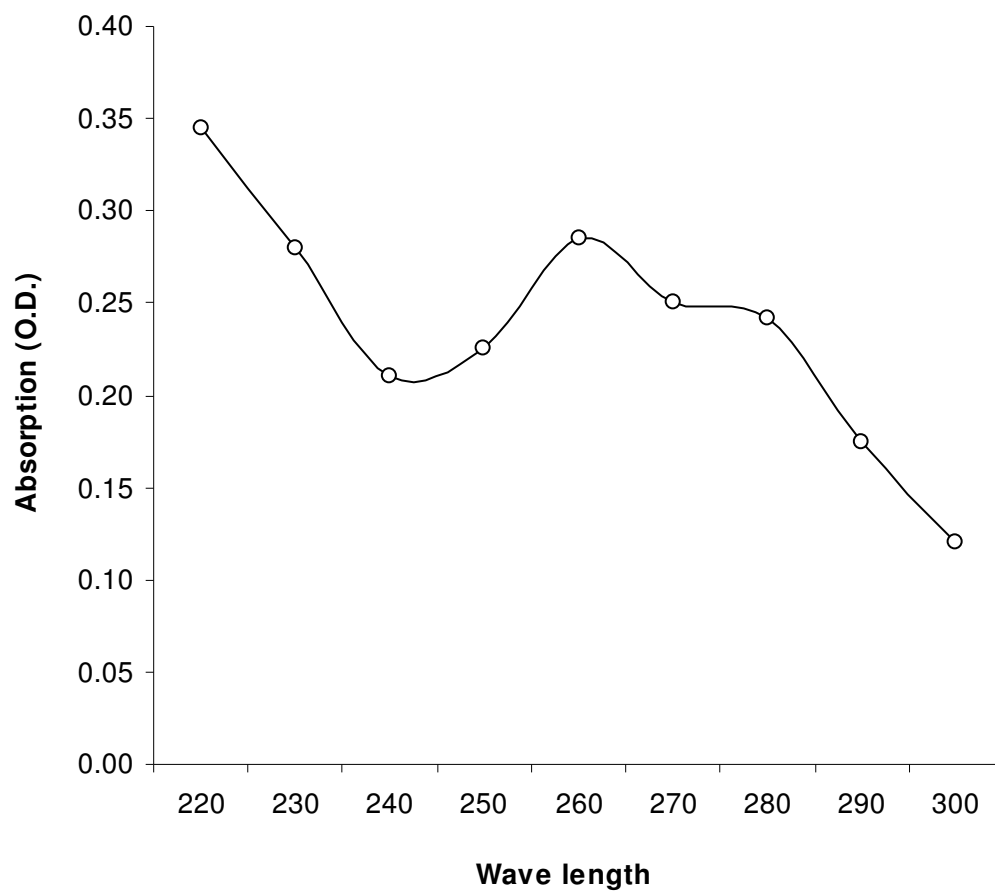


Fig. 5 : UV absorption spectrum of purified preparation of SMV

Table 13: UV absorption of purified preparation of SMV

Wave length	Absorption (O.D.)
220	0.345
230	0.280
240	0.210
250	0.225
260	0.285
270	0.250
280	0.242
290	0.175
300	0.120

The results given in **Table 13** shows that the purified virus preparation has an absorption maximum at 260 nm and a minimum at 240 nm. The 260/280 ratio (uncorrected for light scattering) was 1.17.

4.8 Serology

The results of the microprecipitin tests of the virus with homologous antiserum are given in **Table 14**. The results show that the virus at dilutions of 1/2, 1/4 and 1/ 8 gave very heavy to heavy precipitate with antiserum dilutions of 1/16, 1/32, 1/64 and 1/128. The titre of the homologous antiserum was found to be 1/512. The virus did not react with buffer which was kept as control.

4.9 DAC-ELISA for detection of SMV through polyclonal antiserum

SMV polyclonal antibody was purchased catalog No. SRA 33300 with format ELISA reagent set from Agdia as SMV infected plant sample provide him. The results of the DAC - ELISA for detection of SMV in purified preparations, and leaf are given in **Table 15**. From the results it is observed that the virus could be detected in purified preparations till a dilution of 1: 500. Similarly in the infected leaves, it could be detected to the same level of dilution i.e. 1:500. No reaction was observed in case of healthy soybean leaves. Thus, the result clearly shows that the polyclonal antiserum can be used to detect SMV in leaf samples.

Table 14: Determination of titre of homologous antiserum by microprecipitin tests

Dilution of antiserum	Dilution of antigen							Control (PBS)
	1/2	1/4	1/8	1/16	1/32	1/64	1/128	
1/16	+	+	+	+	+	+	+	-
1/32	+	+	+	+	+	+	+	-
1/64	+	+	+	+	+	+	+	-
1/128	+	+	+	+	+	+	-	-
1/256	+	+	+	+	-	-	-	-
1/512	+	+	+	-	-	-	-	-
1/1024	-	-	-	-	-	-	-	-
Control (Normal serum)	-	-	-	-	-	-	-	-
+	+	+	+	= Very heavy reaction				
+	+	+		= Heavy reaction				
+	+			= Moderate reaction				
+				= Light reaction				
-				= No reaction				

=

Heavy reaction

Table 15 : DAC-ELISA for detection of SMV through polyclonal antiserum

Antigens	Dilutions of Antiserum			
	1:50	1:100	1:250	1:500
Healthy soybean leaves	-	-	-	-
Purified virus	+	+	+	+
Infected leaf samples	+	+	+	+
Buffer	-	-	-	-

Absorbance at A₄₀₅ nm

Grouped as > 1.2 (+ + + +); 0.91- 1.2 (+ + +); 0.61-0.91 (+ + +); 0.31-0.60 (+ +); 0.10-0.30 (+); < 0.10(-)

4.10 Extent of seed transmission by abnormal seeds

Seed transmission studies were carried out using morphologically abnormal seeds for growing on test. The SMV infection affected the seedling emergence in various categories of soybean seeds showing morphological abnormalities. Small shrivelled seeds exhibited 64.0 per cent germination followed by 72.0 per cent germination in large shriveled, 84.0 per cent in oversized seeds, 76.0 per cent in undersized seeds and 88.0 and 76.0 per cent germination in off coloured and mottled seeds, respectively. All the six categories of morphologically abnormal seed, collected from systemically SMV infected plants yielded SMV infection ranging from 9.09 to 78.94 per cent in the growing on test suggesting the transmission of seedborne inoculum of SMV in soybean. A highest of

78.94 per cent seed transmission was obtained in mottled seeds followed by 22.22 per cent in large shrivelled seeds, 18.75 per cent in small shrivelled seeds, 14.28 per cent in oversized seeds and 10.52 and 9.09 per cent seed transmission ,respectively recorded in soybean variety PK-416 (**Table 16 and Fig. 6**).

Presence of seedborne inoculum of SMV in various categories of morphologically abnormal seeds in the same variety was also confirmed using DAC-ELISA test. Results indicate that mottled seeds gave very heavy positive reaction, while small shriveled and large shrivelled seeds gave heavy positive reaction, oversized gave moderate positive reaction, and undersized and off-coloured seeds gave light positive reaction in DAC-ELISA test (**Table 16**) **Plate No. 4. .**

4.10.1 Location of seedborne inoculum of SMV in soybean seeds

The indicator inoculation test was carried out to find out the association of soybean mosaic virus(SMV) with different floral parts i.e. epicalyx, calyx, corolla, androecium and gynoecium and to detect the location of SMV in different seed parts viz. seed coat, cotyledons and embryo to confirm the internally or externally seedborne nature of the virus. The association of SMV with different parts of flower and seed was also confirmed by DAC-ELISA as described in Materials and Methods. Soybean plants of variety PK-416 were used as test plants for indicator inoculation test under glass house conditions.



Plate 4: Morphological abnormalities in soybean seeds collected from systemically SMV infected plant

Table 16 : Extent of seed transmission of soybean mosaic virus through morphologically abnormal soybean seeds as revealed by growing on and ELISA test

Seed Category	No. of seeds sown	No. of Seeds germinated	Per cent seed germination	No. of plants showing SMV infection	Per cent seed transmission	Reaction on ELISA
Small shrivelled	25	16	64	3	18.75	+ + +
Large shrivelled	25	18	72	4	22.22	+ + +
Oversized	25	21	84	3	14.28	+ +
Undersized	25	19	76	2	10.52	+
Off coloured	25	22	88	2	9.09	+
Mottled seed (SMV infected)	25	19	76	15	78.94	+ + + +
Healthy	25	23	92	Nil	Nil	-

+ + + + = Very heavy reaction;
 + + = Moderate reaction;
 - = No reaction

+ + + = Heavy reaction
 + = Light reaction

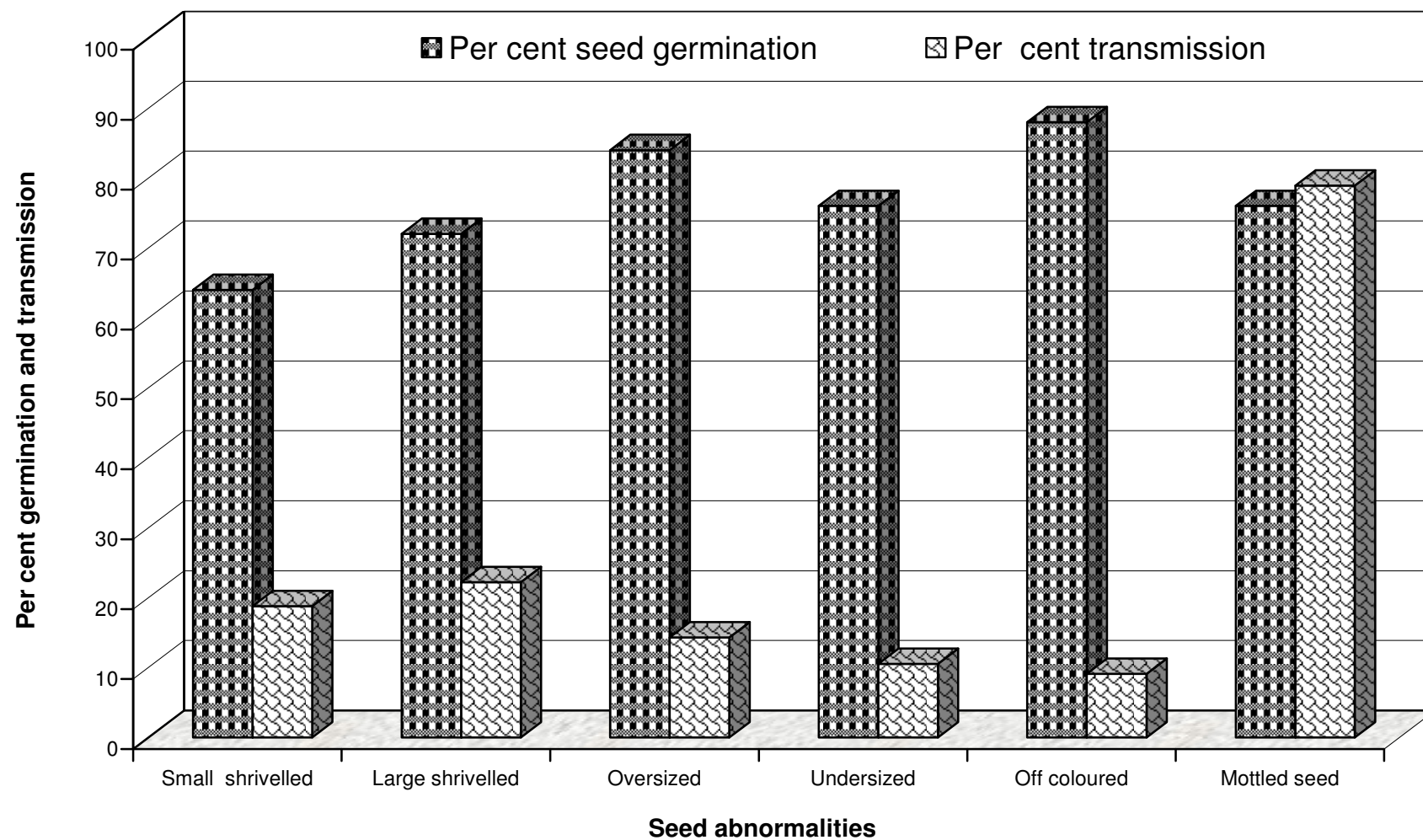


Fig. 6: Effect of morphological abnormalities on transmission of seedborne inoculum of SMV

4.10.1.1 In Flower Parts

The results of the assay of SMV in different floral parts of soybean viz. epicalyx, calyx, corolla, androecium, gynoecium as well as whole flower are given in **Table 17 and Fig. 7**. The results indicate that SMV could be detected in the sap of all the floral parts. The artificial inoculation with sap extracted from different floral parts of soybean exhibited a minimum of 5.32 per cent transmission of SMV on inoculation with sap from epicalyx followed by 6.64 per cent each with sap from calyx and corolla and 13.32 per cent when inoculated with the sap extracted from androecium and gynoecium, respectively. Highest transmission of 17.20 per cent was recorded on inoculation with extracts from whole soybean flower.

The sap extracted from various floral parts also reported positive reaction for SMV infection in DAC-ELISA test, thus indicating the possibilities of SMV infection in all the flower parts (**Table 17 and Fig. 7**).

4.10.1.2 In Seed Parts

The results of the indicator inoculation test for locating of SMV infection in different seed parts viz. seed coat; embryo and cotyledons are presented in **Table 18**. It is clear from the results that indicator plants on inoculation with sap of embryo and cotyledons separately of SMV infected seeds gave a positive reaction under glass house conditions. Data also indicate that there was significant difference in transmission of SMV when inoculated with sap of embryo and

Table 17 : Detection of soybean mosaic virus in different flower parts by indicator inoculation and ELISA test

Flower part	Reaction on indicator plant	No. of plants inoculated	Average no. of plants showing SMV symptom	SMV transmission (%)	ELISA value
Epicalyx	✓	25	1.33(6.62)	5.32(13.33)	+
Calyx	✓	25	1.66(7.40)	6.64(14.93)	+
Corolla	✓	25	1.66(7.40)	6.64(14.92)	+
Androecium	✓	25	3.33(10.51)	13.32(21.42)	++
Gynoecium	✓	25	3.33(10.51)	13.32(21.40)	++
Whole flower	✓	25	4.33(12.00)	17.20(24.50)	+++
Sem±			0.2728	0.1946	
CD at 5%			0.8407	0.5996	

Values given in parenthesis are angular transformed.

++++	=	Very heavy reaction;	+++	=	Heavy reaction
++	=	Moderate reaction;	+	=	Light reaction
-	=	No reaction			

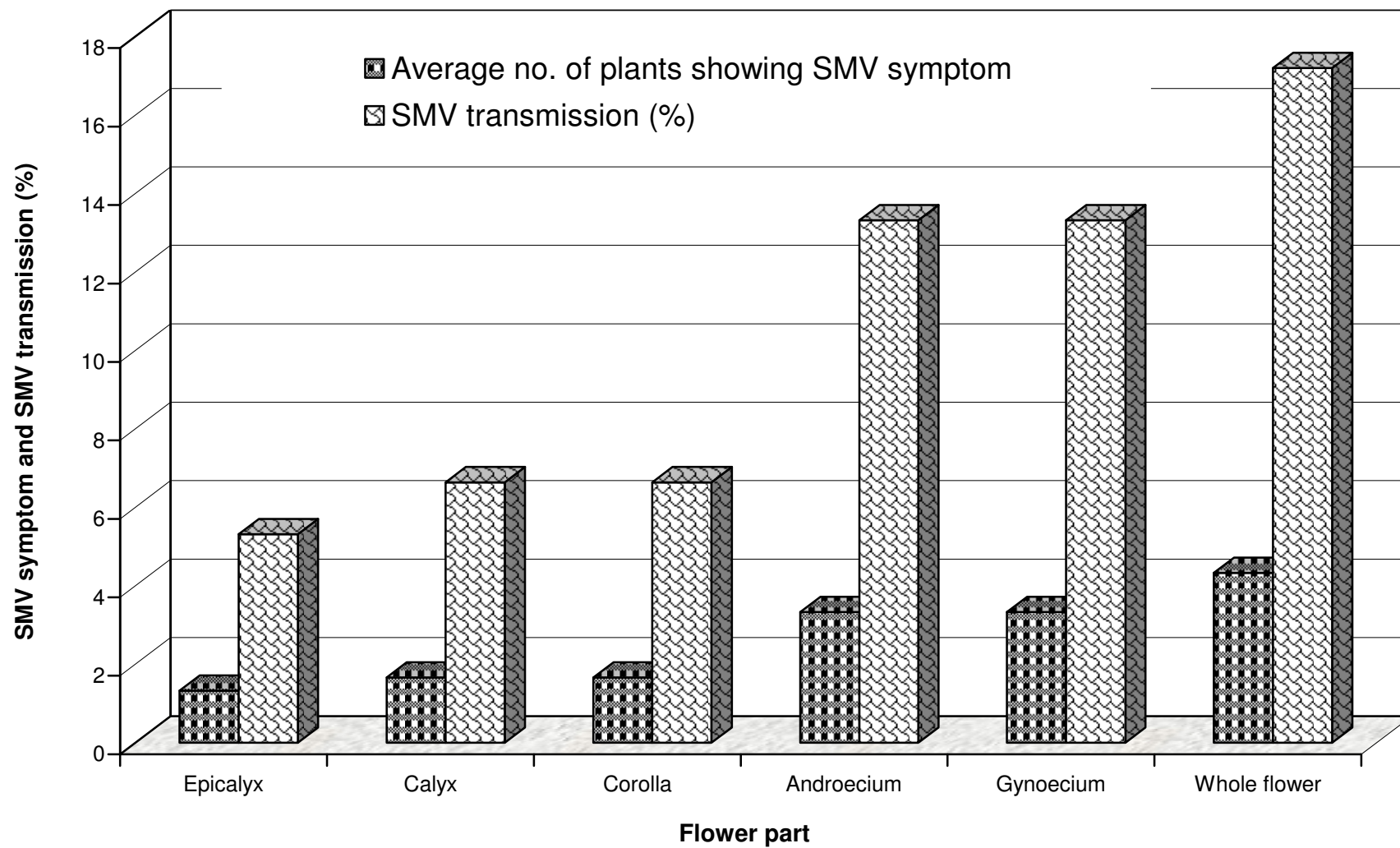


Fig.7 : Detection of soybean mosaic virus in different flower parts

Table18: Detection of soybean mosaic virus by indicator inoculation and DAC-ELISA tests in different seed parts of soybean

Seed part	Reaction on indicator plant	No. of plants inoculated	Average no. of plants showing SMV symptom	SMV transmission (%)	ELISA value
Seed coat	-	25	0.0	0.0	-
Cotyledons	✓	25	3.3	13.32	+
Embryo	✓	25	5.66	22.64	++
Seed surface washings	-	25	0.0	0.0	-

+ + + + = Very heavy reaction
 + + + = Heavy reaction
 + + = Moderate reaction
 + = Light reaction
 - = No reaction

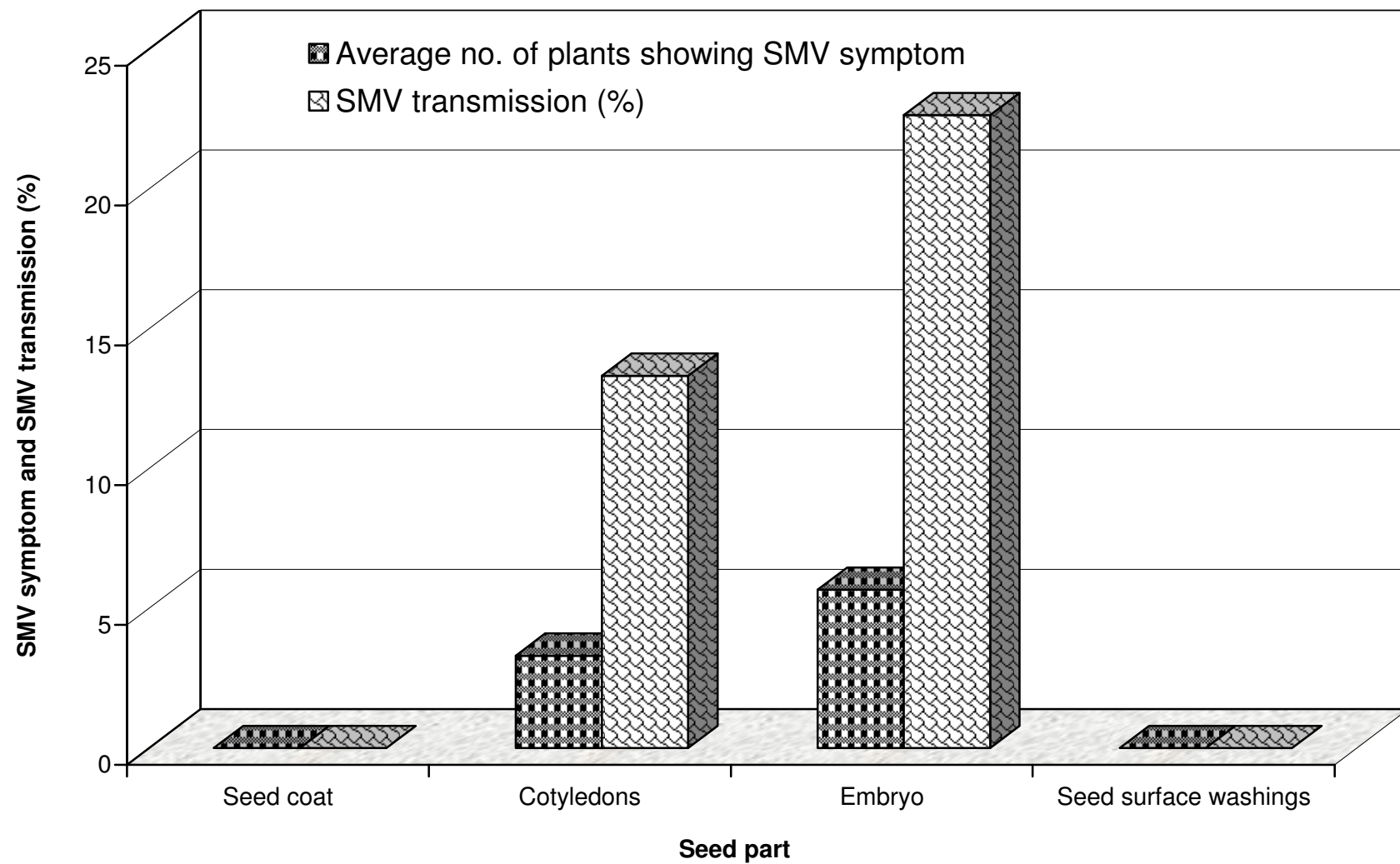


Fig. 8: Detection of soybean mosaic virus in different seed parts

cotyledons, separately. The highest (22.64 %) transmission was recorded when plants were inoculated with sap of embryo, while transmission was 13.32 per cent when inoculated with sap of cotyledons. Inoculation of plants with sap from seed coat did not reveal any symptom of SMV on plants (**Fig. 8**). The sap extracted from cotyledons and embryo part of soybean seed, separately also responded positive reaction in DAC-ELISA test and showed light positive reaction (cotyledon) and moderate positive reaction (embryo), indicating the presence of virus in embryo and cotyledons of soybean seeds. When seed surface washings were used for inoculating soybean plants, no disease appeared, it shows that the SMV is internally seedborne in nature.

4.11 Monitoring of aerial population of aphid adults:

4.11.1 Choice of color:

Three different coloured traps eg. Bright golden yellow, pale yellow, and brown yellow were used for monitoring the aerial populations of aphid as described in para 3.12.1 of materials and methods to see the attractiveness of different colours.

Observations made during July to November are given in **Table 19 and Fig. 9 and Table 20 and Fig. 10** for the *kharif* season of 2007-08 and 2008-09, respectively. Result clearly indicate that bright yellow colour traps were most attracting to aphid followed by pale yellow colour traps while traces of aphid were attracted towards the brown yellow colour traps.

Table 19: Number of aphid adults was trapped weekly by different colored traps (2007-08)

Month	Date	Color of Traps		
		Bright yellow	Pale yellow	Brown yellow
July	16 to 22	80.33	15.00	06.00
	23 to 29	75.66	12.00	03.66
	30 to 5 A	66.33	10.00	02.33
August	6 to 12	77.33	11.00	04.33
	13 to 19	44.66	07.33	01.66
	20 to 26	40.66	07.00	01.00
	27 to 2 S	38.33	05.66	01.00
September	3 to 9	23.00	02.66	00.66
	10 to 16	24.33	06.33	01.33
	17 to 23	36.66	06.33	01.66
	24 to 30	50.66	08.00	02.00
October	1 to 7	56.66	09.66	02.33
	8 to 14	62.33	07.66	01.00
	15 to 21	50.66	05.00	00.66
	22 to 28	40.66	02.66	00.33
	29 to 4 N	34.33	01.66	00.00
November	5 to 11	21.33	01.00	00.00
	12 to 18	05.66	000.00	00.00

*Each value is the mean of three replicates

Table 20: Number of aphid adults was trapped weekly by different colored traps (2008-09)

Month	Date	Color of Traps		
		Bright yellow	Pale yellow	Brown yellow
July	16 to 22	83.00	19.66	07.00
	23 to 29	76.66	14.00	04.33
	30 to 5 A	70.00	12.66	03.66
August	6 to 12	82.00	13.66	06.00
	13 to 19	47.00	06.33	02.66
	20 to 26	41.66	07.66	02.00
	27 to 2 S	36.66	06.33	01.00
September	3 to 9	24.33	13.33	05.33
	10 to 16	26.33	08.00	02.33
	17 to 23	38.66	10.33	03.33
	24 to 30	52.00	11.66	04.00
October	1 to 7	58.33	13.33	05.33
	8 to 14	64.66	15.00	02.00
	15 to 21	52.66	09.33	01.00
	22 to 28	42.33	04.00	01.00
	29 to 4 N	33.66	03.00	00.66
November	5 to 11	23.00	01.66	00.33
	12 to 18	07.33	00.66	00.00

*Each value is the mean of three replicates

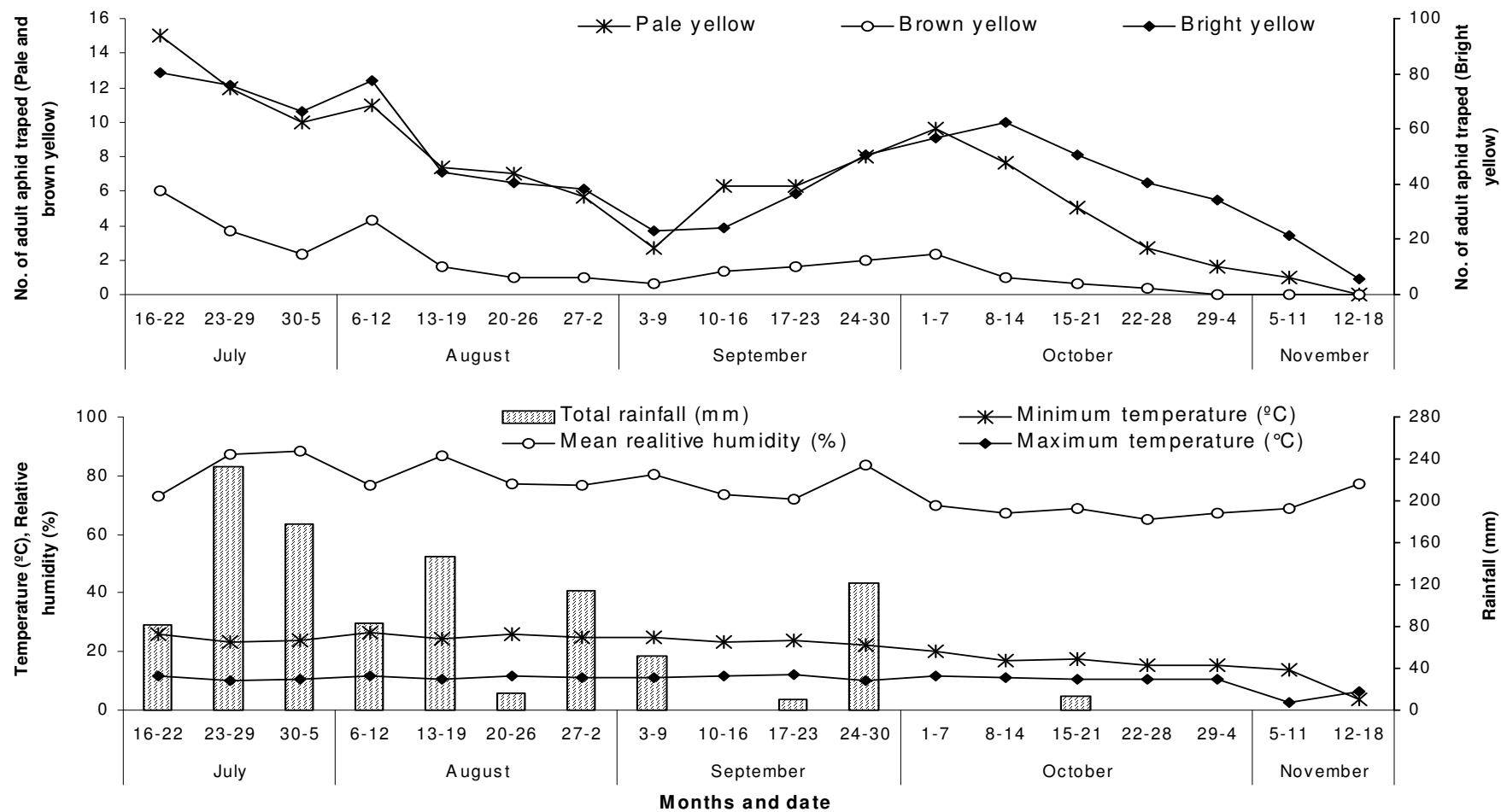


Fig. 9: Trap catches of *Myzus persicae* by different coloured traps with the prevailing weather condition (2007-08)

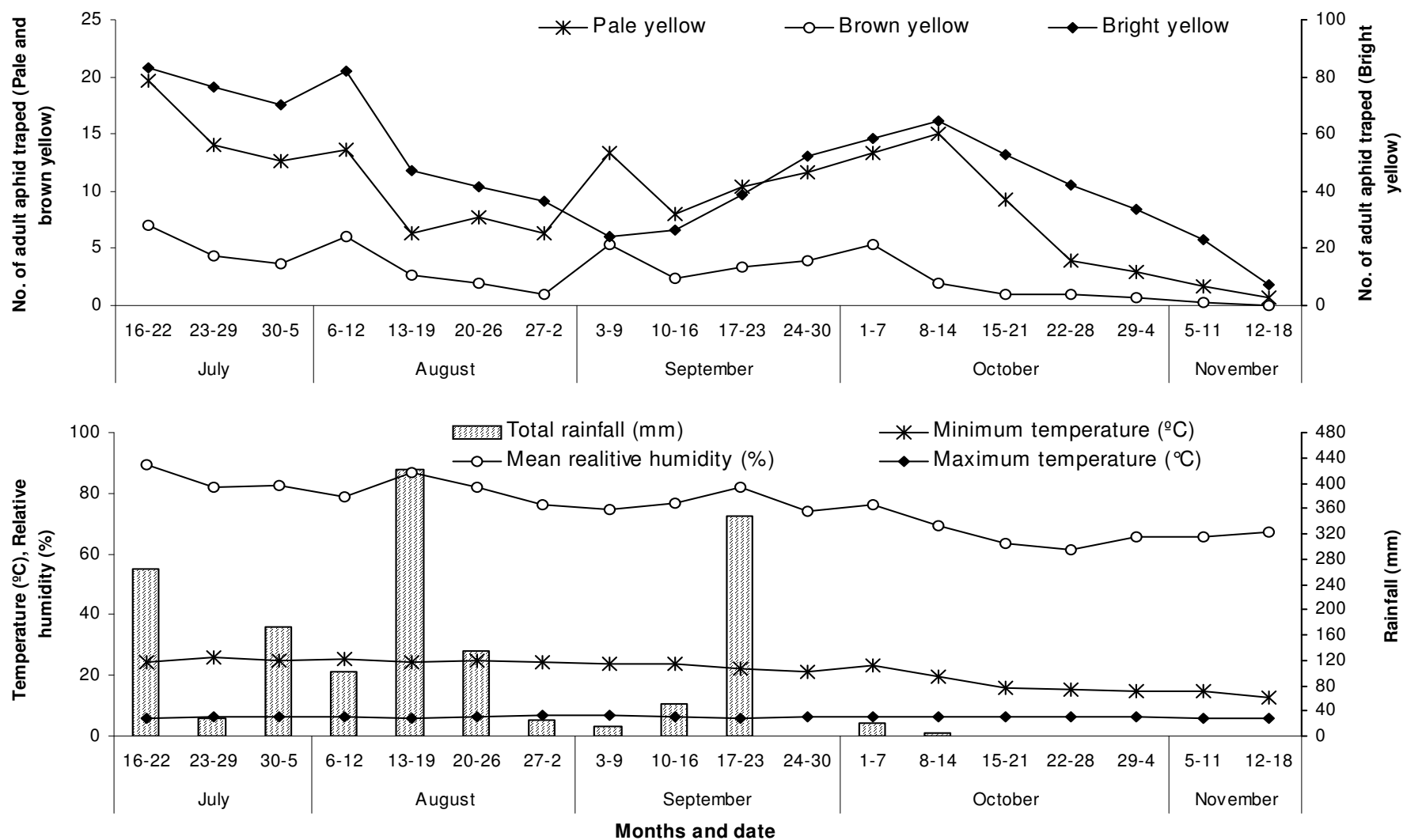


Fig.10: Trap catches of *Myzus persicae* by different coloured traps with the prevailing weather condition (2008-09)

4.11.2 Choice of traps

Two different shaped traps eg. Cylindrical and lamp shape type were traps wrapped with bright golden yellow cellophane paper, were used for monitoring the aerial populations of *Myzus persicae* as described in para 3.12.2.1-2 materials and methods to compare and see the most attractive traps. Fortnight trap count the aphid populations during 2007-08 and 2008-09 cropping season, plotted on graph (**Table 21, Fig. 11 and Fig. 12**) showed that cylindrical trap was most effective in attracting the aphid as compare to lamp shape type.

4.12 Seasonal variations in the aerial populations of aphid adults

Aerial populations of *Myzus persicae* vector was monitored during 2007-08 and 2008-09 with the help of different type of traps in the soybean field. The fortnight trapped populations of aphid were plotted on graph and the metrological data (**Table 22 and Table 23**) were superimposed to ascertain the of weather conditions on the aerial population of aphid as described in para 3.12.3 of materials and methods.

The highest number of aphid adults was trapped during second fortnight of July when the average maximum temperature, average minimum temperature and relative humidity 30.67, 24.64 and 80.87, respectively. Thereafter, a decline in trap catches was observed as the maximum temperature, steady increase and percentage relative

Table 21 : Number of aphid adults was trapped fortnightly by different shaped traps

Month	Date	Shape of traps			
		Lamp shape type trap		Cylindrical trap	
		2007-08	2008-09	2007-08	2008-09
July	16 to 31	68.66	77.33	132.00	193.66
August	1 to 15	65.33	71.66	119.00	156.33
	16 to 31	59.66	64.33	128.66	166.66
September	1 to 15	48.33	45.66	94.66	75.33
	16 to 30	99.33	80.00	141.66	83.66
October	1 to 15	86.33	77.66	137.33	73.00
	16 to 31	50.66	44.66	50.66	48.33
November	1 to 15	14.00	07.33	11.66	05.66
	16 to 30	04.00	02.66	05.33	04.33

*Each value is the mean of three replicates

Table 22: Mean fortnight weather data during the course of investigation year 2007-08

Month	Date	Max. Temp. (°C)	Min. Temp. (°C)	Mean Rel. Hum. (%)	Total Rain Fall (mm)
July	16 to 31	30.67	24.64	80.87	71.93
August	1 to 15	31.23	25.01	84.06	25.30
	16 to 31	31.41	25.28	80.46	07.97
September	1 to 15	31.72	24.24	77.76	03.62
	16 to 30	31.28	23.04	77.16	08.80
October	1 to 15	31.77	18.52	68.53	--
	16 to 31	29.48	16.41	66.87	00.82
November	1 to 15	28.10	13.08	67.60	--
	16 to 30	26.48	10.52	64.43	--

Table 23: Mean fortnight weather data during the course of investigation year 2008-09

Month	Date	Max. Temp. (°C)	Min. Temp. (°C)	Mean Rel. Hum. (%)	Total Rain Fall (mm)
July	16 to 31	28.21	23.34	80.44	20.94
August	1 to 15	30.09	25.12	81.96	24.40
	16 to 31	30.93	24.68	81.00	28.74
September	1 to 15	31.84	23.82	75.80	02.74
	16 to 30	30.34	21.90	77.26	24.88
October	1 to 15	31.02	21.36	72.33	01.62
	16 to 31	29.55	15.12	63.28	--
November	1 to 15	28.90	14.43	65.90	--
	16 to 30	26.18	10.49	65.73	00.16

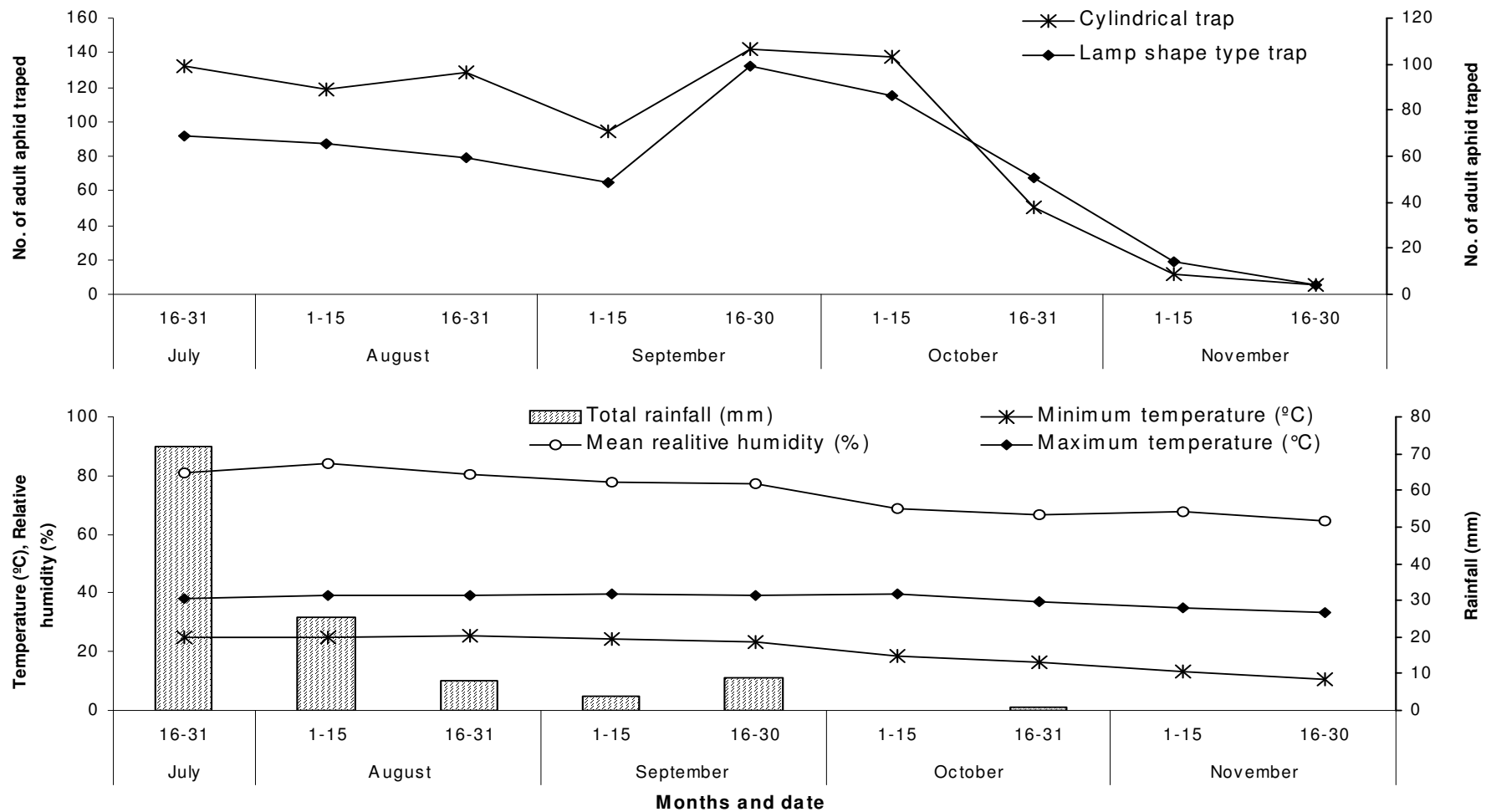


Fig. 11: Trap catches of *Myzus persicae* by different shape traps with the prevailing weather condition (2007-08)

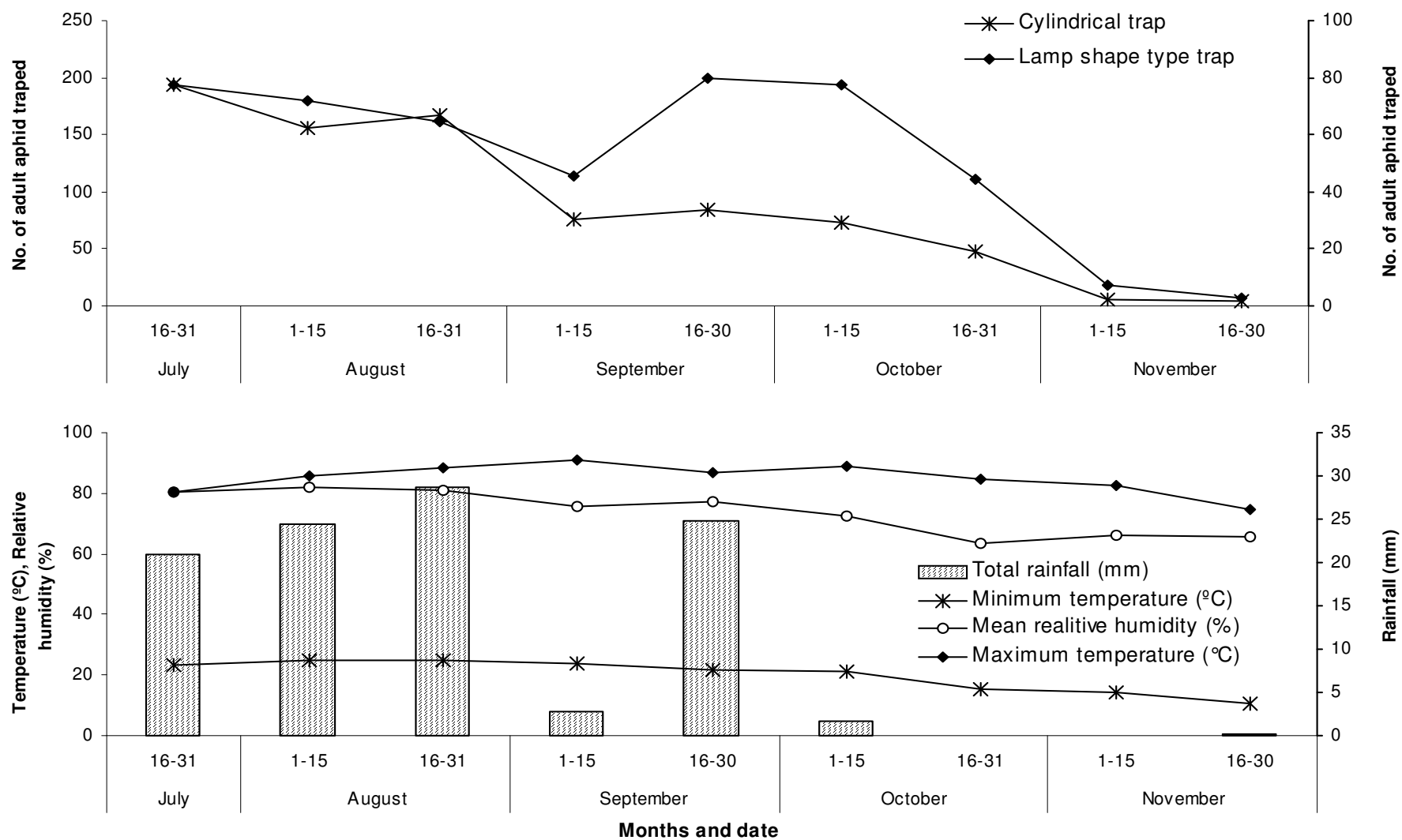


Fig. 12: Trap catches of *Myzus persicae* by different shape traps with the prevailing weather condition (2008-09)

humidity and rainfall steady decrease. Again maximum population of aphid was recorded in second fortnight of September when temperature steadily decline the average maximum temperature, average minimum temperature, and relative humidity 31.28, 23.04 and 77.14 respectively. There was again a steady decline in trap catches from first fortnight of November very few numbers of aphids recorded. During this period average maximum and minimum temperature also decrease (**Table 21**).

Similar trend was observed during 2008-09 *kharif* season soybean crops during course of investigation (**Table 21**).

4.13 Effect of plant age at infection with SMV on yield contributing factors and yield of soybean

The result of SMV infection in relation to plant age and yield contributing factors and yield in soybean are given in **Table 24** and **Table 25** for the *kharif* season of 2007-08 and 2008-09, respectively. Seeds were collected from soybean plants infected at 15, 25, 35, 45 and 65 days interval, separately and were sown under controlled glass house conditions as mentioned in materials and methods. At maturity of the crop, observations were made on number of pods, pod length, no. of seeds per plant. 100 seed weight, yield per plant and percent yield reduction, respectively.

For year 2007-08 **Table 24** and **Fig. 13** reveal that all the above mentioned yield contributing factors were adversely affected as a result of

Table 24 : Loss in yield of soybean due to SMV when infected at different growth stages in var. PK-416 (2007-08)

Plant age at infection(days)	Average number of pods/plant	Average pod length(cm)	Average number of grains/plant	100 seed weight(gm.)	Average yield/Plant (gm.)	% loss in yield
15	5.2	1.7	14.40	4.50	1.42	93.08(74.77)
25	20.2	2.5	36.20	9.34	3.55	83.24(65.86)
35	24.4	2.9	42.00	11.48	7.38	64.10(53.19)
45	30.6	3.6	66.40	12.32	10.69	48.03(43.87)
65	45.2	4.2	94.20	13.10	14.47	29.63(32.96)
Healthy	62.80	4.62	127.6	15.48	20.58	-
Sem±	0.8736	0.5282	1.0214	0.1657	0.1456	0.7285
CD at 5%	2.5501	1.5418	2.9813	0.4837	0.4252	2.1264

Values given in parenthesis are angular transformed

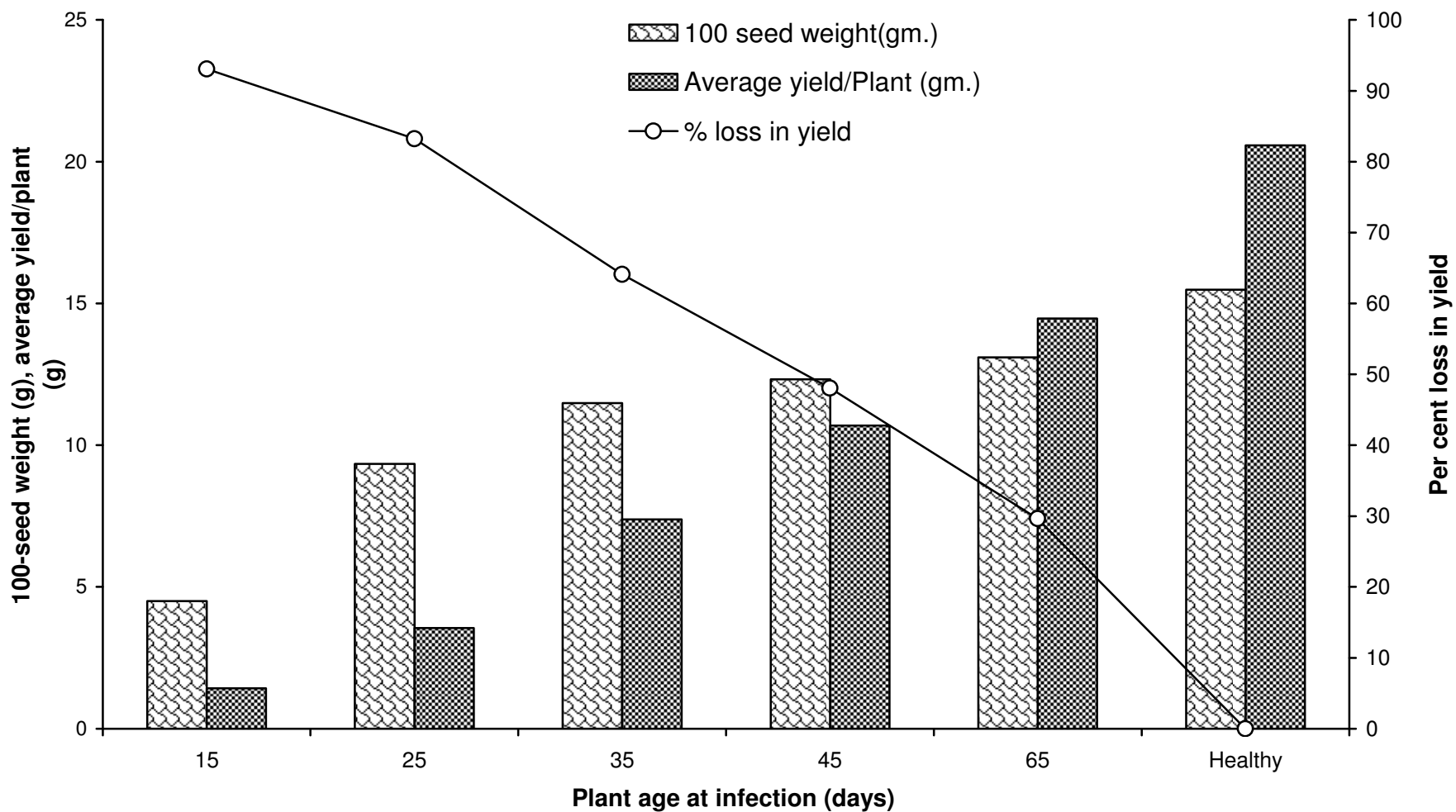


Fig. .13 : Loss in yield of soybean due to SMV when infected at different growth stages in var. PK-416 (2007-08)

SMV. Average number of pods per plant was significantly lower in plant infections where inoculations were commencing from 15 DAS to DAS as compared to healthy plants. A minimum average of 5.2 pods/ plant was found in plants inoculated at 15 DAS, while the maximum average number of pods was 45.2 pods/ plant at 65 DAS. In healthy plants an average of 62.8 pods/ plant was recorded. However, plants when inoculated 15 DAS of planting significant decrease in average pod length 1.7 cm as compared to healthy plants. An average of 14.40 seeds per plant were recorded in case of plants infected at 15 DAS as compared to an average number of 94.20 and 127.6 seeds/ plant from plants infected at 65 DAS and healthy plants, respectively. Hundred seed weight was 4.50 gm in plants infected 15 DAS; 13.10 gm in plants infected at 65DAS and was 15.48 gm when collected from healthy plants. Further, the SMV infection at different stages of plant growth, significantly, affected the seed yield. Average yield/plant was 1.42 gm in plant infected 15 DAS, 14.47 gm in plant infected at 65 DAS and 20.58gm when collected from healthy plant. Maximum yield reduction of 93.08 per cent was found when plant infection occurred at 15 DAS, which was significantly reduced with the delay in infection at 65 DAS where it caused only 29.63 per cent loss in yield. Thus, the yield loss ranged from 29.63 to 93.08 per cent in soybean plants of variety PK-416 depending upon the plant age at the time of infection.

For year 2008-09 **Table 25 and Fig.14** reveal that loss in term of average number of pods /plant was 7.4, 19.6, 22.6, 27.8 and 43.4 in 15 DAS, 25DAS, 35 DAS, 45 DAS and 65 DAS, respectively was recorded

Table 25 : Loss in yield of soybean due to SMV when infected at different growth stages in var. PK-416 (2008-09)

Plant age at infection(days)	Average number of pods/plant	Average pod length(cm)	Average number of grains/plant	100 seed weight(gm.)	Average yield/Plant (gm.)	% loss in yield
15	7.4	1.50	14.00	4.24	1.31	93.24(74.94)
25	19.6	2.10	35.40	10.22	3.34	82.76(65.47)
35	22.6	2.40	41.20	12.26	6.45	66.69(54.75)
45	27.8	3.10	63.00	13.24	10.39	46.66(43.08)
65	43.4	3.92	88.60	15.25	15.62	19.92(26.49)
Healthy	58.40	4.34	126.20	16.42	19.39	-
Sem±	1.0598	0.4711	1.1328	0.1478	0.9022	0.4747
CD at 5%	3.0935	1.3752	3.3065	0.4314	0.2633	1.3858

Values given in parenthesis are angular transformed

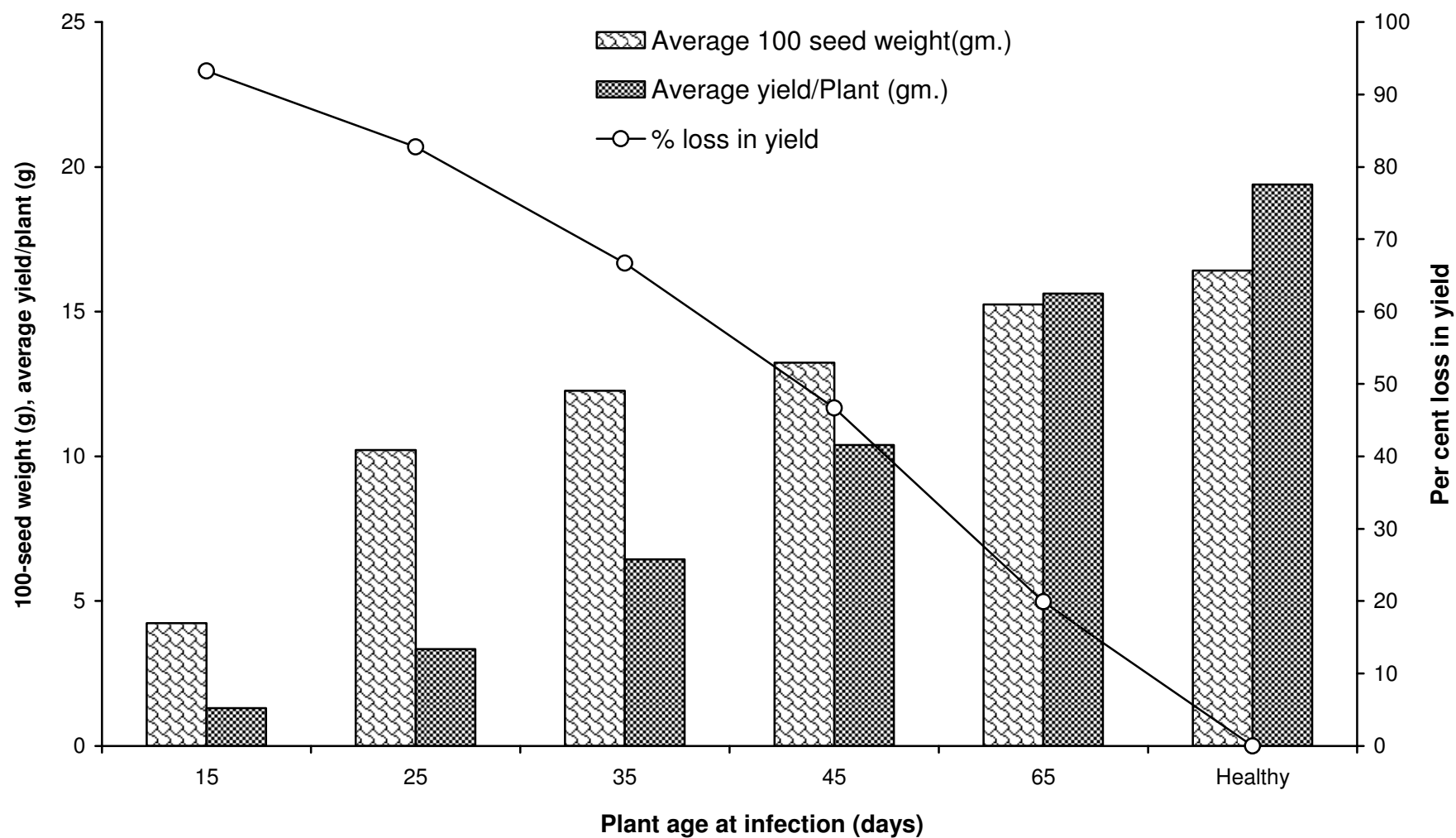


Fig. 14 : Loss in yield of soybean due to SMV when infected at different growth stages in var. PK-416 (2008-09)

while in healthy it was 58.40 pods/plant. In terms of average length of pod a minimum average of 1.50 cm was found in plants inoculated at 15 DAS, while the maximum average length of pods was 3.92 cm at 65 DAS. In healthy plants an average of 4.34 cm was recorded. However, plants when inoculated 15 DAS of planting significant decrease in average number seeds /plant 14.00 seeds per plant as compared to an average number of 88.60 and 126.20 seeds/ plant from plants infected at 65 DAS and healthy plants, respectively. Hundred seed weight was 4.24 gm in plants infected 15 DAS; 15.25 gm in plants infected at 65DAS and was 16.42 gm when collected from healthy plants. Maximum yield reduction of 93.24 per cent was found when plant infection occurred at 15 DAS, which was significantly reduced with the delay in infection at 65 DAS where it caused only 19.92 per cent loss in yield. The results show that there is a progressive decrease in reduction in yield with increase in the age of the plant and virus infection. The findings confirm the highly destructive nature of virus especially when the younger plants get infection **(Plate 5)**.

4.14 Screening for disease resistance

4.14.1 Under field (natural) condition

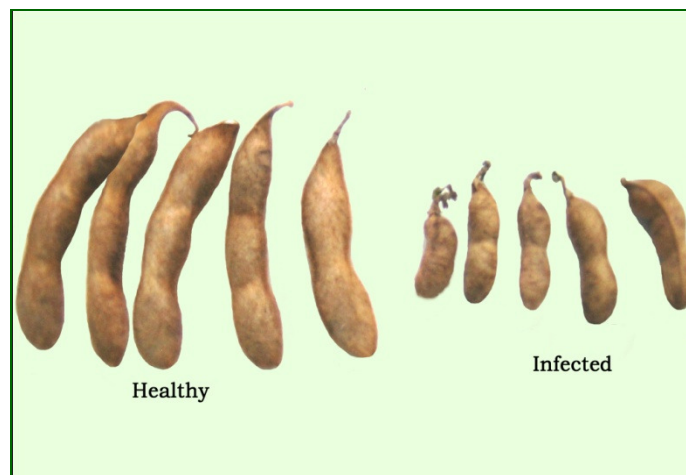
The results of the screening of the soybean cultivars under natural conditions are given in **Table 26 and Table 27** of the *khariif* season of 2007-08 and 2008-09, respectively.



Use of insect proof cages for seed transmission of SMV under field condition in soybean



Puckered, mottled symptoms of SMV on soybean leaves



Effect of SMV on pod development

Plate 5

Table 26: Natural incidence of Soybean Mosaic virus in different cultivars of soybean during *kharif* (2007-08)

Sl. No.	Cultivars	Total Plants	SMV Infected Plant	% SMV infection	SMV Rating (0-5 scale)
01.	Bragg	97	43	44.32	3
02.	Ankur	82	04	04.87	1
03.	Alnkar	117	14	11.96	1
04.	Shilajeet	98	36	36.73	3
05.	PK-262	96	23	23.95	2
06.	PK-327	110	04	03.63	1
07.	PK-416	97	21	21.64	2
08.	PK-564	73	04	05.47	1
09.	PK-472	127	25	19.68	1
10.	PK-308	97	29	29.98	2
11.	Hardee	78	34	47.12	3
12.	PRS-1	58	17	29.31	2
13.	MACS-13	57	13	22.80	2
14.	MACS-450	107	62	57.94	3
15.	Pusa-16	109	46	42.20	3
16.	Pusa-20	104	28	43.58	3
17.	Pusa-24	86	34	39.53	2
18.	MACS-473	113	82	72.56	4
19.	Monetta	85	46	54.11	3
20.	L-32A	128	94	73.43	4
21.	JS-72--280	108	49	45.37	3
22.	NRC-46	114	71	62.28	4
23.	PS-1024	104	28	26.92	2
24.	PS-1042	87	41	39.62	2
25.	PS-1092	129	37	28.68	2
26.	PS-1029	85	14	16.47	1
27.	PS-1241	116	12	10.34	1
28.	PS-1347	107	34	31.77	2
29.	PS-1225	118	34	28.81	2
30.	VLS-52	84	19	22.61	2

Thirty cultivars were screened in *kharif* of 2007-08 (**Table 26**). The cultivars Ankur, Alnkar, PK-327, PK-564, PK-472, PS-1029 and PS-1241 were found to be resistant. PK-327 showed very low incidence of disease at 03.63 per cent. No cultivar, however, escaped infection under natural conditions. The cultivars PK-262, PK-416, PK-308, PRS-1, MACS-13, Pusa-24, PS-1042, PS-1024, PS-1092, PS-1347, PS-1225 and VLS-52 were found to be tolerant. Cultivars such as Bragg, Shilajeet, Hardee, MACS-450, Pusa-16, Pusa-20, Monetta, and JS-72—280 showed susceptibility. While MACS-473, L-32A, and NRC-46 showed moderately susceptibility. No cultivar, however, highly susceptible under natural conditions.

During the *kharif* season of 2008-09 the same thirty cultivars were again screened for resistance. No cultivar, however, highly susceptible under natural conditions. The cultivars L-32A, Monetta and MACS-473 were found to be moderately susceptible (**Table 27**).

Cultivars Bragg, MACS-450, Pusa-20, JS-72-280, PK-308, Hardee, Shilajeet and NRC-46 fell into the category of susceptible cultivars. The tolerant cultivars were PS-1092, PS-1029, Pusa-16, PK-262, VLS-52, PS-1024, Pusa-24, PRS-1, PS-1042 and PS-1347. The cultivars PS-1241 PK-564, PK-472, PK-327, Alnkar, PS-1225, MACS-13, Ankur, and PK-416 were found to be resistant. No cultivars escaped infection in field conditions and thus no cultivar could be classified as Immune in the 30 cultivars that were tested.

Table 27: Natural incidence of Soybean Mosaic virus in different cultivars of soybean during *kharif* (2008-09)

Sl. No.	Cultivars	Total Plants	SMV Infected Plant	% SMV infection	SMV Rating (0-5 scale)
01	Bragg	109	46	42.20	3
02.	PS-1092	94	21	22.34	2
03.	PS-1029	101	23	22.77	2
04.	PS-1241	83	16	19.27	1
05.	MACS-450	121	71	58.67	3
06.	Pusa-16	73	20	27.39	2
07.	Pusa-20	91	38	41.75	3
08.	L-32A	119	87	73.10	4
09.	JS-72--280	109	52	47.70	3
10.	PK-564	112	07	06.25	1
11.	PK-472	110	14	12.72	1
12.	PK-308	89	32	35.95	3
13.	Hardee	115	56	48.69	3
14.	Shilajeet	107	44	41.12	3
15.	PK-262	87	32	36.78	2
16.	PK-327	98	06	06.12	1
17.	Alnkar	93	17	18.27	1
18.	Monetta	102	67	65.68	4
19.	PS-1225	94	18	19.14	1
20.	VLS-52	105	24	22.85	2
21.	NRC-46	95	46	48.42	3
22.	PS-1024	118	32	32.20	2
23.	Pusa-24	96	28	29.16	2
24.	MACS-473	106	78	73.58	4
25.	PRS-1	64	19	29.68	2
26.	MACS-13	85	09	10.58	1
27.	Ankur	85	13	15.29	1
28.	PS-1042	89	27	30.30	2
29.	PK-416	76	08	10.52	1
30.	PS-1347	52	19	36.53	2

14.4.2 Glasshouse (controlled) condition

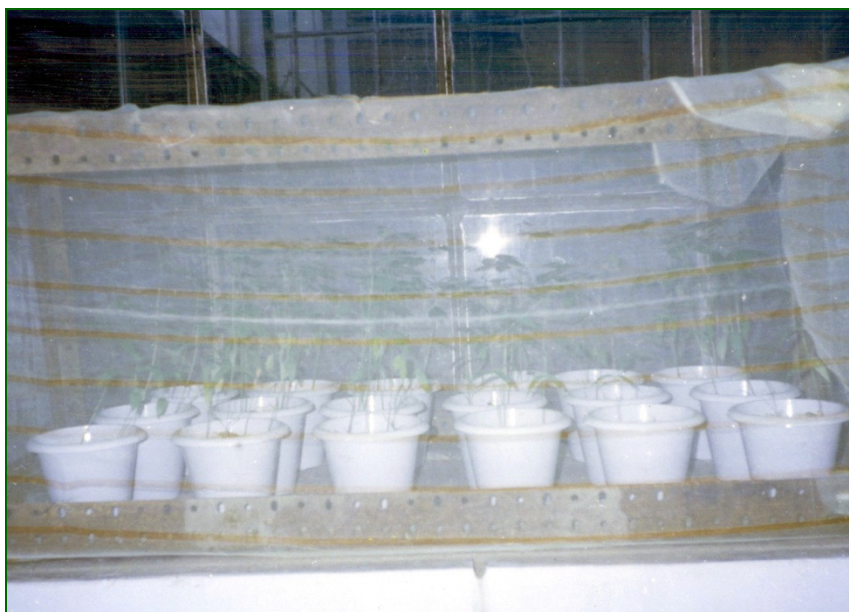
Promising cultivars/lines of soybean which showed resistance/escape under natural conditions were selected and artificially screened. The results were given in **Table 28**. In the artificial screening tests of soybean cultivars/lines Ankur, PK-327, PS-1929 and PS-1241 were categorized as resistant, Alnkar and PS 1225 as tolerant, PK-564, PK-472 and PK -416 and as susceptible and PK -564 and MACS-13 as moderately susceptible based on the disease/reaction rating scale given earlier **Plate 6**.

Table28: Artificial screening of soybean cultivars under glass house condition for resistance against soybean mosaic virus

Sl. No.	Cultivars	Total Plants	SMV Infected Plant	% SMV infection	SMV Rating (0-5 scale)
01.	Ankur	25	4	16	1
02.	Alnkar	25	6	24	2
03.	PK-327	25	3	12	1
04.	PK-564	25	11	44	3
05.	PK-472	25	13	52	3
06.	PS-1029	25	03	12	1
07.	PS-1241	25	02	8	1
08.	PK-564	25	16	64	4
09.	PS-1225	25	07	28	2
10.	MACS-13	25	18	72	4
11.	PK-416	25	14	56	3



(A)



(B)

Plate 6: Soybean germplasm screening under field condition (A) and glass house condition (B)

Discussion

Soybean, *Glycine max* (L.) Merrill a native of Eastern Asia, is an ancient oil crop with hundreds of food, feed and industrial uses. It is known as the “golden bean” of the 20th Century. There are several biotic and abiotic stresses which are responsible for the low production and productivity of soybean. Over one hundred different diseases caused by Viruses, fungi, bacteria and some non infectious diseases are known to affect soybean. Out of these about thirty five diseases are considered to be of economic importance (**Sinclair, 1984**). Soybean is known to be infected naturally by few potyviruses viz. soybean mosaic virus, peanut mottle virus, bean yellow mosaic virus, bean common mosaic virus, blackeye cowpea mosaic virus (**Sinclair, 1984; Tsuchizaki, 1986; and Dijkstra et al., 1987**). Soybean mosaic disease is world wide in distribution (**Anonymous, 1975**). In India, the disease is widely prevalent in states of Madhya Pradesh, Uttar Pradesh, Uttarakhand, Bihar, Maharashtra, Tamil Nadu and Union Territory of Delhi (**Anonymous, 1988**). According to **Dhingra and Chenulu (1980)**, the virus causes heavy losses in soybean year after year and has become highly problematic in India. The soybean mosaic virus (SMV) causes upto 94 per cent reduction in yield (**Anonymous, 1975**) and upto 82 per cent in cv. Bragg (**Dhingra and Chenulu, 1980**). The disease appears in epiphytotic proportions on soybean grown under Uttarakhand condition

almost every year depending on environmental factors and especially when the seeds collected from virus infected plants are sown.

Therefore, present investigation was aimed at gaining the better knowledge of soybean mosaic virus, its maintenance, symptomatology, host range and transmission, stability and detection of seedborne inoculum of soybean mosaic virus through dry seed examination, growing on test, indicator inoculation test and also by Enzyme linked immunosorbent assays (ELISA). Purification, electron microscopy and serology of the virus was done. Study the location of seedborne inoculum of SMV in different floral and seed parts, and establish the seedborne nature of viral inoculum. Experiment were also conducted to find out the more attractive colour and shape of trap for monitoring the aerial population of aphid; yield loss due to the seedborne inoculum, and finally screening of different germplasms /cultivars lines of soybean both under natural and glass house conditions for source of resistance.

5.1 Symptomatology

The virus under investigation caused chief symptoms of systemic mosaic, mosaic mottling and crinkling of the leaf lamina. Distinct puckering, downward curling of leaf margins and necrotic spots were observed in certain cultivars. Reduction of leaf size, slight malformation of leaf shape and stunting in infected plants were commonly observed symptoms. The symptoms caused by the virus by artificial inoculation on soybean under glass house conditions are

identical to those observed under field conditions. The overall symptom expression on soybean due to virus infection is in conformity with those reported by other workers. However, leafy enations were not observed in any of the cultivars tested in glasshouse condition. Since these leafy enations were also not observed in any of the soybean cultivars grown in field under Pantnagar (Uttarakhand) conditions. These findings are similar to **Conover (1948)** and **(Tu, 1992)**. The chief symptoms produced by SMV on soybean were described as mosaic, systemic necrosis, stunting of infected plantlets, rugosity and crinkling of the leaves. Leaves were generally deshaped, showed puckering, reduction in size and occasionally dark green enations along the veins. There is also downward curling of the margins reported by **(Gardner and Kendrick, 1921; Conover, 1948; Ling, 1951; Nariani and Pingaley, 1960; Nikolic and Stakic, 1964; Laviolette, 1968)**. The infected leaflets remain greener for a longer period of time than the non-infected leaflets. Some cultivars show transitory symptoms as yellowish vein clearing **(Gardner and Kendrick, 1921; Conover, 1948; Walters, 1958; Nariani and Pingaley, 1960; Porto and Hagedorn, 1973 and Anonymous, 1975)**.

5.2 Host Range

For many years, soybean was considered to be the only systemic host of SMV **(Pierce, 1955)**. Later, **Quantz (1961)** reported that some

species such as *Dolichos falcatus*, *Hipocrepis multisiliquosa*, *Lupinus albus*, *Phaseolus lathyroides*, *Trigonella coerula* and *T. foenum graecum* were systematically susceptible to SMV. In India, Coffee senna (*Cassia occidentalis*) has been reported as a natural host of SMV (**Singh and Gupta, 1996**). Preliminary host range studies carried out at Pantnagar isolate of SMV indicate similarities with those reported by other workers for SMV. The results revealed that eight out of 24 species tested showed susceptibility to SMV. Systemic symptoms were observed on *Glycine max* L., *Phaseolus vulgaris* L., *Phaseolus lathyroides* L., and *Vigna sinensis* L. Local lesions were obtained on inoculation in *Cyamopsis tetragonoloba* (L.) Taub, *Chenopodium album* Red, *C. amaranticolor* Costa and Reyn, while *Dolichos lablab* L. was found to be symptomless carriers of SMV. Similar findings have been reported by **Naik and Murthy, (1992a)** and **Murayama and Han (1971)**. They reported that the local lesions appeared after 5 days of inoculation on *C. amaranticolor* whereas in present study on SMV local lesions were produced after 15 days of inoculation. The difference in incubation period is either due to the virus strain involved or the difference in climatic conditions. *Dolichos lablab* was found to be a symptomless carrier of SMV as has been reported by **Galvez (1963)**.

5.3 Insect Transmission

The results on insect transmission of SMV revealed that 5 species of aphid *Aphis fabae*, *Aphis craccivora*, *Aphis gossypii*, *Myzus*

persicae and *Lipaphis erysimi* were all capable of transmitting the SMV, but maximum per cent transmission (85%) by *Myzus persicae* while minimum transmission (15%) was reported by *Aphis fabae*. In our study SMV was transmitted most efficiently by *M. persicae* followed by *A. fabae*. Similar study was reported by SMV is transmitted by several species of aphids like *Aphis fragulae*, *A. rhamni*, *A. fabae*, *Macrosiphum solanifoli*, *M. pisi*, *Myzus oronatus*, *Neomyzus circumflexus*, *M. persicae* and *Aulacorthum pseudosolani* (**Heinze and Kohler, 1940; Conover, 1948; Baldacci, 1949; and Ling, 1951**). **Nariani and Pingaley (1960)** reported *A. craccivora*, *M. persicae*, *L. erysimi* and *A. gossypii* as vectors of SMV. Sixteen species of aphids viz., *A. craccivora*, *A. fabae*, *A. fragulae*, *A. glycines*, *A. gossypii*, *A. laburni*, *A. rahmi*, *L. erysimi*, *M. pisi*, *M. solani*, *M. solanifoli*, *M. circumflexus*, *M. oronatus*, *M. persicae*, *R. maidis* and *R. prunifoli* also have been reported as vectors of SMV (**Heinze and Kohler, 1940; Usman et al., 1973 and Anonymous, 1975**).

5.4 Seed Transmission

The present study revealed that the per cent seed transmission ranged from 7.54 to 14.72 in different cultivars while in healthy no symptoms were observed. The maximum seed borne virus transmission was 14.72 per cent and 74 per cent germination was found in cultivar PK-416 while seed borne virus transmission were recorded 13.37, 12.58, and 12.14 percent in PK-308, PK-327 and

Shilajeet, respectively. The minimum seed borne virus transmission was 7.54 per cent with 88.5 per cent seed germination were recorded in cultivar Alnkar in year 2007-08. The per cent seed transmission of SMV ranged from 9.67 to 17.82 in different cultivars recorded in year 2008-09, **(Table 8 and 9)** which is in confirmation to the report by **Ross (1963)** and **Cooper and Kennedy (1966)**. The virus is seed-borne upto 76.9 per cent **(Kendrick and Gardner, 1924; Conover, 1948; Walters, 1963; Ross, 1963; and Anjos et al., 1985)** depending on the cultivar and virus strain involved. It was observed that in a sample of seeds taken from heavily infected plants, 40 per cent of the plants raised were infected **(Heinze and Kohler, 1940)**. Similarly, there was a reduction in germination of the seeds too. The mottled seeds in cultivar PK-416 showed seed transmission of 14.72 per cent with 74 per cent germination as compared to germination in healthy seed (92.5%) which is supported by **Iizuka, 1973**. Our studies also revealed that the Pantnagar isolate of SMV was seed borne and seed transmissible. However, seed transmission varied various cultivars depending upon infection severity and seed viability.

5.5 Stability of the virus in crude sap

Virus under study show TIP between 60-65°C, DEP between 10^{-4} to 10^{-5} and LIV between 4-5 days at room temperature, 7 days at 5-7° C and 15 days at in leaves stored at 5-7° C. These findings are similar to those reported by **Conover (1948), Ling (1951),**

Galvez (1963), Murayama and Han (1971) and Naik and Murthy, 1992. Nariani and Pingaley (1960) reported that the virus can survive upto 7 days at room temperature under desiccated conditions whereas, under present study the virus can survive upto 15 days under similar conditions but at a lower temperature of 5-7°C.

5.6 Purification

The method of Ross (1967) was strictly followed for the purification of the virus. However, the purified virus obtained by the method of **Ross (1967)** contained some green pigments which could not be removed by differential centrifugation and the virus concentration was not adequate. In attempting to remove the green pigments through differential centrifugation, there was loss of virus particles because of aggregation of these particles. The method used for purification in present study was a slight modification of **Ross (1967)** method and consisted of 3 differential centrifugation cycles with low speed centrifugation at 3000 g for 10 min followed by high speed at 80,000 g for 90 min. In the next cycle, the low speed centrifugation was increased from 3000 g to 5000 g for 10 min., followed by high speed at 1,00,000 g for two hours. A highly concentrated and purified virus preparation was obtained after the differential centrifugation cycles. To counter the loss of particles due to aggregation, the use of 0.2 M urea was successfully made (the use of 0.5 M or more urea resulted in the breakage of the particles). In the

present modified method, initial low speed at 3000 g for 10 min and the later low speed at 5000 g for 10 min along with 0.2 M urea was found effective in sedimenting most of the host material. This gave a clear amber colored supernatant. The pellets obtained after the differential centrifugation cycles were found to be whitish in colour without any green pigments.

5.7 Electron Microscopy

The morphology of the present virus in crude sap and purified preparation as examined under electron microscope revealed the presence of long, filamentous, flexuous particles of size 680 x 12.5 nm. This is almost similar to those reported by **Galvez (1963)**, **Liu (1963)**, **Quinions and Dunleavy (1970)**, **Soong and Milbrath (1980)**, **Anjos et al. (1985)** and **EI. Ahdal, 1996**.

5.8 UV absorption studies

In the present study, the UV absorption spectrum of the purified virus preparation showed an absorption maximum at 260 nm and a sharp absorption minimum at 240 nm. The results are in conformity with those reported by **Ross (1967)** and **Soong and Milbrath (1980)**. The A₂₆₀/A₂₈₀ ratio of the present virus was found similar to that reported by **Han and Murayama (1971)** but differs from the one reported by **Soong and Milbrath (1980)**.

5.9 Serology

5.9.1 Titre Determination

The titre of homologous antiserum of the present virus as determined by the microprecipitin test was found to be 1:512. However, **Quinions and Dunleavy (1970)**, **Gerdeichuk and Sokolova (1972)** and **Anjos *et al.* (1985)** have, reported a titre of 1:2048, 1:128 and 1:1024, respectively of homologous antisera of SMV in microprecipitin tests .

5.9.2 Development of Polyclonal Antiserum

In the present investigations the virus was detected following the basic procedure described by **Clark and Adams (1977)**. Detection of SMV using ELISA has been reported earlier by **Lister (1978)**, **Moore *et al.* (1982)**, **Chen *et al.* (1982)**, **Iwai *et al.* (1985)**, **Yu *et al.* (1989)**, **Dong *et al.* (1997)**, and **Sun *et al.* (2008)**. Indirect ELISA has been used to establish new strains of SMV (**Almeida *et al.*, 1995**). Within field, spread of SMV has also been quantified using strain specific monoclonal antibodies (**Nutter *et al.*, 1998**). Serological relationship between different viruses has been also worked out through ELISA (**Jain *et al.*, 2000**). The extraction buffer and Tris-EDTA with 2 per cent PVP and 0.2 per cent ovalbumin successfully extracted the virus for its detection. In present study the homologous antiserum with a titre of 1:512 were used. For dilutions of the antiserum 1:50, 1:100, 1:250 and 1:500 were used. The virus could be detected from infected leaf samples even at a dilution of

1:500. Several workers have previously encountered the problem of background reaction. This is because of a non-specific reaction between the sample from healthy plants and the antiserum. This problem was countered by cross absorption of the antiserum with samples from healthy plant so that there is no background reaction later on. A_{405} value was read after 2 hours and 18 hours of incubation in order to have a confirmatory assessment. It was found that A_{405} value did not increase considerably after 18 hours of incubation. It is therefore sufficient to finally assess the reaction, after 2 hours of incubation. **Lister (1978)** had used the homologous antiserum with a titre of 1:256 as determined by the microprecipitin tests.

5.10 Location of seed inoculum of SMV in different flower and seed parts of soybean:

In present investigation the morphologically abnormal seeds showing reduced seed size, seed coat mottling and shrivelling have been known to cause a higher percentage of transmission of virus. Seeds of variety PK-416 of soybean with systemic infection of SMV showed certain adverse effect on seed morphology. A close and careful visual observation of seeds collected from systemically SMV infected seeds of variety PK-416 revealed the presence of six categories of morphologically abnormal seed, collected from systemically SMV infected plant. All the above-mentioned categories of morphologically abnormal seeds viz. small shrivelled, large shrivelled, oversized seeds, undersized seeds, off-coloured, and mottled seeds obtained

from systemically SMV infected plants. Small shrivelled seeds exhibited 64.0 per cent germination followed by 72.0 per cent germination in large shriveled, 84.0 per cent in oversized seeds, 76.0 per cent in undersized seeds and 88.0 and 76.0 per cent germination in off coloured and mottled seeds, respectively. All the six categories of morphologically abnormal seed, collected from systemically infected plants yielded SMV infection ranging from 9.09 to 78.94 per cent in the growing on test suggesting the transmission of seedborne inoculums of SMV. A highest of 78.94 per cent seed transmission was obtained in mottled seeds followed by 22.22 per cent in large shrivelled seeds, 18.75 per cent in small shrivelled seeds, 14.28 per cent in oversized seeds and 10.52 and 9.09 per cent seed transmission, respectively recorded in soybean variety PK-416. Presence of seedborne inoculum of SMV in various categories of morphologically abnormal seeds in the same variety was also confirmed using DAC-ELISA test. Results indicate that mottled seeds gave very heavy positive reaction, while small shriveled and large shrivelled seeds gave heavy positive reaction, oversized gave moderate positive reaction, and undersized and off-coloured seeds gave light positive reaction in DAC-ELISA test **(Table 16)**. **Bowers and Goodman (1979)** detected SMV in flowers, green pods immature seeds, testa, cotyledons and embryos. **Anjos et al. (1985)** reported the presence of SMV in leaves, stems and pods but not in the roots during all stages of growth using serological tests. **Iwai et al. (1985)**

reported that both mild and severe strains of SMV were detected in seed coat and embryo, but only mild strain was detected in the cotyledons using ELISA.

Indicator inoculation test and serological test (DAC-ELISA) were performed to study the location of infection in soybean seed. The indicator plants when sap inoculated at primary leaf stage with the extracts prepared from different flower and seed parts resulted in the transmission of SMV infection. The assay results of different floral parts i.e. epicalyx, calyx, corolla, androecium and gynoecium obtained from systemically SMV infected plants showed the presence of SMV in all floral parts (**Table 17**). The presence of virus in the various seed components viz., seed coat, cotyledons and embryo was also detected in indicator inoculation test (**Table 18**). Symptoms of SMV started appearing on the second trifoliate leaf stage onwards. The third trifoliate, generally showed light green colour and increased size followed by conspicuous crinkling of the upper surface. The crinkling usually became evident at 15 days after inoculation. Subsequently produced trifoliate also showed similar symptoms. The indicator inoculation test has successfully been performed to find out the presence of SMV in different parts of soybean seed by **Anjos *et al.* (1985)** and **Bowers and Goodman (1979)**. Similar report were reported by **Iizuka (1973)** reported that the virus is pollen transmitted and present in immature seed coats and embryos of infected seeds. It was also demonstrated that embryos and cotyledons

of infected seeds can transmit the virus whereas seed coat can not **(Porto and Hegedon, 1973). Nicolaescu (1987)** developed a simple method of detection of SMV by individuals testing of embryos from seed. **Bawja and Pacumbaba (1996)** reported that SMV was detected in individual embryo of both mottle and nonmottled soybean seed. It was not detected in individual stained and non stained seed coats and in endosperms of either type of seeds. However, virus was detected in individual hypocotyls in post germinated seeds. **Dong et al. (1997)** suggested that the principle route of soybean embryo infection was by invasion of ovules.

However it is difficult to estimate the per cent seed infection, unless individual seed extract is tested for assay. This method would be useful especially for quarantine purpose where it may be important to know only the presence or absence of the virus with the seeds. It may, therefore, be inferred on the basis of indicator inoculation test that the SMV was recoverable from the infected floral and seed parts in quantities sufficient to perform assay.

To investigate whether the virus was present on seed surface as a contaminant, seeds of variety PK -416 of SMV from systemically SMV infected plants were used. The location of the SMV in soybean seeds has further been supported by experiment on seed surface washings. The test plants when inoculated with seed washings did not develop disease symptoms at any growth stage of the plant indicating

the absence of virus in seed washings. DAC-ELISA using seed surface washings as antigen also did not give any indication of the presence of virus in such washings. Moreover, seeds with or without seed coat, when planted also showed more or less the same transmission (13.32%), while infected embryo inoculated gave 22.64 per cent transmission, thus indicating that the virus is internally present in the SMV seeds. All the three tests when considered together strongly indicate that SMV is internally seedborne, located within the embryo and cotyledons of the soybean seeds and it is not present as surface contaminant. Similar results have been shown, in pea seed borne mosaic virus (**Stevenson and Hagedorn, 1973**), in cowpea aphid borne mosaic virus (**Pathak, 1974**), and bean common mosaic virus in French bean (**Ekpo and Saettler, 1974**) and in soybean **Bawja and Pacumbaba (1996)**.

5.11 Monitoring aerial population of aphid (*Myzus persicae*), the vector SMV

5.11.1 Evaluation of trap colour

Bright golden colour traps attracted maximum aphid adults followed by pale yellow colour traps. Very few of the aphids were attracted towards the brown yellow colour. Similar result has already been reported by several workers. **Mohanti and Basu (1991)** used golden yellow water pan traps for monitoring aerial population of white flies.

5.11.2 Shape of traps

Observations were made during two consecutive years clearly showed that cylindrical shape traps were more effective in attracting aphids as compared to lamp shape. Effectiveness of cylindrical traps may be due to more reflection of sun rays as compared to lamp shaped trap.

5.12 Seasonal variations in the aerial populations of aphid

Observations, made during two consecutive years clearly showed that maximum aphids trapped in second fortnight of July and September. The number of aphid increased as the temperature slightly decreased and per cent relative humidity decreased. Less number of aphid adults was trapped during first fortnight of September and second fortnight of November due to decrease in relative humidity, while in November due to low temperature. **Nene (1973), Gill and Rataul (1986) and Mohanti and Basu (1991)** also reported that rapid multiplication and activity of *B. tabaci* largely was controlled by rains particularly when there were regular showers with strong winds. The subsequent decline in trap catches and their total absence during peak winter reasonably be concluded due to low temperature (**Nene, 1973 and Mohanti and Basu (1991)**).

5.13 Yield losses in soybean due to the soybean mosaic virus

A number of parameters such as pods per plant, pod length, number of seeds per pod, yield per plant, 100 seed weight, and yield reduction were studied in PK-416 variety of soybean. As revealed in

table, deterioration of all the yield contributing factors and yield in SMV infected plants was observed as compared to healthy plants. For year 2007-08 resulted revealed that all the above mentioned yield contributing factors were adversely affected as a result of SMV infection. Average number of pods per plant was significantly lower in plant infections inoculated at 15 DAS to 65 DAS as compared to healthy plants. A minimum average of 5.2 pods/ plant was found in plants inoculated at 15 DAS, while the maximum average number of pods was 45.2 pods/ plant at 65 DAS. In healthy plants an average of 62.8 pods/ plant was recorded. However, plants when inoculated 15 DAS of planting significant decrease in average pod length 1.7 cm was observed as compared to healthy plants. An average of 14.40 seeds per plant were recorded in case of plants infected at 15 DAS as compared to an average number of 94.20 and 127.6 seeds/ plant from plants infected at 65 DAS and healthy plants, respectively. Hundred seed weight was 4.50 gm in plants infected 15 DAS; 13.10 gm in plants infected at 65 DAS and was 15.48 gm when collected from healthy plants. Further, the SMV infection at different stages of plant growth, significantly, affected the seed yield. Average yield/plant was 1.42 gm in plant infected 15 DAS, 14.47 gm in plant infected at 65 DAS and 20.58gm when collected from healthy plant. Maximum yield reduction of 93.08 per cent was found when plant infection occurred at 15 DAS, which got significantly reduced with the delay in infection at 65 DAS where it caused only 29.63 per cent loss in yield.

Thus, the yield loss ranged from 29.63 to 93.08 per cent in soybean plants of variety PK-416 depending upon the plant age at the time of infection.

For year 2008-09 results revealed that the average number of pods /plant was 7.4, 19.6, 22.6, 27.8 and 43.4 in plant inoculated with SMV after 15 DAS, 25DAS, 35 DAS, 45 DAS and 65 DAS, respectively. In healthy plant it was 58.40 pods/plant (**Table 25**). In terms of average length of pod a minimum average of 1.50 cm was found in plants inoculated at 15 DAS, while the maximum average length of pods was 3.92 cm at 65 DAS. In healthy plants an average of 4.34 cm was recorded. However, plants when inoculated 15 DAS a significant decrease in average number seeds /plant (14.00 seeds/plant) as compared to an average number of seeds (88.60 and 126.20 seeds/ plant) from plants infected at 65 DAS and healthy plants. Hundred seed weight was 4.24 gm in plants infected 15 DAS; 15.25 gm in plants infected at 65DAS and was 16.42 gm when collected from healthy plants. Maximum yield reduction of 93.24 per cent was found when plant infection occurred at 15 DAS, which was significantly reduced with the delay in infection at 65 DAS where it caused only 19.92 per cent loss in yield. The results show that there is a progressive decrease in reduction in yield with increase in the age of the plant and virus infection.

Critical analysis of results indicates that the reduction in number of pods per plant is the most important single factor which affected the

yield adversely. Pods per plant were reduced because the SMV infection has been shown to cause considerable malformation of the inflorescence and pollen sterility (**Mohammad et al., 2003**). The variation in yield losses reported by different workers may be due to different environmental conditions at their work place, stage of infection and presence of insect vectors in an area causing secondary spread. Various workers have also reported the effect of SMV on yield contributing parameters and yield. **Dhingara and Chenulu, 1980, Tu, 1989** and **Haque et al., 1993**.

5.14 Screening for Resistance

In the present study, 30 cultivars of soybean were screened for resistance to SMV. The screening was done under field conditions in the *kharif* season of 2007-08 and 2008-09. The cultivars were divided into 6 categories depending upon their reaction to the disease. The significant observation was that no cultivar was found to be immune. Out of these cultivars PK - 327, PK - 472, PK - 564, PK - 416, Alnkar, and Ankur showed resistant reaction of disease (**Table 20, 21 and 22**). Some initial studies have been done by **Ross (1969)**, **Sundaresh et al. (1984)** and **Suteri (1986)**. **Zhuang et al. (1996)** have reported high levels of resistance in perennial wild soybean (*G. canescens*, *G. clandestina* and *G. tomentella*). **Ren et al. (2000)** have reported that increased pubescence is positively correlated with resistance to SbMV in soybean. Markers to identify Rsv 1 the gene responsible for

resistance to SMV have been developed (**Hayes and Maroof, 2000**). **Zheng et al. (2000)** have screened 348 soybean accessions for resistance to SMV.

Out of 11 promising germplasm /cultivars (which showed resistance/escape under field conditions) tested Ankur PK-327, PS-1929 and PS-1241 showed resistance under artificial screening with SMV virus.

This indicates that this screening could be used as a reliable instrument of identifying sources of resistance to SMV.

Summary and Conclusion

One third of all known plant viruses are now recognized as potyvirus and a current estimate is that more than 180 definitive and possible members belong to this group. The inherent diversity among members within this group has created considerable difficulties in characterizing and establishing relationships between many of its members. In India, although more than 30 potyviruses are reported, their identification is mainly based on symptomatology and host range studies. These criteria cannot provide correct identification of viruses and their strains, which is important to know, their distribution, epidemiology and for breeding crop plants for disease resistance. Thus, the present investigations were carried out on detection (biologically and serologically), transmission, host range, and resulting losses. Soybean mosaic virus (SMV) has now become a potential threat to the cultivation of soybean in India. The results obtained during present studies have been summarized below:

1. This virus culture was obtained from naturally infected soybean. A single local lesion culture from a local lesion host *Chenopodium amaranticolor* was maintained on the systemic host *Glycine max*.
2. The chief symptoms caused by the virus on artificially inoculated cv. PK-416 were systemic mosaic, puckering, mosaic mottling,

reduction in leaf size and stunting of infected plantlets. Similar symptoms were observed in field conditions too.

3. The host range studies revealed that the virus infected systemically *Glycine max*, *Phaseolus vulgaris*, *P. lathyroides* and *Vigna sinensis* causing general symptoms of systemic mosaic mottling, reduction of leaf lamina, and stunting of plants. *Cyamopsis tetragonoloba*, *C. amaranticolor* and *Chenopodium amaranticolor* were the local lesion hosts of the virus. The virus causes 1-2 mm sized dark brown to blackish necrotic local lesions on *Cyamopsis tetragonoloba* and 2-3 mm reddish brown local lesions on *Chenopodium amaranticolor*, which soon coalesce to form necrotic patches. *Dolichos lablab* found to be symptomless carriers of the virus.
4. The virus was readily sap as well as aphid transmissible. The results showed that 5 species of aphid *Aphis fabae*, *Aphis craccivora*, *Aphis gossypii*, *Myzus persicae* and *Lipaphis erysimi* were all capable of transmitting the SMV. However, maximum per cent transmission of 85% was by *Myzus persicae* while minimum transmission of 15% was by *Aphis fabae*. The virus was transmitted by different aphid species in a non-persistent manner.
5. In the relative efficacy tests, *Myzus persicae* was found to be the most efficient vector of the virus. Ten aphids each were given a pre acquisition fasting of 2 hrs, virus acquisition feeding period

of 5 min and inoculation access period of 12 hours. *Myzus persicae* gave the highest i.e. 78.33% virus transmission. It was followed by *Aphis craccivora* with 61.66%.

6. The investigations revealed that there is a strong relationship between the aphid population and disease. At higher aphid populations, there is a greater incidence of the disease. However, the investigations indicate that the primary source of inoculum remains the infected seeds.
7. The per cent seed transmission in collected from infected plants ranged from 7.54 to 14.72 in year 2007-08 while in 2008-09 it 9.67 to 17.82 recorded in different cultivars, respectively with no symptom in plant raised from healthy seeds. The maximum seed borne virus transmission was 14.72 per cent with 74 per cent germination in cultivar PK-416.
8. Studies on stability of the virus in crude sap consisted of determination of TIP, DEP and LIV. The virus had a TIP between 60-65 ° C, DEP between 10^{-4} to 10^{-5} and LIV between 4-5 days at room temperature, 7 days at 5-7° C, and 15 days in leaves stored at 5-7 °C.
9. The virus was successfully purified employing a modification of Ross's method (1967). The method consisted of 3 cycles of differential centrifugation followed by sucrose density gradient centrifugation. The final purified virus preparation was highly concentrated without pigments.

10. Purified virus preparations gave an absorption maxima at 260 nm and absorption minima at 245 nm indicating a typical nucleoprotein spectrum in the UV spectrophotometer.
11. Long flexuous filamentous particles of average size 680 x 12.5 nm were observed in crude sap as well as in purified preparations when they were examined under the electron microscope.
12. Polyclonal antiserum of the virus purchased catalog no. SRA 33300 with format ELISA reagent set from Agdia Chemicals with purified virus preparations. The titre of the antiserum was found to be 1:512 by microprecipitin, tests.
13. The morphologically abnormal seeds showing reduced seed size, seed coat mottling and shrivelling have been known to cause a higher percentage of transmission of SMV. Seeds of variety PK-416 of soybean with systemic infection of SMV showed certain adverse affect on seed morphology.
14. All the six categories of morphologically abnormal seed, collected from systemically SMV infected plants yielded SMV infection ranging from 9.09 to 78.94 per cent in the growing on test suggesting the transmission of seedborne inoculum of SMV in soybean.
15. DAC-ELISA test could successfully be employed for detection of SMV. Mottled seeds gave very heavy positive reaction, while small shriveled and large shrivelled seeds gave heavy positive

reaction, oversized seeds gave moderate positive reaction, and undersized seeds and off-coloured seeds gave light positive reaction in DAC-ELISA test.

16. The indicator plants when sap inoculated at primary leaf stage with the extracts prepared from different flower and seed parts resulted in the transmission of SMV infection. The assay results of different floral parts i.e. epicalyx, calyx, corolla, androecium and gynoecium, obtained from systemically SMV infected plants showed the presence of SMV in all floral parts. The presence of virus in the various seed components viz., seed coat, cotyledons and embryo was also detected in indicator inoculation test and DAC- ELISA.
17. Bright golden colour traps attracted maximum aphid adults followed by pale yellow colour traps. Very few of aphids were attracted towards the brown yellow colour.
18. Cylindrical shape traps were more effective in attracting aphids as compare to lamp shape. Effectiveness of cylindrical traps may be due to more reflection of sun rays as compare to lamp shape trap.
19. The highest number of aphid adults was trapped during second fortnight of July when the average maximum and minimum temperature between 30.67 and 24.64 °C, respectively with 80.87 % relative humidity. Thereafter, a decline in trap catches was observed.

20. A minimum average of 5.2 pods/ plant was found in plants inoculated at 15 DAS, while the maximum average number of pods was 45.2 pods/ plant at 65 DAS. In healthy plants an average of 62.8 pods/ plant was recorded. Hundred seed weight was 4.50 gm in plants infected 15 DAS; 13.10 gm in plants infected at 65DAS and was 15.48 gm when collected from healthy plants.
21. Out of the total of 30 cultivars tested in the *kharif* of 2007-08 and 2008-09, six were found to be resistant in both the years. These are viz. PK - 327, PK - 472, PK - 564, PK - 416, Alnkar, and Ankur.
22. Out of 11 promising germplasm /cultivars which showed resistant reaction or escape under field conditions were Ankur, PK-327, PS-1929 and PS-1241 showed resistance under artificial screening with SMV virus.

*Literature
Cited*

LITERATURE CITED

- Akhtar, A., Sher, H., Ali, A., and Hassan, S. (1992).** Biological characterization of soybean mosaic virus. *Sarhad J. of Agriculture*. **8** (5) : 555-561.
- *Almeida, A.M.R. (1995).** Evaluation of soybean tolerance to soybean mosaic virus. *Fitopato.-Bras.* **20** (1) : 24-29.
- Almeida, A.M.R., Almeida, L.A., and Kiihl, R.A.S. (2001).** Screening of soybean genotypes for resistance to two strains of soybean mosaic virus. *Fitopatologia Brasileira*. **26**(1) : 99.
- Almeida, A.M.R, Almeida, L.A. de, Kuhl, RAdS., Domit, L.A., De Almeida, L.A. (1995).** Identification of a new strain of soybean mosaic virus in Brazil. *Arquivos de Bialogi e Technologia*. **38** (4) : 1095-1100.
- *Anjos, J.RN. Lin, M.T., and Kitajima (1985).** Characterization of a Brazilian isolate of soybean mosaic virus. *Fitapatol Bras.* **10** : 143-157.
- Anonymous (1948).** Plant diseases. Notes contributed by the biological branch. *Agr. Gaz. New South Wales* **59** : 527-530.
- *Anonymous (1962).** Record of research work carried out in 1960. Deptt of Agr. Tanganyika. pp116.
- *Anonymous (1972).** Annual report, East. African Agr. and For. Res. Org., record of research for 1971, Nairobi, Kenya.
- Anonymous (1988).** All India Coordinated Research Project on soybean. 19th Annual workshop held at TNAU, Coimbatore. pp136.
- Anonymous (1975).** Compendium of soybean disease. The American phytopathological society, Inc. pp 69.
- Anonymous (2008).** Soybean and soy oil price forecast 2008-09. A *JRG commodities research publication*. pp 1-16.
- Armer, C.A., Wiedenmann, R.N. and Irwin, M.E. (1999).** Effects of soybean mosaic virus on the facultative phytophagous predator *Orius insidiosus* (Heteroptera : Anthocoridae). *Environmental Entomology*. **28**(6) :1036-1043.

- Bajwa, M.A. and Pacumbaba, R.P. (1996).** Location of soybean mosaic virus in seed-parts of individual dormant and germinating mottled and non-mottled soybean seeds. *Journal of Phytopathology*. **144**(3):151-155.
- *Baldacci, E. (1949).** Soybean mosaic in Italy. *Olearia*. **3** : 111-112.
- *Baudys, E. (1931).** Phytopathological notes VII. *Ochrana Rostlin*. **11** : 178-197.
- Bellardi, M.G., Marani, F., and Bertaccini, A. (1993).** Detection of soybean mosaic virus (SMV) in *Chrysanthemum frutescens*. *Phytopathologia Mediterranea*. **32**(2) :156-158.
- Bensch, D., Pappu, S.S., Niblett, C.L., Varón de Agudelo, F., Morales, F., Hodson, E., Alvarez, E., Acosta, O., and Lee, R.F.(1996).** A strain of soybean mosaic potyvirus infecting *Passiflora* spp. in Colombia. *Plant Disease*. **80** (3) :258-262.
- *Bilyk, L.H. and Legunkova, R.M. (1966).** Electron microscope studies on the soybean mosaic virus. *Mykrobiol. Zh.* **28** : 39-42.
- *Bilyk, L.H.(1965).** Distribution of soybean virus in Ukraine. *Mykrobiol. Zh.* **27** : 40-45.
- Blottenberg, H. and Irwin, M.E. (1992).** Using mixed cropping to limit seed mottling induced by soybean mosaic virus. *Plant Disease*. **76** (3) : 304-306.
- Bos, L. (1972).** Soybean mosaic virus. CMI/AAB Descriptions of Plant Viruses No, **93**.
- Bowers, G.R, Jr. and Goodman, R.M. (1979).** Soybean mosaic virus Infection of soybean seed parts and seed transmission. *Phytopathology*. **69** : 569-572.
- Brandes, J. and Wetter, C. (1959).** Classification of elongated plant viruses on the basis of particle morphology. *Virology*. **8** : 99-115.
- Bryant, G.R., Hill, J.H., Bailey, T.B., Tachibana, H., Durand, D.P., and Bennet, H.I. (1982).** Detection of soybean mosaic virus in seed by solid-phase radioimmunoassay. *Plant Disease*. **66** (8) :693-695.
- Burrows, M.E.L, Boerboom, C.M., Gaska, J.M, and Grau, C.R.(2005).** The relationship between *Aphis glycines* and Soybean mosaic virus incidence in different pest management systems. *Plant Disease*. **89** (9) : 926-934.

- Castano, M. and Morales, F.J. (1983).** Seed transmission of soybean mosaic virus in *Phaseolus vulgaris* L. *Fitopatologia Brasileira*. **8**(1):103-107.
- Chen, C., Durand, D.P. and Hill, J.H. (1982).** Detection of pathogenic strains of soybean mosaic virus by Enzyme Linked Immunosorbent Assay with polystyrene plate and beads as the solid phase. *Phytopathology*. **72** : 1177-1181.
- *Chen, Y. Chu, M., and Sun, J. (1981).** Identification of a mosaic disease of soybean in Jiangsu. *Acta Phytopathologica Sinica*. **11** : 31-36.
- Chen, Z.Y., Ruan, Y.L, and Jin, D.D. (1984).** Rapid diagnosis of soybean mosaic disease by electron microscopy in Szechwan. *Shanghai Agricultural Science and Technology*. No. **6** :26.
- Cho, E.K., Choi, S.H. and Hwang, C.Y. (1984).** Epidemiology of soybean mosaic virus diseases. *Korean J. Plant Protection*. **23** : 197-202.
- Cho, E.K., and Goodman, R.M. (1979).** Strains of soybean mosaic virus: classification based on virulence in resistant soybean cultivars. *Phytopathology*. **69**(5) :467-470.
- Cho, E.K., Chung, B.J., and Lee, S.H. (1977).** Studies on identification and classification of soybean virus diseases in Korea. II. Etiology of a necrotic disease of *Glycine max*. *Plant Disease Reporter*. **61** (4): 313-317.
- Clark, M.F. and Adams, A.N. (1977).** Characteristics of the microtitre plate method of enzyme linked immunosorbent assay for the detection of plant viruses. *J. Gen. Viral*. **34** : 475-483.
- Clark, M.F. and Bar Joseph, M. (1984).** Enzyme immunosorbent assays in plant virology. In : Methods in Virology (Eds. Maramorsch, K. and Roprowski, H. Vol. VII. pp. 51-85. Academic Press, New York.
- *Clinton, G.P. (1916).** Notes on plant diseases of Connecticut. Agr. Expt. Sta. Ann. Rpt., pp 421-451.
- Close, D.C., Watson, D.R. and Dingley, J.M. (1970).** Soybean disease in New Zealand. New Zealand. *J. Agr.* **120** :79.

- Conover, RA. (1948).** Studies of two viruses causing mosaic disease in soybean. *Phytopathology*. **38** : 724-735.
- Cooper, R.L. and Kennedy, B.W. (1966).** Soybean mosaic linked to seed coat mottling. *Crops and Soils*. **19** : 10.
- Costa, A.S.; Da Costa, Lima Neto V., Almeida, L.D., and Bulisani, E. (1978).** Systemic invasion of bean varieties of certain groups by the soybean mosaic virus. *Fitopatologia Brasileira*. **3** :27-37.
- De Guerpel, H. (1942).** The pests and diseases of soybean. *Rev. Bot. Appl and Agr. Trap*. **17** : 195-201.
- *De Vasconcelos, F.A.T. (1964).** A contribution to the study of soybean mosaic virus. *Anais. Inst. Sup. Agran. Univ. Tech. Lisb*. **26** : 181-223.
- Demski, J.W. and Jellum, M.D. (1975).** Single and double virus infection of soybean : plant characteristics and chemical composition. *Phytopathology*. **65**(10) :1154-1156.
- Demski, J.W. and Kuhn, C.W. (1989).** Soybean mosaic virus. In : Sinclair JB, Backman PA, eds. *Compendium of Soybean Diseases* 3rd ed. St Paul Minn., USA : APS Press, 55-57.
- Dhingra, K.L. and Chenulu, V.V. (1980).** Effect on soybean mosaic virus on yield and nodulation of soybean cv. Bragg. *Indian Phytopathology*. **33** : 586-590.
- Dhingra, K.L. and Chenulu, V.V. (1981).** Studies on the transmission of urd leaf crinkle and chickpea leaf reduction viruses by *Aphis craccivora* Koch. *Indian phytopath.* **34** : 38-42.
- Diaco, R; Hill, E.K.; Hill, J.H.; Tachibana, H. and Durand, D.P. (1985).** Monoclonal antibody based biotin-avidin ELISA for the detection of soybean mosaic virus in soybean seeds. *J. Gen.Viral*. **66** : 2089-2094.
- *Dickson, B.T. (1924).** Mosaic studies IV. *Phytopath.***14**: 346.
- Dijkstra, J.; Bos, L.; Bourneester, H.J.; Hadistono, T. and Lohmis, H. (1987).** Identification of blackeye cowpea mosaic from germplasm of yard long bean and from soybean and relationships between blackeye cowpea mosaic virus and cowpea aphid borne mosaic virus. *Neth. J. Plant Pathol*. **93** :113-114.

- Dilghton, F.C. and Tinsleey, T.W. (1958).** Notes on some plant virus diseases in ghana and Sierra Leonon. *West African Sci. Assoc.* **4** : 4-8.
- Domier, L.L.; Latorre, I.J.; Steinlage, T.A.; McCoppin, N. and Hartman, G.L. (2003).** Variability and transmission by *Aphis glycines* of North American and Asian Soybean mosaic virus isolates. *Archives of Virology.* **148** (10) : 1925-1941.
- Domier, L.L.; Steinlage, T.A.; Hobbs, H.A.; Wang, Yi.; Herrera, Rodriguez, G.; Haudenshield, J.S.; Mc Coppin, N. and Hartman, G.L. (2007).** Similarities in seed and aphid transmission among Soybean mosaic virus isolates. *Plant Disease.* **91** (5) : 546-550.
- *Dong, H.; Pu, Z.j Pu, Z.; Dong, H.P.; Pu, Z.M. and Pu, Z.Q. (1997).** On the soybean embryo invasion by soybean mosaic virus. *Journal of Nanjing Agricultural Univ.* **20** (1) : 35-38.
- Dunleavy, J.M.; Quiniones, S.S. and Krass, C.J. (1970).** Poor seed quality and rugosity of leaves of virus-infected Hood soybeans. *Phytopathology.* **60** : 883-886.
- Ekpo, E.J.A. and Saettler, A.W. (1974).** Distribution pattern of bean common mosaic virus in developing bean seeds. *Phytopathology.* **64** : 269-270.
- El-Ahdal, A.M.B. (1996).** Purification of soybean mosaic virus for antiserum preparation. *Annals of Agr. Sci. Cairo.* **41** (1) : 197-205.
- El-Amrety A.A.; El-Said, H.M. and Salem, D.E. (1985b).** Effect of soybean mosaic virus interaction on quality of soybean seed. *Agricultural Research Review.* **63**(2) : 155-164.
- El-Amrety, A.A.; El-Said, H.M. and Salem D.E. (1985a).** Evaluation of response of different soybean cultivars to soybean mosaic virus infection. *Agricultural Research Review.* **63** :145-153.
- Fereres, A.; Kampmeier, G.E. and Irwin, M.E. (1999).** Aphid attraction and preference for soybean and pepper plants infected with Potyviridae. *Annals of the Entomological Society of America.* **92** (4): 542-548.
- Galvez, G.B. (1963).** Host-range, purification and electron microscopy of soybean mosaic virus. *Phytopathology.* **53** : 388-393.

- *Garbowski, L. and Juraszkowna, H. (1933).** Diseases of useful plants in the period of 1926-1930. A summary of reports of the plant protection stations. *Rocznik Ochrony Roslin Sect. A1* : 97-235.
- Gardner, M.W. and Kendrick, J.B. (1921).** Soybean mosaic. *J. Agr. Res.* **22** : 111-114.
- *Gerdeichuk, A.I. and Sokolova, E.E. (1972).** Preparation of diagnostic antisera for soybean mosaic virus. *Vest. Sei. Khoz. Naubi.* **17** : 101-103.
- Gill, C.K. and Rataul, H.S. (1986).** A note on the incidence, estimation of losses and symptomology of yellow mosaic virus on soybean, *Glycine max.* *Journal of Entomology.* **48** : 524-526.
- Goodman, KM. and Oard, J.H. (1980).** Seed transmission and yield losses in tropical soybeans infected by soybean mosaic virus. *Plant Disease.* **64** : 913-914.
- Goodman, KM.; Bowers, G.K, Jr and Paschal, E.H. (1979).** Identification of soybean lines and cultivars with low incidence of soybean mosaic virus transmission through seeds. *Crop Science.* **19** : 264-267.
- Gough, K.H. and Shukla, D.D. (1981).** Coat protein of potyviruses 1. Comparison of kur Australian strains of sugarcane mosaic virus. *Virology.* **111** : 455-462.
- *Grancini, P. (1941).** II Mosaic" delta soja. *Avvenire Agr. Milano.* **49** : 231-235.
- Guo, Z.; Gai, J.; Guo, Z. and Gai, J.Y. (1997).** Growth and morphogenesis of leaf culture in vitro from soybean plants inoculated with soybean mosaic virus (SMV). *Acta Phytopath. Sinica.* **27** (2) : 125-128.
- Halbert, S.E. and Irwin, M.E. (1981).** Effect of soybean canopy closure on landing rates of aphids with implications for restricting spread of soybean mosaic virus. *Ann. Appi. Biol.* **98** (1) : 15-19.
- Halbert, S.E.; Irwin, M.E. and Goodman, KM. (1981).** A late aphid species and their relative importance as field vectors of soybean mosaic virus. *Ann. Appi. Biol.* **98** : 1-9.
- *Han, Y.H. and Murayama, D. (1971).** Studies on soybean mosaic virus I. Separation of virus strains by different host. *J. Fac. Agro. Hokkaido Univ.* **56** : 303-310.

- *Han, Y.S. (1959).** Soybean diseases in Taiwan. *Agr. Assoc. China (Taiwan).J.* **26** : 31-38.
- *Hansford, C.G. (1934).** Annual report of mycologist. *Deptt. Agr. Uganda Ann. Rept.* **2** : 48-51.
- Haque, G.; Arif, M.; Hassan, S.; Ali, A. and Khan, M. (1993).** Assessment of yield losses in soybean due to soybean mosaic virus. *Sarhad Journal of Agriculture.* **9**(3) :227-229.
- Hayes, A.J. and Maroof, M.A.S. (2000).** Targeted resistance gene mapping in soybean using modified AFLPS. *Theoretical and Applied Genetics.* **100** (8) : 1279-1283.
- Heath, K.G. (1958).** Annual report of the department of agriculture, Federation of Malaya, for the year 1957.
- *Heinze, K. and Kohler, E. (1940).** Die mosaik-krankheit der sojabohne und ihre ubertragung durch insekten. *Phytopath Z.* **13** : 207-242.
- Hepperly, P.R.; Bowers, G.R.Jr.; Sinclair, J.B. and Goodman, R.M. (1979).** Predisposition to seed infection by *Phomopsis sojae* in soybean plants infected by soybean mosaic virus. *Phytopathology.* **69** (8) : 846-848.
- Hill, E. K.; Hill, J.H. and Durand, D.P. (1984).** Production of monoclonal antibodies to viruses in the potyvirus group : use in radioimmunoassay. *J. General Virology.* **65** (3) 525-532.
- Hill, J.H.; Lucas, B.S.; Benner, H.I.; Tachibana, H.; Hammond, R.B. and Pedigo L.P. (1980).** Factors associated with the epidemiology of soybean mosaic virus in Iowa. *Phytopathology.* **70**(6) :536-540.
- Hill, S.A. (1984).** Methods in plant virology. Blackwell Scientific Publications, Oxford, pp 167.
- Hill, J.H., and Benner, H.I. (1980a).** Properties of soybean mosaic virus ribonucleic acid. *Phytopathology.* **70**(3) :236-239.
- Hill, J.H. and Benner, H.I. (1980b).** Properties of soybean mosaic virus and its isolated protein. *Phytopathologische Zeitschrift.* **97**(3) :272-281.
- Hobbs, H.A.; Hartman, G.L.; Wang, Y.; Hill, C.B.; Bernard, R.L.; Pedersen, W.L. and Domier, L.L. (2003).** Occurrence of seed coat mottling in soybean plants inoculated with Bean pod mottle virus and Soybean mosaic virus. *Plant Disease.* **87** (11) : 1333-1336.

- Hodgson, E.W.; Koch R.L. and Ragsdale D. W. (2005).** Pan Trapping for Soybean Aphid (Homoptera Aphididae) in Minnesota Soybean Fields. *J. Entomo Society*. **4** : 409-419.
- Hopkins, J.C.F. (1932).** A list of plant diseases occurring in Southern Rhodesia. Supplement 2. (June, 1931-May, 1932). *Rhodesia Agr. J.* **29** : 462-467.
- Hunst, P.L. and Tolin, S.A. (1982).** Isolation and comparison of two strains of soybean mosaic virus. *Phytopathology*, **72**(7) :710-713.
- *Iizuka, N. (1973).** Seed transmission of viruses in soybean. *Bulletin Tohoku Agricultural Experiment Station*. **46** :131-141.
- Irwin, M.E. (1999).** Implications of movement in developing and deploying integrated pest management strategies. *Agricultural and Forest Meteorology*. **97** : 235-248.
- Irwin, M.E. and Schultz, G.A. (1981).** Soybean mosaic virus. *Plant Protection Bulletin*. FAO. **29**(3/4) :41-55.
- *Iwai, H.; Ito, T.; Sato, K. and Wakimoto, S. (1985).** Distribution patterns of soybean mosaic virus strains B and D in soybean seeds at different growth stages. *Annals of the Phytopathological Society of Japan*. **51**(4) : 475-481.
- Jain, K K.; Lahiri, L. and Varma, A. (2000).** Peanut stripe potyvirus : prevalence, detection and serological relationship. *Indian Phytopathology*. **53** (1) : 14-18.
- Jayaram, C.; Deusen, KA. Van.; Eggenberger, A.L.; Schwabacher, A.W.; Hill, J.H. and Van-Deusen, KA. (1998).** Characterization of a monoclonal antibody recognizing a DAG-containing epitope conserved in aphid transmissible potyviruses. *Virus Research*. **58** (1-2) : 1-11.
- Jayaram, C.; Hill, J.H. and Miller, W.A. (1992).** Complete nucleotide sequences of two soybean mosaic virus strains differentiated by responses of soybean containing the Rsv resistance gene. *J. gen. Viral*. **73** : 2067-2077.
- Johnson, H.W. and Chamberlain, D.W. (1953).** Bacteria, fungi and viruses on soybeans. In : Plant Diseases, year-book of agriculture, pp 238-247. U S Deptt. Agr. ARS, Washington, D.C.

- Kendrick, J.B. and Gardner, M.W. (1924).** Soybean Mosaic : seed transmission and effect on yield. *J. Agr. Res.* **27** : 91-98.
- Kennedy, B.W. and Cooper, R.L. (1967).** Association of virus infection with mottling of soybean seed coats. *Phytopathology.* **57** : 35-37.
- Kennedy, B.W. and Cooper, R.L. (1966).** Influence of virus infection on seed coat discoloration in soybean. *Phytopathology.* **56** :884.
- Khetarpal, R.K.; Parakh, D.B.; Singh, S.; Singh, S. and Nath, R. (1992).** ELISA detection of soybean mosaic virus in testas, embryos and seedlings from mottled and unmottled seeds of imported soybean germplasm. *Indian Journal of Virology.* **8** (2): 106-110.
- Kim, Y.; Roh, J.; Kim, M.; Lm, D. and Hur, L. (2000).** Seasonal occurrence of aphids and selection of insecticides for controlling aphids. *Korean J. Crop. Science.* **45** (6) : 353-355.
- Kim, Y.H.; Noh, J.H.; Kim, M.K.; Im, D.J.; Lee, B.H. and Lee, S.C. (1996).** Effects of SMV-G5H strain on plant growth and seed chemical composition of soybean cv. Danyeobkong. *Korean Journal of Crop Science.* **41**(3) :340-347.
- Klessner, P.J. (1954).** New records of legume virus diseases in England. *Plant Path.* **3** : 84.
- Kline, A.S.; Anderson, E.J. and Smith, E.B. (1997).** Occurrence of cowpea stunt disease causing viruses on wild bean in Arkansas. *Plant Disease.* **81**(2) :231.
- Kosaka, Y.; Fukunishi, T.; Komada, H.; Kiritani, K. and Bay, P.J. (1990).** Control of soybean mosaic disease with an attenuated soybean mosaic virus. *Proceedings of the International Seminar "Biological control of Plant disease and virus vectors"*, Japan pp 103-110.
- *Koshimizu, Y. and Izuka, N. (1963).** Studies on soybean virus diseases in Japan. *ToJwku Natl. Agrl. Expt. Stn. Bull.* **27** : 1-103.
- *Kwon, S.H. and Oh, J.H. (1983).** Induced mutation for soybean mosaic virus disease resistance on soybean. In : Induced mutations for disease resistance in crop plants II. International Atomic Energy Agency, Vienna, Austria, 183-191.

- Laguna, I.G.; Truol, G.A.M. and Nome, F. (1987).** Relation between "saddle spotted" soybean seed and seed transmission of soybean mosaic virus. *Fitopatología*. **22**(2) :71-76.
- Leviolette, F.A. (1968).** New comprehensive manual modern soybean production. *Viruses Diseases*. **38**(9) : 196-198.
- Li, W.M. and Pu, Z.Q. (1991).** Population dynamics of aphids and epidemics of soybean mosaic virus in summer sown soybean fields. *Acta. Plzytopnt/IOlnctcn Sinicn*. **18** (3) : 123-126.
- Liao, L.; Liu, Y.; Sun, D.; Tian, P.; Liao, L.; Liu, Y.Z.; Sun, D.M. and Tian, P.Z. (1995).** Top necrosis symptoms in soybean plants infected with soybean mosaic virus. *Acta Agronomica Sinica*. **21** (6): 707-710.
- Ling, L. (1951).** Bibliography of soybean diseases. *Plant Dis. Reprtr. Suppl.* **204** : 109-173.
- Linhell, D. (1939).** Estonia : Plant disease new to the country. *Internatl. Bull. Plant Protect.* **13** : 105-106.
- Lister, R.M. (1978).** Application of enzyme linked immunosorbent assay for detecting viruses in soybean seed and plants. *Phytopathology*. **68** : 1393-1400.
- *Liu, Y. (1963).** A preliminary study on the diagnosis of soy bean mosaic virus. *Acta. Phytopath. Sinica*. **6** : 209-213.
- *Lu, W.; Zhang, M. and Zhang, Z.(1981).** Relationship between soybean mottling seed coat and strains of soybean mosaic virus. *Acta. Phytopathologia Sinica*. **11** : 31-36.
- Luan, X. and Luan, X.Y. (1997).** Inheritance of soybean plant resistant to No. III (173) strain of soybean mosaic virus. *Soybean Science*. **16** (3) : 223-226.
- Lucas, B.S. and Hill, J.H. (1980).** Characteristics of the transmission of three soybean mosaic virus isolates by *Myzus persicae* and *Rhopalosiphum maidis*. *Phytopnth. Z.* **99** : 47-53.
- Ma, D.; Xu, S.; Zhang, Z.; Hu, W.; Zhang, C.; Song, S. and Zhao, R. (1982).** Detection of soybean mosaic virus with enzyme-linked immunosorbent assays (ELISA). *Acta Phytopathologica Sinica*. **12**(2): 47-52.

- Madden, L.V.; Hughes, G. and Irwin, M.E. (2000).** Coupling disease-progress curve and time of infection functions for predicting yield loss of crops. *Phytopathology*. **90** (8) : 788-800.
- Martyn E.E. (1942).** Disease of plant in Jamaica. Jamaica Deptt. Sci and Agr. Bull (NS.) pp 32-43.
- Mehta, S.L. (1987).** Relevance of improving protein quality in cereals. *Everyman's science*. **22**: 1-10.
- Mohammad, A.; Muazam, S. and Sher, H. (2003).** Effect of soybean mosaic potyvirus on growth and yield components of commercial soybean varieties. *Pakistan Journal of Plant Pathology*. **1**(2) : 54-57.
- Mohanti , A.K. and Basu A.N. (1991).** Seasonal variations in the aerial populations of white fly vectors, *B. tabaci* under Delhi conditions. *Indian Phytopathology*. **44** :494 -496.
- Moore, D.L.; Lister, RM.; Abney, Js. and Athow, K.L. (1982).** Evaluation of virus contents in soybean by Enzyme linked Immunosorbent Assay. *Plant Disease*. **66** : 790-793.
- Morales, F.J.; Niessen, A.I.; Castano, M. and Calvert, L. (1990).** Detection of a strain of soybean mosaic virus affecting tropical forage species of *Centrosema*. *Plant Disease*. **74** (9) :648-651.
- *Mullar, A.S. (1941).** Survey of diseases of cultivated plant in Venezuela. *Soc. Venezolana da Ciene. Natl. Bol.* **7** : 99-113.
- *Murata, T. (1925).** Soybean mosaic. *J. Plant Protection (Tokyo)*. **12** : 451-452.
- *Murayama, D. and Han., Y.H. (1971).** Occurrence of soybean mosaic virus in Taiwan. *Plant Proto Bull. Taiwan*. **13** : 75-86.
- Naik, RG. and Murthy, RV.K. (1992).** Studies on relationship of soybean mosaic virus on soybean with its vector *Myzus persicae* Sulz. *Legume Research*. **15** (1) : 24-28.
- Naik, R.G. and Murthy, R.V.K. (1992 a).** Characterization of virus causing mosaic on soybean (*Glycine max* L. Merr.) from Karnataka. *Legume Research*. **15**(1) : 24-28.
- Nakano, M.; Usugi, T. and Shinkai, A. (1988).** Effect of inoculation time of soybean mosaic virus on yield and seed quality of soybean. *Proceedings of the Association for Plant Protection of Kyushu*. **34**(63) :13-16.

- Nariani, T.K. and Pingaley, K.V. (1960).** A mosaic disease of soybean [Glycine max (L.) Merr.]. *Indian Phytopath.* **13** : 130-136.
- Nene, Y.L. (1973).** Viral diseases of some warm weather pulse crops in India. *Plant Dis. Repr.* **57** : 463-467.
- Nicolaescu, M. (1987).** Research on the detection of soybean mosaic virus on soybean seeds by the embryo test. *Analele. Inst. de Cercetori Pentru Protectia Plantelor.* **20** : 11-14.
- Nikolic, C.V. and Stakic, D. (1964).** Soybean mosaic virus in Yugoslavia.
- Noordam, D. (1973).** Identification of plant viruses. Oxford and IBH Publishing Co., New Delhi. pp 207.
- Nutter, F.W. Jr.; Schultz, P.M. and Hill, J.H. (1998).** Quantification of within field spread of soybean mosaic virus in soybean using strain specific monoclonal antibodies. *Phytopathology.* **88** (9) : 895-901.
- Pathak, H.C. (1974).** Seedborne plant viruses identification and diagnosis in seed health testing. *Seed Sci. and Techno.* **2** : 3-155.
- Patil, R.G. and Byadgi, A.S. (2005).** Transmission studies of soybean mosaic virus. *Karnataka Journal of Agricultural Sciences.* **18**(1) : 52-54.
- Pierce, W.H. (1955).** The identification of certain viruses affecting leguminosae plants. *J. Agr. Res.* **51** : 1017-1039.
- Pietersen, G. and Garnett, H.M. (1992).** Properties and strain identity of soybean mosaic virus isolates from the Transvaal, South Africa. *Phytophylactica.* **24**(3) :279-283.
- *Pin Kevich, E.V. and Krylov, A.V. (1977).** Isolation and some physical properties of soybean mosaic virus. *Biologicheskie Nanki.* **20** : 26-31.
- Porto, M.D.M. and Hagedorn, D.J. (1973).** Some characteristics of Brazilian isolate of soybean mosaic virus. 2nd Internatl. Congo Plant Path. Abstrs. 0924.
- Porto, M.D.M. and Hagedorn, D.J. (1974).** Susceptibility of *Phaseolus lathyroides* to soybean mosaic virus. *Plant Dis. Repr.* **58** : 322-326.

- Porto, M.D.M., and Hagedorn, D.J. (1975a).** Seed transmission of a Brazilian isolate of soybean mosaic virus. *Phytopathology*. **65**(6) : 713-716.
- Porto, M.D.M. and Hagedorn, D.J. (1975b).** Effects of temperature and fertility on a Brazilian soybean mosaic. *Plant Disease Reporter*. **59** (12) :947-950.
- Provvidenti, R.; Gonsalves, D. and Ranali, P. (1982).** Inheritance of resistance to soybean mosaic virus in *Phaseolus vulgaris*. *The Journal of Heredity*. **73** :302-303.
- Quantz, L. (1961).** Studies on bean common mosaic virus and soybean mosaic virus. *Phytopath. Z.* **43** : 79-101.
- Quimio, G.M. and Calilung, V.J. (1993).** Survey of flying viruliferous aphid species and population build up of *Aphis glycines* Matsumura in soybean fields. *Philippine Entomologist*. **9** (1) : 52-100.
- Quiniones, S.S. and Dunleavy, J.M. (1970).** Identify of a soybean mosaic virus isolated from *Glycine max* variety Hood. *Plant Dis. Repr.* **54** : 301-305.
- Quiniones, S.S., Dunlevy, J.M., and Fisher, J.W. (1971).** Performance of three soybean varieties inoculated with soybean mosaic virus and bean pod mottle virus. *Crop Science*. **11** (5) : 662-664.
- Ren, Q.; Pfeiffer, T.W. and Ghabrial, S.A. (2000).** Relationship between soybean pubescence density and soybean mosaic virus field spread. *Euphytica*. **111** (3) : 191-198.
- Ren. Q.; Pfeiffer, T.W. and Ghabrial, S.A. (1997).** Soybean mosaic virus incidence level and infection time : interaction effects on soybean. *Crop Science*. **37** (6) : 1706-1711.
- Reyes, G.M. (1957).** Diseases of soybeans. *Philippines Bur. Plant Indus. Plant Indus. Dig. fan* 1957, p. 61.
- *Rieuf, P. (1960).** Pathogenic and saprophytic organisms of plants in Morocco. Morocco Servo. de la Rech. Agron. et de l' Ensieh. *Cahiers de la Rech. Agron.* **9**. pp 359.
- Rose, J.P. (1963).** Interaction of soybean mosaic and bean pod mottle viruses infecting soybean. *Phytopathology*. **53** : 883.

- Ross, J.P. (1967).** Purification of soybean mosaic virus for antiserum production. *Phytopathology*. **57** : 465-467.
- Ross, J.P. (1968).** Effect of single and double infections of soybean mosaic virus and bean pod mottle virus on soybean yield and seed characters. *Plant Disease Reporter*. **52** :344-348.
- Ross, J.P. (1969).** Pathogenic variation among isolates of soybean mosaic virus. *Phytopathology*. **59** :829-832.
- Ross, J.P. (1969b).** Effect of time and sequence of inoculation of soybeans with soybean mosaic and bean pod mottle viruses on yield and seed characters. *Phytopathology*. **59** :1404-1408.
- Ross, J.P. (1970).** Effect of temperature on mottling of soybean seed caused by soybean mosaic virus. *Phytopathology*. **60** :1798-1800.
- Ruesink, W.G. and Irwin, M.E. (1986).** Soybean mosaic virus epidemiology : a model and some implications. In : McLean GD, Garrett RG, Ruesink WG, eds. *Plant Virus Epidemics : Monitoring, Modelling and Predicting Outbreaks*. Academic Press Australia pp. 295-313.
- Schultz, G.A.; Irwin, M.E. and Goodman, R.M. (1983).** Factors affecting aphid acquisition and transmission of soybean mosaic virus. *Annals of Applied Biology*. **103** (1) : 87-96.
- Schultz, G.A.; Irwin, M.E. and Goodman, R.M. (1985).** Relationship of aphid (Homoptera : Aphididae) landing rates to the field spread of soybean mosaic virus. *Journal of Economic Entomology*. **78** (1) :143-147.
- Shi, Ainong; Chen, Pengyin; Zheng, Cunling; Hou, Anfu and Zhang, Bo (2008).** A PCR-based marker for the Rsv1 locus conferring resistance to soybean mosaic virus. *Crop Science*. **48** (1) : 262-268.
- Sinclair, J.B. (1984).** Compendium of soybean diseases (2nd Edition). *The American Phytopathological Society*, U.S.A. pp 104.
- Singh, B.R. and Gupta, S.P. (1996).** Natural occurrence of soybean mosaic virus in coffee senna. *Indian Journal of Virology*. **12** (1): 71-72.
- Singh, B.B. and Saxena, M.C. (1979).** Soybean production in India. *Indian Farming*. **28** (10) : 7-9.

- Soong, M.M. and Milbrath, G.M. (1973).** Effect of light and buffer solutions on total lesion production in Top Crop bean by soybean mosaic virus. 2nd Internatl. *Congo Plant Path.* 02-95.
- Soong, M.M. and Milbrath, G.M. (1980).** Purification, partial characterization and serological comparison of soybean mosaic virus and its coat protein. *phytopathology.* **70** : 388-391.
- Sovulescu, T. (1935).** Phytosanitary conditions in Rumania during the year 1933-34. *Inst. Cere. Agron. Romaniei Publ.* **24** : 1-59.
- Steere, R.L. (1956).** Purification and properties of tobacco ring spot virus. *Phytopathology.* **46** : 60-69.
- Stevenson, W.R. and Hagedorn, D.J. (1973).** Further studies on seedtransmission of pea seedborne mosaic virus in *Pisum sativum*. Plant disease reporter. **57** :248-252.
- Sun, H.; ShenTu, S.; Xue, F.; Duns, G. and Chen, J. (2008).** Molecular characterization and evolutionary analysis of soybean mosaic virus infecting *Pinellia ternata* in China. *Virus Genes.* **36** (1) : 177-190.
- Sundaresh, H.N.; Janardhan, A.; Hiremath, P.E. and Ponnappa, K.M. (1984).** Varietal reaction of soybean to mosaic virus. *Current Research.* **12** : 34.
- Suteri, B.D. (1981).** Effect of soybean mosaic virus on seed germination and its transmission through seeds. *Indian Phytopath.* **34** : 370-371.
- Suteri, B.D. (1986).** Resistance in soybean cultivars to two strains of soybean mosaic virus. *Indian Phytopath.* **39** : 578-580.
- *Tsuchizaki, T. (1986).** Partial characterization and serological relationships of three potyviruses isolated from leguminous crop in Thailand. International symposium on virus disease of rice and leguminous crops in tropics. *Trop. Agril.Res.* Series No. **19** : 115-120.
- Tu J.C. (1989).** Effect of different strains of soybean mosaic virus on growth, maturity, yield, seed mottling and seed transmission in several soybean cultivars. *Journal of Phytopathology.* **126**(3) : 231-236.
- Tu, J.C. (1977).** Effects of soybean mosaic virus infection on ultrastructure of bacteroidal cells in soybean root nodules. *Phytopathology.* **67**(2) :199-205.

- Tu, J.C. (1992).** Symptom severity, yield, seed mottling and seed transmission of soybean mosaic virus in susceptible and resistant soybean : the influence of infection stage and growth temperature. *Journal of Phytopathology*. **135**(1) :28-36.
- Tu, J.C. (1989).** Effect of different strains of soybean mosaic virus on growth, maturity, yield, seed mottling and seed transmission in several soybean cultivars. *Journal of Phytopathology*. **126** (3) :231-236.
- Usman, K. M.; Rangnathan, K.; Kandaswami, T.K.; Damodaran, A.P.S. and Ayyavoo, R. (1973).** Studies on a mosaic disease of soybean. *Madras Agriculture Journal*. **60** : 472-474.
- Van Nieberk, B.P. and Lombard, M. (1967).** Soybean mosaic and mottled seed. *Farming S. Africa*. **43** : 7-9.
- Vance, V.B. and Beachy, R.N. (1984).** Translation of soybean mosaic virus RNA in vitro : Evidence of protein processing. *Virology*. **132** : 271-281.
- Vroon, C.W.; Pietersen, G. and Tonder, H.J. (1988).** Seed transmission of soybean mosaic virus in *Lupinus albus* L. *Phytophylactica*. **20**(2):169-175.
- *Vui-Yui, D. (1961).** The mosaic disease of soybean varieties in Bulgaria. *Rast. Zasht. (Bulgaria)*. **9** : 20-26.
- Walters, H.J. (1958).** A virus disease complex in soybean in Arkansas. *Phytopathology*. **48** :346.
- Walters, H.J. (1963).** Leguminous hosts of soybean mosaic virus. *Plant Dis. Repr.* **47** : 726-728.
- Wang, R.Y.; Kritzman, A.; Hershman, D.E. and Ghabrial, S.A. (2006).** *Aphis glycines* as a vector of persistently and nonpersistently transmitted viruses and potential risks for soybean and other crops. *Plant Disease*. **90**(7) : 920-926.
- Wang, R.Y. and Ghabrial, S.A. (2002).** Effect of aphid behavior on efficiency of transmission of soybean mosaic virus by the soybean-colonizing aphid, *Aphis glycines*. *Plant-Disease*. **86**(11) : 1260-1264.
- Warshall Peter and Imhoff Dan (1999).** Soybean of happiness –A 3000 year history of our most modern oilseed. Whole Earth Review, Spring 1999. Pointfoundation ([http://wholeearth.com/issue/96/aticle/78/soybean of happiness](http://wholeearth.com/issue/96/aticle/78/soybean%20of%20happiness)).
- Wilcox, J.R. and Laviolette, F.A. (1968).** Seed coat mottling response of soybean genotypes to infection with soybean mosaic virus. *Phytopathology*. **58** : 1446-1447.

- Woodworth, C.M. and Cole, L.J. (1924).** Mottling of soybeans. *J. Hered.* **15** : 349-354
- Xu, Z.; Cockbain, A.J.; Woods, R.D. and Roberts, E.L. (1986a).** Viruses of *Vicia faba* in China. The selected works of virus diseases on leguminous crops (1968-1985). Nanjing Agricultural University.
- Yu, M.H.; Frenkel, M.J.; Mckern, N.M.; Shukla, D.D.; Strike, P.M and Ward, C.W. (1989).** Coat protein of potyviruses. 6. Amino acid sequences suggest watermelon mosaic virus 2 and soybean mosaic virus N are strains of the same potyvirus. *Archives of Virology.* **105**(1-2) : 55-64.
- Yu, Seok; Moon, Jung Kyung; Hwang, Tae-Young; Kim, Yul Ho; Kim, Hwan Mook and Jeong, Soon Chun (2005).** Genetic and sequence analysis of the RAPD fingerprint containing a Rsv3-linked marker rapidly identified by using RILs in soybean. *Korean Journal of Genetics.* **27**(2) : 127-132.
- *Yu, T.F. (1939).** A list of plant viruses observed in China. *Acta. Phytopath. Slnica.* **1** : 1-18.
- Zhang, M.H.; Wei, P.W.; Guo, J.Q. and Hao, S.H. (1988).** Factors for the epidemics of soybean mosaic virus and integrated measures for its control. *Acta. Phytophylactica Sinica.* **15** (4) : 223-228.
- Zheng, C.M.; Bing, T.; Gao, F.L. and Wu, Z.P. (1998).** Study on the changes of free amino acid and soluble protein in seed coat of soybeans with different resistance to seed coat mottling after infection with soybean mosaic virus. *Acta Phytopatologica Sinica.* **28**(3) :227-231.
- Zheng, CuiMing; Chang, RuZhen; Qiu, LiJuan; Chen, P.Y.; Wu. XiaoLei and Chen, ShouYi (2003).** Identification and characterization of a RAPD/SCAR marker linked to a resistance gene for soybean mosaic virus in soybean. *Euphytica.* **132**(2) : 199-210.
- Zheng, Z.; Chang, R.; Qui, L.; Wu, Z.; Gao, F.; Zheng, C.M.; Chang, R.Z. and Qiu, L.G. (2000).** Identification of the resistance of soybean germplasm to SMV3. *Soybean Science.* **19**(4) : 299-306.
- Zhuang, B.; Sun, Y.; Lu, Q.; Wang, Y.; Xu, B.; Zhuang, B.C.; Sun, Y. J.; Lu, Q.H.; Wang, YM. and Xu, B. (1996).** A study on resistance to soybean mosaic virus and *Aphis glycines* of perennial wild soybean. *Soybean Genetics Newsletter.* **23** : 66-69.

Appendix

Appendix

Standard Meteorological week's average weather data of 2007-08

Month	Date	Max. Temp. (°C)	Min. Temp. (°C)	Mean Rel. Hum. (%)	Total Rain Fall (mm)
July	16 to 22	33.1	26.0	73.0	82.0
	23 to 29	28.2	23.4	87.5	233.2
	30 to 5 A	29.2	23.9	88.5	178.4
August	6 to 12	33.3	26.6	76.5	83.6
	13 to 19	30.2	24.1	87.0	146.2
	20 to 26	32.3	25.9	77.5	15.6
	27 to 2 S	30.5	24.8	76.5	114.2
September	3 to 9	31.3	24.9	80.5	52.0
	10 to 16	32.7	23.2	73.5	00.0
	17 to 23	34.0	24.0	72.0	10.8
	24 to 30	28.1	22.2	83.5	121.0
October	1 to 7	32.2	19.9	70.0	00.0
	8 to 14	31.4	16.9	67.0	00.0
	15 to 21	29.1	17.6	69.0	13.2
	22 to 28	29.7	15.6	65.0	00.0
	29 to 4 N	29.4	15.3	67	00.0
November	5 to 11	27.8	13.7	69.0	00.0
	12 to 18	17.9	03.7	77.5	00.0

Standard Meteorological week's average weather data of 2008-09

Month	Date	Max. Temp. (°C)	Min. Temp. (°C)	Mean Rel. Hum. (%)	Total Rain Fall (mm)
July	16 to 22	28.6	24.3	89.5	264.2
	23 to 29	30.9	25.9	82.0	028.2
	30 to 5 A	30.3	24.8	82.5	172.0
August	6 to 12	31.3	25.5	79.0	102.2
	13 to 19	28.5	24.5	87.0	422.8
	20 to 26	31.5	24.8	82.0	135.4
	27 to 2 S	32.3	24.5	76.0	024.2
September	3 to 9	32.4	23.9	74.5	016.4
	10 to 16	31.6	24.0	76.5	050.4
	17 to 23	28.6	22.0	82.0	347.6
	24 to 30	31.3	21.3	74.0	000.0
October	1 to 7	30.9	23.1	76.0	019.6
	8 to 14	31.0	19.8	69.5	004.8
	15 to 21	30.1	15.9	63.5	000.0
	22 to 28	29.3	15.1	61.5	000.0
	29 to 4 N	29.4	14.7	65.5	000.0
November	5 to 11	29.0	14.8	65.5	000.0
	12 to 18	27.5	12.6	67.0	000.0

VITA

The author was born on 11th November 1982 at village Madia, near Chunar in District Mirzapur (Uttar Pradesh). He completed his High School and Intermediate from P.D.N.D. inter college, Chunar (Mirzapur), in the year 1998 and 2000 respectively, from U.P. Board, Allahabad. He completed his B. Sc. (Ag.) with 1st division from Udai Pratap Autonomous College, Varanasi which affiliated to V.B.S. Purvanchal University of Jaunpur in 2004. He was the recipient of Rajya Krishi Utpadan Mandi Samiti Uttar Pradesh merit scholarship during the B.Sc. (Ag.) degree programme. After that he joined C.S. Azad University of Agriculture and Technology, Kanpur for his Master's degree in Plant Pathology and completed in the year 2006.

Thereafter, he joined G.B.P.U. & T., Pantnagar and admitted for Ph. D. (Plant Pathology) in year 2006-07. He was the recipient of University fellowship during his Ph. D. degree programme.

He served as Joint Secretary and General Secretary of Plant Pathology Society, Pantnagar during the year 2006-07 and 2008-2009, respectively.

Address for Correspondence:-

Vikram Singh

S/O Late Shri Baleshwar Singh

Vill. & Post- Madia

Block – Sikher,

Thana-Chunar

Distt.- Mirzapur (Uttar Pradesh)

Pin – 231304

Mob. : +91- 9557117401

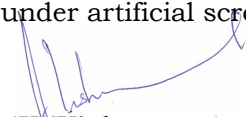
E-mail: <vikrampatho@gmail.com>

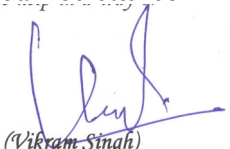
< vikramsinghpatho@yahoo.com >

Name	:	Vikram Singh	Id. No.	:	34018
Sem. & year of admission	:	1 st , 2006-07	Degree	:	Ph.D.
Major	:	Plant Pathology	Deptt.	:	Plant Pathology
Thesis title	:	“Soybean Mosaic Virus (SMV): Methods for detection and mechanism of transmission in Soybean (<i>Glycine max</i> L. Merrill).”			
Advisor	:	Dr. (Mrs.) K. Vishunavat			

ABSTRACT

The present investigations were carried out on host range, detection techniques, location and transmission of the seedborne inoculum of soybean mosaic virus (SMV), which has now become a potential threat to the cultivation of soybean (*Glycine max* L. Merrill) in India and is responsible for substantial yield losses. This virus culture was obtained from naturally infected soybean. A single local lesion culture from a local lesion host *Chenopodium amaranticolor* was maintained on the systemic host *Glycine max*. The host range studies revealed that the virus infected systemically *Glycine max*, *Phaseolus vulgaris*, *P. lathyroides* and *Vigna sinensis* causing general symptoms of systemic mosaic mottling, reduction of leaf lamina, and stunting of plants. *Cyamopsis tetragonoloba*, *C. amaranticolor* and *Chenopodium amaranticolor* were the local lesion hosts of the virus. The virus was readily sap as well as aphid transmissible but maximum per cent transmission 85% by *Myzus persicae* while minimum transmission 15% by *Aphis fabae* was recorded, respectively. In the relative efficacy tests *Myzus persicae* gave the highest i.e. 78.33% virus transmission. It was followed by *Aphis craccivora* with 61.66%. The per cent seed transmission ranged from 7.54 to 14.72 in year 2007-08 while in 2008-09 it 9.67 to 17.82 recorded in different cultivars respectively. The maximum seed borne virus transmission was 14.72 per cent and 74 per cent germination was found in cultivar PK-416. The determination of TIP, DEP and LIV the virus had a TIP between 60-65 ° C, DEP between 10⁻⁴ to 10⁻⁵ and LIV between 4-5 days at room temperature, 7 days at 5-7° C and 15 days at in leaves stored at 5-7 ° C were recorded. The virus was successfully purified employing a modification of the method consisted of 3 cycles of differential centrifugation followed by sucrose density gradient centrifugation. The final purified virus preparation was highly concentrated without pigments and gave absorption maxima at 260 nm and absorption minima at 245 nm indicating a typical nucleoprotein spectrum in the UV spectrophotometer. Long flexuous filamentous particles of average size 680 x 12.5 nm were observed in under the electron microscope. Six categories of morphologically abnormal seed, collected from systemically SMV infected plants yielded SMV infection ranging from 9.09 to 78.94 per cent. Small shrivelled seeds exhibited 64.0 per cent germination followed by 72.0 per cent germination in large shriveled, 84.0 per cent in oversized seeds, 76.0 per cent in undersized seeds and 88.0 and 76.0 per cent germination in off coloured and mottled seeds, respectively. SMV in various categories of morphologically abnormal seeds was also confirmed using DAC-ELISA test. The assay results of different floral parts i.e. epicalyx, calyx, corolla, androecium and gynoecium and seed components viz., seed coat, cotyledons and embryo was detected in indicator inoculation test and DAC- ELISA obtained from systemically SMV infected plants. Bright golden colour traps attracted maximum aphid adults followed by pale yellow colour traps, while cylindrical shape traps were more effective in attracting aphids as compare to lamp shape. However, the highest number of aphid adults was trapped during second fortnight of July. Hundred seed weight was 4.50 gm in plants infected 15 DAS; 13.10 gm in plants infected at 65DAS and was 15.48 gm when collected from healthy plants. Out of the total of thirty cultivars screened under natural condition six were found to be resistant in both the years. These are viz. PK - 327, PK - 472, PK - 564, PK - 416, Alnkar, and Ankur, while out of 11 promising germplasm /cultivars tested Ankur, PK-327, PS-1929 and PS-1241 showed resistance under artificial screening with SMV virus.


(K. Vishunavat)
Advisor


(Vikram Singh)
Author