MOSAIC DISEASE OF *Dolichos biflorus* L., TRANSMISSION, HOST RANGE AND EFFECT OF THE VIRUS ON THE HOST

BY

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THESIS

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C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bonafide research work conducted by Shri. V.P. Sukumara Dev under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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INTRODUCTION

INTRODUCTION

Horsegram is extensively cultivated in South India, especially in Mysore, Andhra and Madras States. It is known as a poor man's pulse crop.

A severe disease characterised by mosaic mottling was observed at the Agricultural College Farm, Vellayani, during 1965-'66. A review of the relevant literature revealed that a mosaic of Dolichos biflorus had been reported from Bombay State by Uppal (1931) who also observed about 25 per cent seed transmission of the disease. Later, Capoor and Varma (1948) in their studies on double bean yellow mosaic virus showed that the mosaic of Dolichos biflorus was actually caused by the above virus. They also studied the modes of transmission and host range of the virus. A virus disease of Phaseolus aureus was subsequently reported by Nariani (1960) who observed Dolichos biflorus as an additional host of the virus. He, however, considered it to be distinct from double bean yellow mosaic virus, because of the difference in host range, eventhough, the methods of transmission of both the diseases were similar.

Since two distinct viruses are known to cause mosaic disease of Dolichos biflorus in two different states of India, it was thought worthwhile to investigate the mosaic of the above plant observed in this area in regard to the modes of transmission as well as host range with a view to determine the identity of the causal virus.

It is known that infection alters the host metabolism to a considerable extent. Variations in carbohydrate and nitrogen contents are commonly noted in diseased plants which ultimately tell on the C/N ratio. An attempt is therefore made to determine the extent to which the carbohydrate and nitrogen contents of the horsegram plant are affected as a result of infection.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Iwanowsky (1899) first reported a mosaic disease of bean from Russia. Since then a number of virus diseases of leguminaceous crop plants have been reported from various parts of the world. In India, virus diseases have been reported on double bean, lima-bean, french bean, indian bean, pea, cowpea, chinese sarson, horsegram etc.

Uppal (1931) reported a mosaic disease of Dolichos biflorus in the oretwhile Bombay State, resembling bean (Phaseolus vulgaris) mosaic and characterised by chlorosis, clearing of veins, blistering and downward cupping of the trifoliate leaves, shedding of flowers and extensive sterility of the pods. The disease was reported to be very serious and generally prevalent in the Bombay Presidency and was of late found to be causing heavy damage to Dolichos lablab in Gujarat. Capoor & Varma (1948) remarked that this disease has since been shown to be caused by the same virus which affects the double bean plants. In their studies on yellow mosaic of Phaseolus lunatus they observed that apart from D.biflorus the disease could be transmitted to

Phaseolus limensis; P.vulgaris; P.aureus, and Canavalia ensiformis by artificial inoculation. They named the virus as double bean yellow mosaic virus. Nariani(1960) while working on yellow mosaic of Phaseolus aureus observed that the disease could be transmitted to D.biflorus, Glycinemax, P.lathiroides, P.aconitifolius and P.acutifolius by the agency of white-fly, Bemisia tabaci. He named the disease as yellow mosaic of Phaseolus aureus.

Insect transmission.

Kirkpatrick (1930) while experimenting with cotton leaf curl virus reported for the first time that plant viruses could be transmitted by white-flies. So far, six species of white-flies (four belonging to the Genus Bemisia and one each to Aleurocanthus and Trialeurodes) are known to act as vectors of plant viruses. According to Varma (1962) twenty three virus diseases of plants are known to be transmitted by white-flies, out of which, at least 12 have been reported from India. Yellow vein mosaic of bhindi, cassava mosaic, tobacco leaf curl, tomato leaf curl, double bean yellow mosaic and mosaic of Dolichos lablab are some of the important plant virus diseases that are known to be transmitted by the agency of white-flies in India.

Virus-vector relationships have been investigated only in a few cases of white-fly transmitted plant virus diseases. Varma (1952) while working on yellow vein mosaic of bhindi observed that in order to produce 100 per cent infection at least 10 white-flies are necessary. The above author (1962) reported that while single white-fly is able to transmit the virus, greater transmission occurred by using a large number. The virus causing yellow vein mosaic of pumpkin could be acquired by its vector, B. tabaci within a feeding period of 15 minutes (Annonymous, 1954). Bird (1957) reported that B. tabaci required a period of two hours to acquire the virus causing mosaic of Jatropha gossypifolia. Varma (1962) reported that the minimum time required by white-flies for acquisition of the virus of yellow vein mosaic of bhindi is in the neighbourhood of 30 minutes; the proportion of insects that became infective increased with increasing time on infected plants. Though viruliferous white-flies can transmit in as short a a feeding interval as 10 minutes most require longer period. He further reported that in the majority of the viruses transmitted by white-flies, a minimum incubation period in their vectors is necessary, which varied between 2-8 hours. Bawden (1963) stated that in white-flies there is a latent period of few hours to a day

or more between acquiring and becoming able to transmit the virus. Varma (1955) reported that white-flies fed on yellow vein mosaic affected bhindi plants for 12-24 hours remained infective for life. He, however, stated that the persistence of infectivity depended on the duration of feeding.

Seed transmission.

Of the several hundreds of plant virus diseases that have been described so far, only a few have been shown to be transmitted through seeds. In most of these cases, the percentage of seed transmission has been found to be rather low.

Seed transmission seems to be characteristic of most of the viruses affecting leguminaceous plants and the common bean mosaic furnishes the best known example of the above. McClintock (1917) and Reddick and Stewart (1919) reported that bean mosaic virus will be transmitted through the seeds of Phaseolus vulgaris and P.lunatus. Dickson (1922) observed seed transmission in crops like Pisum sativum, Trifolium prutense, T.hybridum and Melilotus alba. Similar findings have been reported by Kendrick and Gardner (1924) in soybeans and Ogilvie (1924) in string beans. Racicot(1930)

found that 55 percent of the seeds of P.vulgaris collected from a mosaic affected crop had virus in them. Nelson (1932) reported 13-50 per cent infection through the seeds of P.vulgaris. Uppal (1931) reported 25 per cent seed transmission in the mosaic disease of D.biflorus. However, Capoor and Varma (1948) who showed this disease to be caused by double bean yellow mosaic virus could not show the evidence of seed transmission of the disease with 472 double bean seeds collected from diseased plants. Snyder (1942) reported that asparagus bean virus will be transmitted through the seed to the extent of 37 per cent. Doolittle and Jones (1925), however, failed to get evidence of seed transmission in pea mosaic. Similarly Pierce (1934) found that bean mosaic virus 2 was not seed-borne though bean mosaic virus 1 was seed-borne.

Nelson (1932) observed that in an infected plant of P.vulgaris the same pod might contain infected as well as virus free seeds and that proportion of infected seeds depended on the time of infection of the plants by the virus. Fajardo (1930) observed that the amount of seed transmission depended upon the season, duration and age of the crop. The percentage of seed

transmission was less in early varieties and those affected in bloom. Harrison (1935) observed that pods formed early could transmit the mosaic disease of beans to a larger proportion of the seed during the first 19 days from the date of flowering than those set during later period of blossoming. Crowley (1957) opined that transmissibility is not a property of either the virus or the host alone, but a combination of the two. He found that the temperature before fertilization had influenced the percentage of seed transmission by bean mosaic virus. When temperature exceeded 65°F no transmission occurred. Bawden (1963) reported that the seed transmissibility also depended upon the physiology of the host at the time of flowering. He found that all the seeds of the same plant and even in the same pod may not carry the virus.

Transmission of a virus through the seeds of one host species but not through the seeds of another also occurs. Doolittle and Walker (1925) observed that the virus causing cucumber mosaic is transmitted through the seeds of wild cucumber but not through the seeds of cultivated cucumber. Henderson (1931) reported that the virus of cowpea mosaic is transmitted by the seed of the

asparagus bean, but not of cowpea, and tobacco ring spot virus is not carried in the seed of tobacco plant but is present in the seed of Petunia sp. Broadbent (1958) showed that the lettuce mosaic virus is passed through the seeds of Senecio vulgaris, which rarely exhibited symptoms but from which the virus could be transmitted to lettuce by the agency of aphids. Gorgan and Walker (1948) showed that seed transmission is a characteristic of the virus, rather than the host. They found that common mosaic of beans was transmitted through the seed, while yellow mosaic was not.

Bennett (1940) distinguished three groups of viruses viz. those restricted to the phloem, parenchyma and both phloem and parenchyma. Absence of seed transmission with viruses restricted to phloem was attributed to the lack of vascular connections between the embryo and mother plant, whereas those of the parenchyma is due to the inability of the virus to enter or remain active in micro and megaspores of the pollen. As evidence to this, the association of seed transmission with pollen infection in all such cases has been pointed out by Caldwell, 1934; Bennett and Esau, 1936; and Gratia and Manil, 1936.

Effect of virus disease on the host plant.

(1) Carbohydrate content.

Reduction in carbohydrate content of the host plant is a characteristic of many virus diseases. Matsumoto (1922) showed a reduction in the accumulation of starch and probably sugar in mosaic affected leaves of the Azuki bean (P. vulgaris var; auren). Similar results have been reported by Brewer et al (1926) in tomato affected with mosaic and double streak viruses and by Cordingly et al (1934); Dimofte (1942) and Rischkov (1943) in mosaic affected tobacco. Holmes (1931) reported decreased production and accumulation of carbohydrates in the local lesions of Nicotiana glutinosa caused by tobacco mosaic virus. Dunlap (1930) suggested that viruses causing mosaic type diseases will decrease the carbohydrate content in the diseased leaves, while the reverse was true in the case of yellows.

Infected plants having a higher carbohydrate content was shown by Murphy (1923); Camp bell, 1925; and Thung, 1928 in potato leaf roll, Rosa (1927) in western yellow blight of tobacco, Sastri (1930) in spike disease of sandal, Rhiakhovsky and Fedulaer (1941) in cereals affected with wheat mosaic virus and by Watson and Watson (1953) in sugar beet yellows. Sastri (1930) attributed high carbohydrate

content in the leaves to the defective translocation of carbohydrates. Watson and Watson (1953), attributed this to the increased resistance to the movement of sugars through the leaf lamina.

(2) Nitrogen content.

Perhaps the most remarkable change in a virus infected plant is in the nitrogen metabolism. Cempbell (1925) reported a higher content of total nitrogen in potato affected with leaf roll. Similar observations were made by Spencer, 1930; Cordingly et al, 1934; Commoner et al, 1953; Best and Gallus, 1953; and Eskerous, 1964 in tobacco affected by mosaic disease. A higher content of total nitrogen in infected plants is also reported by Brewer et al (1926) in tomato infected with mosaic and in spike disease of sandal by Naresimhamoorthy et al, 1929; and Sreenivasaya et al, 1929. Dunlep (1930) suggested that viruses causing mosaic type diseases will increase the nitrogen content in the diseased leaves while the reverse was true in the case of yellows. John (1963) reported that in the case of Dolichos lablab plants infected with Dolichos enation mosaic virus and tomato infected with tobacco mosaic virus the various fractions of nitrogen viz. total nitrogen, protein nitrogen, soluble nitrogen and nitrate nitrogen showed

striking disparity between healthy and infected plants. He further observed an increase in the free aminoacid content of the infected shoots of D.lablab and tobacco.

(3) C/N ratio.

It has long been recognised that virus infection usually change the carbon:nitrogen ratio in the leaves; while some viruses increase it, others decrease it. Campbell (1925) reported that virus infection increased the C/N ratio, but Brewer et al (1926), Dunlap (1930) and Bawden (1960) reported decrease in the C/N ratio due to virus infection.

(4) Root Development.

Lucas (1939) and Soding & Funke (1941) reported poor development of root systems in virus infected plants. Similar results have been obtained by John (1959) in D.lablab infected with Dolichos enation mosaic virus.

Lucas (1939) and Soding and Funke (1941) attributed the poor development of the root system to a low auxin content in the roots affected by potato degeneration disease.

(5) Yield.

Kendrick and Gardner (1924) observed reduction in yield varying from 30 to 75 per cent in soybeans infected by mosaic. Racicot (1926) while comparing the yield difference in healthy and mosaic affected beans obtained an average yield of 20 bushels per acre in healthy plants against 15 bushels in the case of diseased plants. Boning (1928) indicated that bean mosaic incurred a loss of 5 to 10 per cent of the crop under normal conditions. Snyder (1934) reported that the loss from pod distortion in mosaic infected peas might amount to 10 per cent or more of the crop. Hartar (1936) reported considerable reduction in yield in lima beans affected by mosaic disease in Merryland. Chamberlain (1937) observed that garden peas with a small percentage of mosaic suffered a reduction in yield up to 47.7 per cent. Newton and Peiris (1953) reported that yellow mosaic of Pigeon pea did not seriously reduce the yield in Ceylon. Chenulu et al (1966) reported that the loss in yield due to groundnut mosaic varied from 29-100 per cent in terms of kernel weight and 22-97 per cent in terms of pod weight, depending upon disease intensity.

MATERIALS AND METHODS

MATERIALS AND METHODS

1. Seed material.

A local variety of horsegram obtained from the College Farm was used in the present study.

2. Culture of the virus.

The culture of the virus on horsegram plants was maintained in the field by natural transmission, by raising the crop at frequent intervals.

3. Test plants.

The following 26 plant species belonging to six different families were used in the study of the host range of the virus.

1.	French bean.	Phaseolus vulgaris L.,
2.	Lima bean	Phaseolus lunatus L.,
3.	Sword bean	Canavalia ensiformis (L.)D.C.,
4.	Indian bean	Dolichos lablab L.,
5.	Cluster bean	Cyamopsis psoroloides D.C.,
6.	Soy bean	Glycine max (L.)Merr.,
7.	Black gram	Phaseolus mungo L.,
8.	Green gram	Phaseolus aureus Roxb.,
9.	Cow pea	Vigna sublong Walp.,
10.	Sann hemp	Crotalaria juncea L.,

11.	Crotalaria	*Crotalaria striata* D.C.,
12.	Kolinji	*Tephrosia purpurea* Pers.,
13.	Petunia	*Petunia hybrida* Hort.,
14.	Tobacco	*Nicotiana tabacum* L.,
15.	Tomato	*Lycopersicon esculentum* Mill.,
16.	Datura	*Datura stramonium* L.,
17.	Brinjal	*Solanum melongena* L.,
18.	Physalis	*Physalis minima* L.,
19.	Chillies	*Capsicum annum* L.,
20.	Zinnia	*Zinnia elegans* Jacq.,
21.	Emelia	*Emelia sonchifolia* D.C.,
22.	Ageratum	*Ageratum conyzoides* L.,
23.	Hollyhock	*Althea rosea* L.,
24.	Bhindi	*Abelmoschus esculentus* W. & A.,
25.	Chenopodium	*Chenopodium amaranticolor* Costa & Reyn.,
26.	Cucumber	*Cucumis sativus* (L)Hoench.,

4. Transmission studies.

All transmission studies were done under insect-proof conditions.

(a) Insect transmission.

The white-fly, *Bemisia tabaci* Gen., was used as the vector. Virus free cultures of the insect were reared on healthy tobacco plants inside the insect-proof house.

The test insects were first fed on diseased plants for a specified length of time and then released on healthy plants and kept there for a specified length of time. Glass chimnies of size 2" x 2.5" x 5.5" with one side covered by a muslin cloth were used to keep the insects confined to the test plants. The insects were first released inside the chimnies which were then inverted over diseased plants in pots and allowed to feed. After specified acquisition feeding times, the plants in the chimnies were disturbed by introducing a small iron wire through the muslin cloth in order to remove the insects from the plants. The chimnies were then lifted with the insects adhering on the inner side, and were placed over healthy plants for infection feeding. After the specified infection feeding period, the chimnies were removed and the plants were disturbed to ward off any adhering flies.

The following acquisition feeding periods were tried.

15 minutes, 30 .., 45 .., 60 .., 75.., 90..,
105 .., 2 hrs, 4 hrs, 6 hrs, 8 hrs, 12 hrs,
16 hrs and 24 hrs.

The insects were straight away released on healthy test plants soon after the acquisition feeding.

Minimum number of white-flies required to cause infection were also tried using a minimum of one white-fly and to a maximum of 10.

(b) Sap transmission.

Sap transmission was done using concentrated as well as standard sap with and without using carborundum as an abrasive.

The concentrated sap was prepared by crushing and extracting the juice of infected leaves. The expressed juice thus obtained was applied on the leaves of young and vigorously growing test plants by rubbing with a small piece of cotton wool without causing injury to leaves. When carborundum was used, it was either dusted on the leaves before smearing the sap or used along with the sap.

The standard sap was prepared by adding 1 cc of sterilized distilled water to every gram of diseased plant material used for expressing the sap.

(c) Seed transmission.

Seeds were collected both from naturally infected and artificially inoculated plants, in which the infection has occurred when young. The seeds were sown in pots under insect-proof conditions.

(d) Transmission by grafting.

Grafting was done by inarching.

5. Chemical analysis.

(a) Collection of samples.

Sampling was done at four stages of growth of both healthy and inoculated plants as given below.

1st sample	On the date of inoculation i.e. when the plants were in the first trifoliate leaf stage.
2nd sample	20 days after inoculation.
3rd sample	40 days after inoculation.
4th sample	60 days after inoculation.

The experiment was done on potted plants with one plant in a pot. The pots used were of the size 4½" x 6" filled with the same potting mixture and plants were raised under same manurial and irrigational treatments. Infected plants required

were obtained by artificial inoculation. Inoculation was done when the plants were 14 day's old. The shoot and root systems of the plants selected for analysis were collected separately. Fifty plants were taken as a sample irrespective of the age of the plant.

Samples of shoot and root were taken from the same lot of plants. The plant material for the estimation of total sugar was dried at 60°C for 72 hrs, and that for other estimations at 105°C for 6 hrs., in a hot air-oven. The percentage of moisture and dry matter were also recorded. The dried plant materials were labelled and stored in desiccator.

(b) Estimation of total sugars.

Two grams of the moisture free plant material was taken in an Erlenmeyer flask to which 180 ml of water and 20 ml of concentrated hydrochloric acid were added. The flask was provided with a reflux condenser and boiled for two and a half hours, cooled and neutralized with sodium hydroxide initially and then with sodium carbonate. It was then filtered and the filtrate was made up to 200 ml and the quantity of total sugars was estimated by titration against Fehling's solution.

Ten ml of Fehling's solution (5 ml of A and 5 ml of B) was measured into a conical flask, diluted with 20 ml of water, heated to boiling and then the filtrate was added from a burette. When a faint precipitate was obtained 2-5 drops of methylene blue was added and the titration was completed at the stage of discoloration of methylene blue. The percentage of total sugars were calculated as follows.

$$\text{Percentage of total sugar.} = \frac{\text{Factor for Fehling's solution} \times 200 \times 100}{\text{Titre value} \times \text{weight in grams.}}$$

(c) Estimation of crude fibre.

Two gram of the moisture free plant material was taken in an Erlenmeyer flask. About 200 ml of 1.25 percent sulphuric acid was added and the solution was heated to boiling. After about 30 minutes digestion the contents of the flask were filtered through a linen supported in a flatted funnel and washed free from acid with boiling water. The residue on the linen was transferred completely to the flask and 200 ml of 1.25 percent boiling sodium hydroxide was added to it through the linen and the contents of the flask was digested for 30 minutes.

Afterwards it was filtered through the same linen and washed free of alkali with boiling water and later washed twice with alcohol. The residue was scraped into a silica crucible, dried free of moisture and weighed after cooling. Then the contents of the crucible was ignited to white ash in a muffle at 600°C and was weighed again. The difference between the two weights gave the weight of crude fibre and was expressed as percentage.

(d) Estimation of total carbohydrates.

For the determination of total carbohydrates the sugars and crude fibre estimated separately were added and represented as per cent.

(e) Estimation of total nitrogen.

Total nitrogen was estimated according to the method given by Piper (1950).

One gram of moisture free plant material was transferred into a Kjeldahl digestion flask containing 20 ml of concentrated sulphuric acid to which was added 10 g of Potassium sulphate and one gram of copper sulphate and digested. After the completion of digestion as evidenced by the appearance of a light blue colour the flask was cooled and water

extracts of the digested material was transferred into a 800 ml distillation flask. About 100 ml of 40 percent sodium hydroxide was added to it and distillation was carried out. The distillate was collected in an Erlenmeyer flask containing a known excess of 0.1 normal sulphuric acid. When about 150-160ml of distillate was collected, distillation was stopped and the excess acid was back titrated with 0.1 normal sodium hydroxide using methyl red as indicator.

Titre value for calculation is the volume of 0.1 normal sulphuric acid used for neutralization of with the alkali formed by distillation. The volume of acid not used during distillation was determined by titration with 0.1 normal sodium hydroxide. The differences in the volume of acid taken to the volume determined by titration, gave the volume of acid used for neutralization during distillation. This value become the titre value.

$$\text{Percentage of nitrogen in the plant material.} = \frac{\text{Titre value} \times 0.0014 \times 100}{\text{Weight of material in grams}}$$

6. Determination of root development in diseased and healthy plants.

The length of root systems, wet weight and dry weight of three months old healthy and diseased plants were determined. Comparative observations of 10 plants each were made.

7. Effect of disease on yield.

The number of pods formed, weight of pods, weight of seeds and weight of bhusa were determined. Comparative observations for 50 plants of both healthy and diseased were made throughout the life of the plants.

SYMPTOMATOLOGY

SYMPTOMATOLOGY

The symptoms of the disease first appear as light yellow patches on the leaflets. These gradually enlarge and develop into bright yellow patches, often covering most of the leaf area. Occasionally, the entire leaflet becomes chlorotic. No change in the size and shape of the leaf is noticed. The roots of the infected plants are poorly developed with poor nodulation.

Fig. 1. Symptoms of mosaic on horsegram.

FIG 1

Fig. 2. Photograph showing symptoms of mosaic on horsegram.

1. Typical mosaic

2. Chlorosis

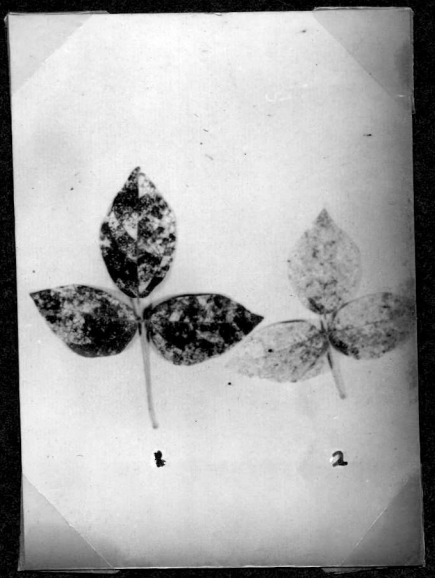


FIG. 2.

RESULTS

R E S U L T S

I. TRANSMISSION STUDIES.

A. Insect transmission.

(i) Effect of giving different acquisition feeding time.

It was found that the vector can acquire the virus with a minimum feeding time of 15 minutes. The infectivity increased when a feeding time of two hours or more was given up to 24 hours.

An incubation period of 10-20 and rarely up to 28 days was noted in the plants before symptom expression.

A corresponding increase in the percentage of infection or reduction in the incubation period in plants was not seen with increased acquisition feeding time (Table 1).

(ii) Number of insects required for transmission.

Infection could not be obtained when a single insect was used. None of the fifty test plants on which single insect transmission was done took infection. Infection was obtained when six or more insects were used per plant.

TABLE 1.

Effect of giving different acquisition feeding times on the transmissibility of the horsegram mosaic virus by Bemisia tabaci.

No. of white flies.	Period of acquisition feeding.	No. of plants inoculated.	Duration of inf. feeding	No. of plants infected.
10	15 min.	8	24 hr.	2
..	30 ..	8	..	1
..	45 ..	6	..	1
..	60 ..	8	..	3
..	75 ..	9	..	3
..	90 ..	7	..	2
..	105 ..	7	..	2
..	2 hr.	14	..	10
..	4 ..	14	..	8
..	6 ..	8	..	5
..	8 ..	8	..	4
..	12 ..	8	..	7
..	16 ..	8	..	5
..	24 ..	9	..	8

B. Sap transmission.

The virus could not be transmitted by sap inoculation.

C. Seed transmission.

Seed transmission of the disease was not noted. Out of 884 seedlings produced by sowing seeds collected from infected plants, none showed symptoms of the disease. These plants were kept under observation for two months.

D. Transmission by grafting.

The disease was readily transmissible by grafting. The symptoms on the grafted plants appeared in the young axillary shoots and top shoots in 25 to 28 days. All the leaves on the new growth showed typical yellow and green areas characteristic of the disease.

II. HOST RANGE.

Of the twenty six species of plants tested, transmission of the virus was possible only on frenchbean (Phaseolus vulgaris). The symptoms on frenchbean were different from those on horsegram. A downward curling of the leaflet from the place of attachment to the petiole was noted. This downward curling was not prominent on the first trifoliate leaf. Mottling of

Fig. 3. Symptoms of the disease on frenchbean.

FIG 3

yellow green and dark green areas were more distinct on the third and fourth trifoliate leaves than on the first. Mottling of yellow and green areas on the foliage was very conspicuous without any puckering. The yellow areas enlarged until the whole plant turned chlorotic.

Back transmission from the infected french bean plants to horsegram plants using white-flies produced typical symptoms of the disease on the latter.

III. CHEMICAL ANALYSIS.

(a) Estimation of percentage of moisture and dry matter in healthy and infected plants.

The percentage of moisture and dry matter in the healthy and infected shoots were more or less similar. The percentage of moisture decreased as the plants became older with a corresponding increase in the percentage of dry matter. The percentage of moisture on the date of inoculation was 87.21 which fell to 77.35 in the healthy plant and to 77.11 per cent in the infected plant when the plants were 74 days old. This indicates that infection did not affect the moisture content of shoots (Table 2), Fig. 4.

TABLE 2

Percentage of moisture and dry matter of healthy and inoculated shoots of Dolichos biflorus.

Age of the plant.	Days after inoculation.	Percentage of moisture		Percentage of dry matter.	
		Healthy shoot	Inoculated shoot	Healthy shoot	Inoculated shoot.
14 days	On the date of inoculation.	87.21	87.21	12.79	12.79
34 days	20 days after inoculation.	84.33	83.01	15.67	16.99
54 days	40 days after inoculation.	82.65	82.50	17.35	17.50
74 days	60 days after inoculation.	77.35	77.11	22.65	22.89

In the healthy as well as in the diseased plants the percentage of moisture in the roots decreased as the plants become older with a corresponding increase in the dry matter (Table 3). The moisture content of the roots of infected plants was however found to be lower than that of the healthy plants. The moisture

content of the roots of 74 days old healthy plants was 80.17 percent while that of infected plants was 72.52 percent.

TABLE 3

Percentage of moisture and dry matter of healthy and infected roots of Dolichos biflorus

Age of the plant.	Days after inoculation	Percentage of moisture		Percentage of dry matter	
		Healthy root	Infected root	Healthy root	Infected root.
14 days	On the date of inoculation.	84.70	84.70	15.30	15.30
34 days	20 days after inoculation.	79.89	73.26	20.11	26.74
54 days	40 days after inoculation.	85.41	77.44	14.59	22.56
74 days	60 days after inoculation.	80.17	72.52	19.83	27.48

(b) Total Sugars.

The sugar content in the shoot system of the infected plant was found to be lower than that in the

healthy plant, on dry as well as on fresh basis. The sugar contents in the shoots of 74 days old healthy and diseased plants on dry basis were of 10.64 percent and 7.37 per cent respectively, the corresponding percentage on fresh basis being 2.40 and 1.68.

In healthy shoots, the percentage of sugar on dry basis, was not much affected by the age of the plant. Fourteen days old plants had a sugar content of 10.35 per cent which showed a slight increase as the plant became older but ultimately it fell to 10.64 per cent, when the plants were 74 days old. But on fresh basis, the percentage of sugar gradually increased as the plant became older. The initial percentage of 1.32 in the 14 days old plants increased gradually and rose up to 2.40 per cent when the plants were 74 days old.

In the infected shoot there was a fall in the percentage of total sugar on dry basis. The sugar content fell from 9.02 to 5.94 per cent and this was subsequently followed by an increase to 7.37 per cent when the plants were 74 days old. The sugar content of infected shoot when calculated on fresh basis, however, showed a slight increase from the initial percentage of

1.32 to 1.68 after 60 days of inoculation when the plants were 74 days old. This increase was very low when compared to that in the healthy shoot.

The sugar content of root system of infected plants was also found to be lower than that of the healthy plant on dry basis. At the age of 74 days the percentage of sugar in the healthy root was 5.22 while that in the infected root was only 4.23. However, when calculated on fresh basis the sugar content of the infected root was found to be slightly higher than that of the healthy root being 1.16 per cent and 1.03 per cent respectively.

In healthy as well as in the infected roots there was an initial increase in the sugar content and subsequently when the plants became older there was a fall. In healthy roots the sugar content rose from 4.55 per cent to 9.73 per cent on dry basis and from 0.69 to 1.95 per cent on fresh basis between 14 days and 34 days of growth which at the age of 74 days fell to 5.22 per cent and 1.03 per cent respectively. The sugar content in infected roots also rose from 4.55 to 8.93 per cent on dry basis and on fresh basis from 0.69 to 2.38 per cent between 14 and 34 days of plant growth which at the age of 74 days fell to 4.23 per cent and 1.16 per cent respectively (Table 4), FIG: 6 & 7.

TABLE 4

Percentage of total sugars in shoot and root systems of both healthy and infected Dolichos biflorus.

Age of the	Days after	Percentage of total sugar							
		Healthy shoot		Inoculated shoot		Healthy root		Infected root	
		Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis
14 days	On the date of inoculation.	10.35	1.32	10.35	1.32	4.55	0.69	4.55	0.69
34 days	20 days after inoculation.	10.60	1.66	9.02	1.53	9.73	1.95	8.93	2.38
54 days	40 days after inoculation.	11.20	1.94	5.94	1.03	8.60	1.25	7.07	1.59
74 days	60 days after inoculation.	10.64	2.40	7.37	1.68	5.22	1.03	4.23	1.16

(c) Crude fibre.

The percentage of crude fibre in the root systems of infected plant was found to be lower than that in the healthy plant. This was apparent only on dry basis being 39 per cent and 22 per cent on 74 days old healthy and diseased plants respectively. The difference was not, however, very marked on fresh basis being 7.73 per cent and 6.04 per cent in healthy and infected roots respectively.

No appreciable difference was noted in the crude fibre content of the healthy and infected shoots (Table 5).

(d) Total carbohydrates.

The total carbohydrate content in the infected shoots was found to be lower than that of the healthy shoot on dry as well as on fresh basis. At the age of 74 days the healthy shoot recorded 32.64 per cent total carbohydrates on dry basis whereas in infected shoots it was only 28.37 per cent. On fresh basis the corresponding figures were 7.38 per cent and 6.48 per cent.

TABLE 5

Percentage of crude fibre in shoot and root systems of both healthy and infected Dolichos biflorus.

Age of the plant.	Days after inoculation.	Percentage of Crude fibre							
		Healthy shoot		Inoculated shoot		Healthy root		Infected root	
		Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis
14 days	On the date of inoculation	30.00	3.83	30.00	3.83	24.00	3.67	24.00	3.67
34 days	20 days after inoculation.	27.50	4.30	22.50	3.82	30.00	6.03	19.00	5.08
54 days	40 days after inoculation.	32.00	5.55	25.00	4.55	40.00	5.83	32.00	7.21
74 days	60 days after inoculation.	22.00	4.98	21.00	4.80	39.00	7.73	22.00	6.04

The total carbohydrates in the healthy and infected shoots, on dry basis, decreased as the plants became older. An initial percentage of 40.35 on the date of inoculation decreased to 32.64 and 28.37 in 74 days old healthy and infected shoots respectively. But on fresh basis a definite increase was noticeable from 5.15 per cent on the date of inoculation to 7.38 per cent and 6.48 per cent in the healthy and infected shoots respectively in 74 days old plants.

The total carbohydrates in the infected roots were found to be lower than those of the healthy roots on dry basis being 26.23 per cent and 44.22 per cent respectively in 74 days old plants.

In the healthy roots the total carbohydrates on dry basis showed a definite tendency to increase in the early stages of growth followed by a slight fall when the plants were 74 days old. The carbohydrate content rose from 28.55 per cent in 14 days old plants to 48.60 per cent in 54 days old plants and then fell to 44.22 per cent when the plants were 74 days old. But no such definite pattern was noticeable in the case of the root system of infected plants (Table 6), Fig. 8 & 9.

TABLE 6

Percentage of total carbohydrates in shoot and root systems of both healthy and infected Dolichos biflorus.

Age of the plant.	Days after inoculation.	Percentage of total carbohydrate							
		Healthy shoot		Inoculated shoot		Healthy root		Infected root	
		Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis
14 days	On the date of inoculation	40.35	5.15	40.35	5.15	28.55	4.36	28.55	4.36
34 days	20 days after inoculation	38.10	5.96	31.52	5.35	39.73	7.98	27.93	7.46
54 days	40 days after inoculation.	43.20	7.49	31.94	5.58	48.60	7.08	39.07	8.80
74 days	60 days after inoculation	32.64	7.38	28.37	6.48	44.22	8.76	26.23	7.20

(e) Total Nitrogen.

The nitrogen content in the shoot system of the infected plant was found to be higher than that in the healthy plant both on dry and fresh basis. When the healthy shoots recorded 2.99 per cent at the age of 74 days, the infected shoots recordad 3.25 per cent on dry basis. On fresh basis, the corresponding figures were 0.67 per cent and 0.74 per cent.

The nitrogen content on dry basis decreased as the plant became older and this tendency was noticeable in the healthy as well as in the diseased shoots. The initial percentage of 4.49 fell to 2.99 in the healthy and to 3.25 in the infected shoots.

The nitrogen content of the healthy shoot on fresh basis showed slight fluctuations as the plant became older but in the infected shoot it showed a definite tendency to increase from 0.57 per cent on the date of inoculation to 0.74 per cent when the plants were 74 days old.

The nitrogen content in the root systems of infected plant was also found to be higher than that in the healthy plant on dry as well as on fresh

basis. The nitrogen content of 74 days old healthy and infected plants were 1.78 per cent and 2.74 per cent on dry basis. The corresponding figures on fresh basis were 0.35 per cent and 0.67 per cent.

The nitrogen content of the healthy roots on dry basis showed a tendency to decrease with increasing age of the plant being 2.90 per cent in 14 days old plants and 1.78 per cent when the plants were 74 days old. But on fresh basis a slight increase was initially noted, from 0.44 per cent to 0.51 per cent, which decreased later on to 0.35 per cent. A similar tendency was noted in the infected root also. (Table 7), FIG. 10 & 11.

(f) Carbohydrate/Nitrogen ratio.

A narrow C/N ratio was noted in the infected plants both in the shoot and root systems. At the age of 74 days the C/N ratio of healthy shoot was 10.92:1 while that of the infected shoot was 8.73:1. Similarly healthy roots recorded a C/N ratio of 24.84:1 as against 10.62:1 in the infected roots of the same age.

TABLE 7

Percentage of total Nitrogen in shoot and root systems of both healthy and infected Dolichos biflorus.

Age of the plant.	Days after inoculation.	Percentage of total Nitrogen.							
		Healthy shoot		Infected shoot		Healthy root		Infected root	
		Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis	Dry basis	Fresh basis
14 days	On the date of inoculation.	4.49	0.57	4.49	0.57	2.90	0.44	2.90	0.44
34 days	20 days after inoculation.	3.26	0.51	3.45	0.58	2.54	0.51	2.74	0.73
54 days	40 days after inoculation.	3.05	0.52	3.77	0.65	2.34	0.34	2.62	0.59
74 days	60 days after inoculation.	2.99	0.67	3.25	0.74	1.78	0.35	2.47	0.67

The C/N ratio of healthy shoots widened initially till the plants were 54 days old from 9:1 to 14.16:1 and then it again narrowed down to 10.92:1 by the 74th day.

The C/N ratio of inoculated shoots remained almost steady during different stages of plant growth.

A gradual widening of the C/N ratio was observed in the root system of healthy plants when the plants become older. The initial ratio of 9.84:1 recorded when the plants were 14 days old widened gradually to 24.84:1 when they were 74 days old. But no such definite pattern was noticeable in the case of the infected plants. The C/N ratio of the roots of infected plants, was however, much narrower than that of the healthy roots at all stages of growth. While in the healthy roots of 74 days old plants the C/N ratio was 24.84:1, that in the infected root was only 10.62:1 (Table 8), FIG. 12.

TABLE 8

Carbohydrate/Nitrogen ratio of both shoot and root systems of healthy and inoculated Dolichos biflorus.

Age of the plant.	Days after inoculation.	Carbohydrate/Nitrogen Ratio			
		Healthy shoot	Inoculated shoot	Healthy root	Infected root
14 days	On the date of inoculation.	9:1	9:1	9.84:1	9.84:1
34 days	20 days after inoculation.	11.69:1	9.14:1	15.64:1	102:1
54 days	40 days after inoculation.	14.16:1	8.50:1	20.77:1	14.91:1
74 days	60 days after inoculation.	10.92:1	8.73:1	24.84:1	10.62:1

IV. ROOT DEVELOPMENT IN HEALTHY AND INFECTED PLANTS.

There was significant difference between the total length of healthy and infected roots. The roots of healthy plants were longer with normal nodulation.

In infected plants the roots were shorter with poor nodulation (Table 9).

TABLE 9

Variations in length and weight of healthy and diseased plants of Dolichos biflorus.

Sl.No.	Source	Healthy	Infected
1.	Mean length of 10 plants.	45.60 cm.	34.05 cm.
2.	Mean wet weight of 10 plants.	12.40 g	3.76 g

V. YIELD OF HEALTHY AND INFECTED PLANTS.

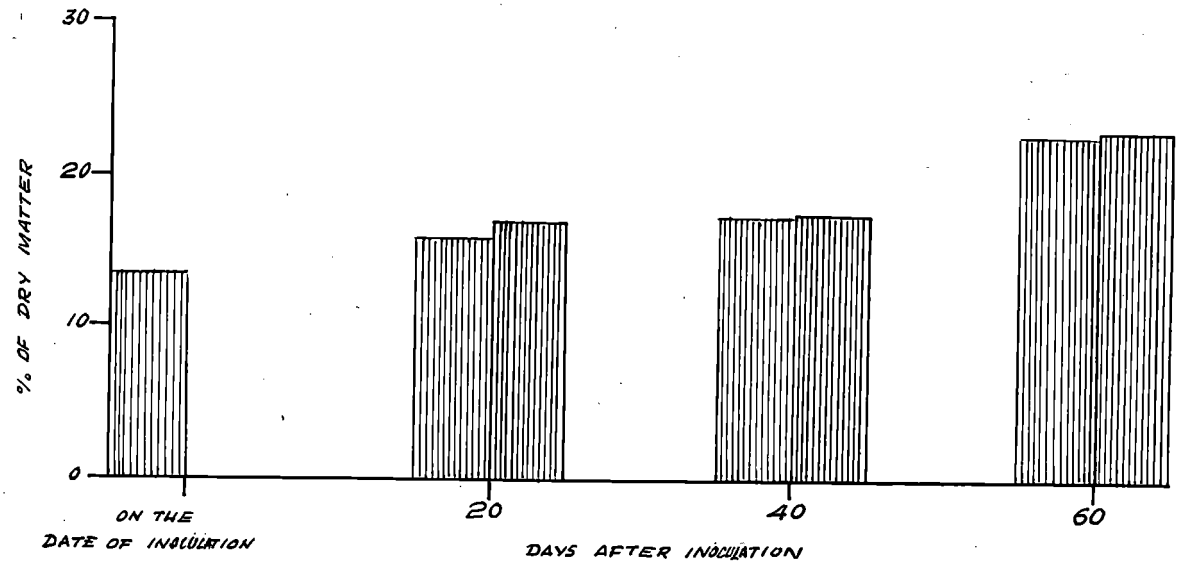
There was significant difference in the number and weight of pods, weight of seeds and weight of bhusa of healthy and infected plants. Infected plants recorded lesser pod formation with decreased weight of pods, seeds and bhusa (Table 10).

TABLE 10

Yield difference between healthy and infected plants of Dolichon biflorus.

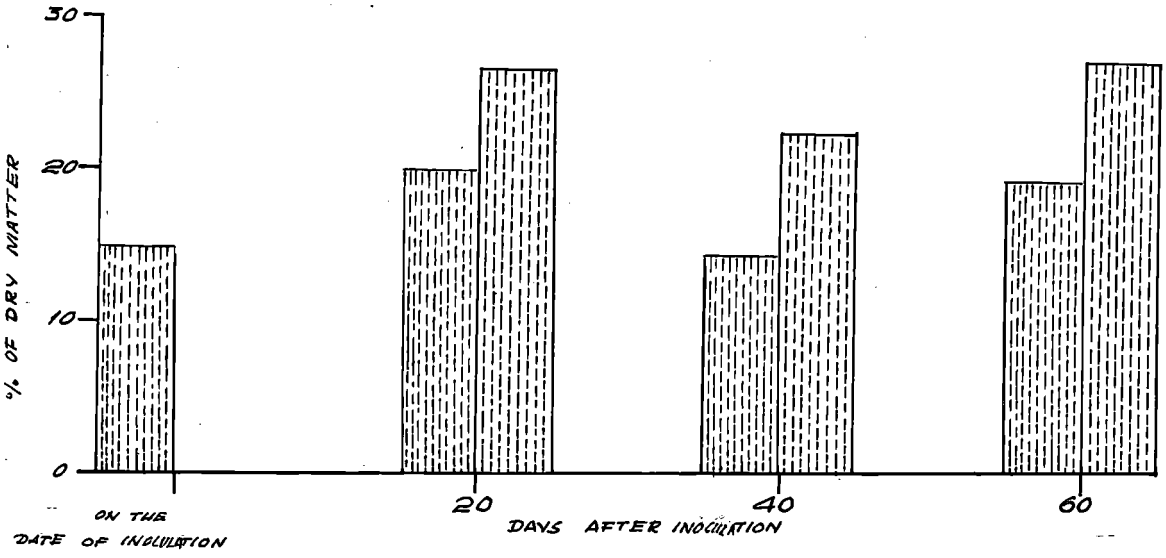
Sl.No.	Source	Healthy/ Infected	Mean of 50 plants	Difference between Means
1.	Number of pods.	Healthy	34.92	20.34**
		Infected	14.58	
2.	Weight of pods	Healthy	6.89	4.85**
		Infected	2.04	
3.	Weight of seeds.	Healthy	5.21	3.72**
		Infected	1.49	
4.	Weight of bhusa	Healthy	1.67	0.98**
		Infected	0.69	

● The significance was tested by appropriate test criterion.



PERCENTAGE OF DRY MATTER IN HEALTHY AND INFECTED SHOOT SYSTEM OF D. BIFLORUS

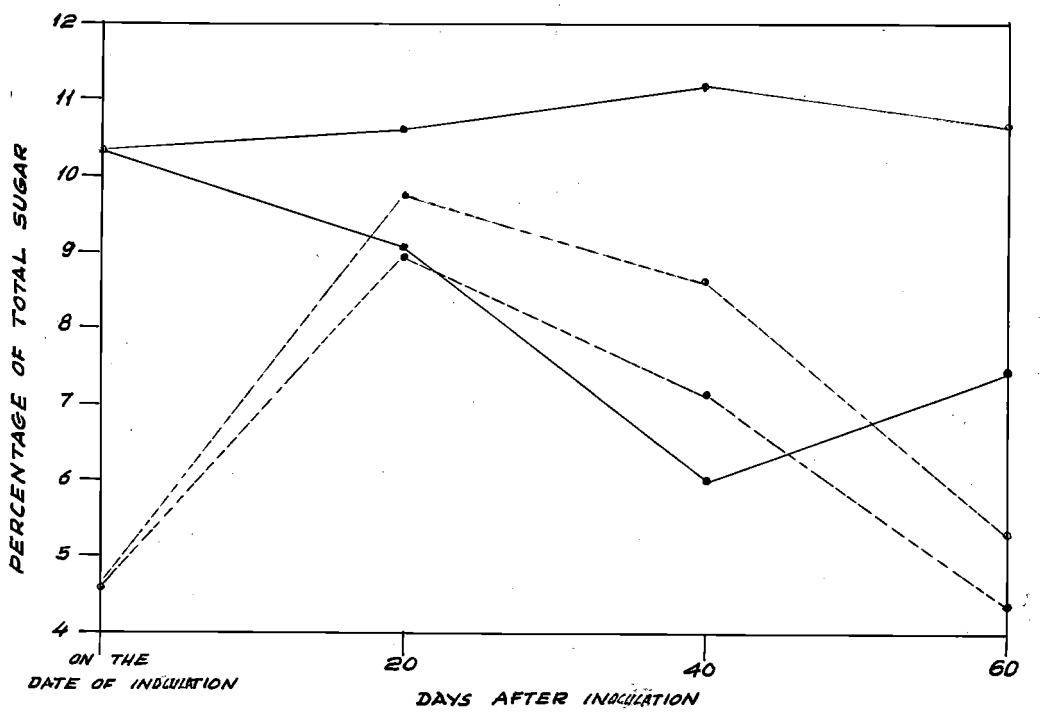
FIG 4



PERCENTAGE OF DRY MATTER IN HEALTHY AND INFECTED ROOTS OF D. BIFLORUS

FIG 5

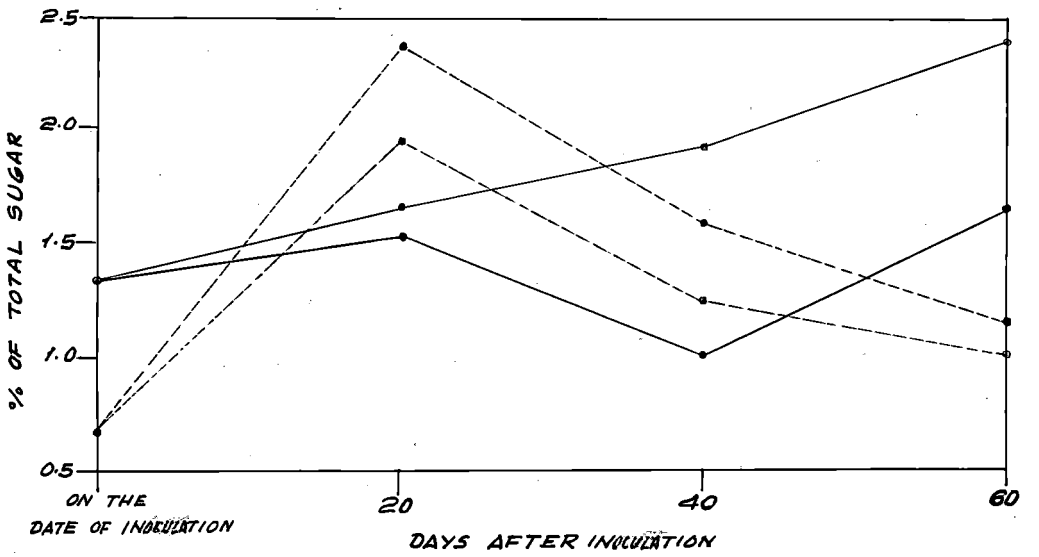
HEALTHY SHOOT HEALTHY ROOT INFECTED SHOOT. INFECTED ROOT.



TOTAL SUGAR

(DRY WT BASIS)

FIG 6

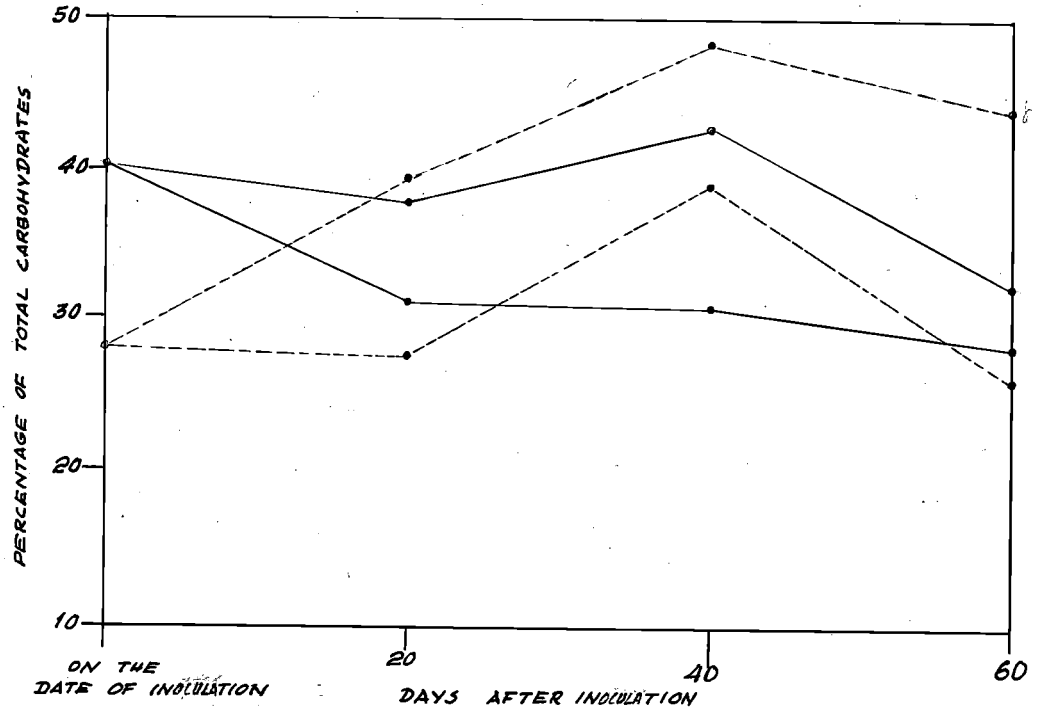


TOTAL SUGAR

(FRESH BASIS)

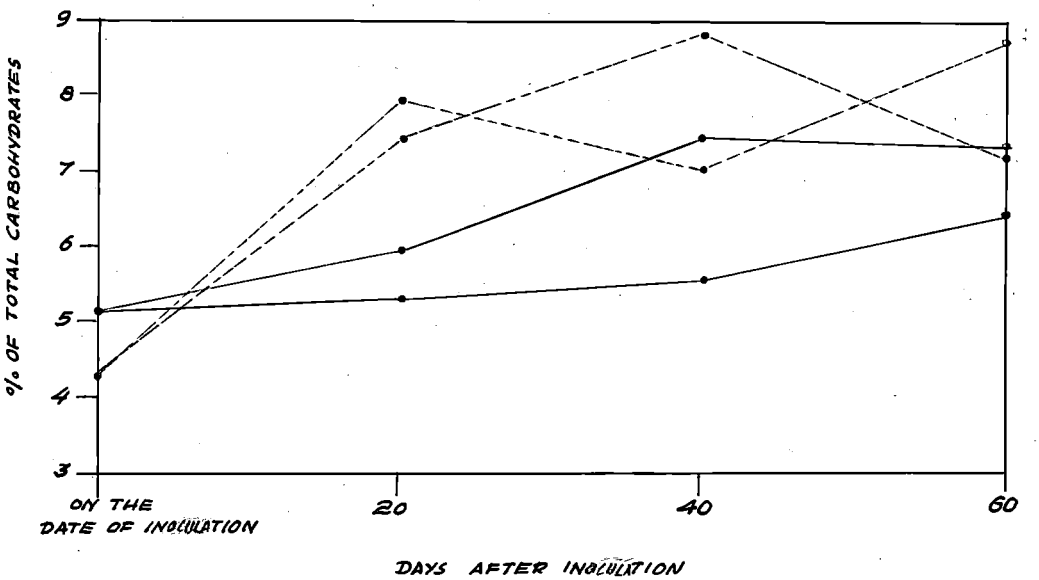
FIG 7

——HEALTHY SHOOT-----HEALTHY ROOT.—— INFECTED SHOOT.----INFECTED ROOT.



TOTAL CARBOHYDRATES
(DRY Wt BASIS)

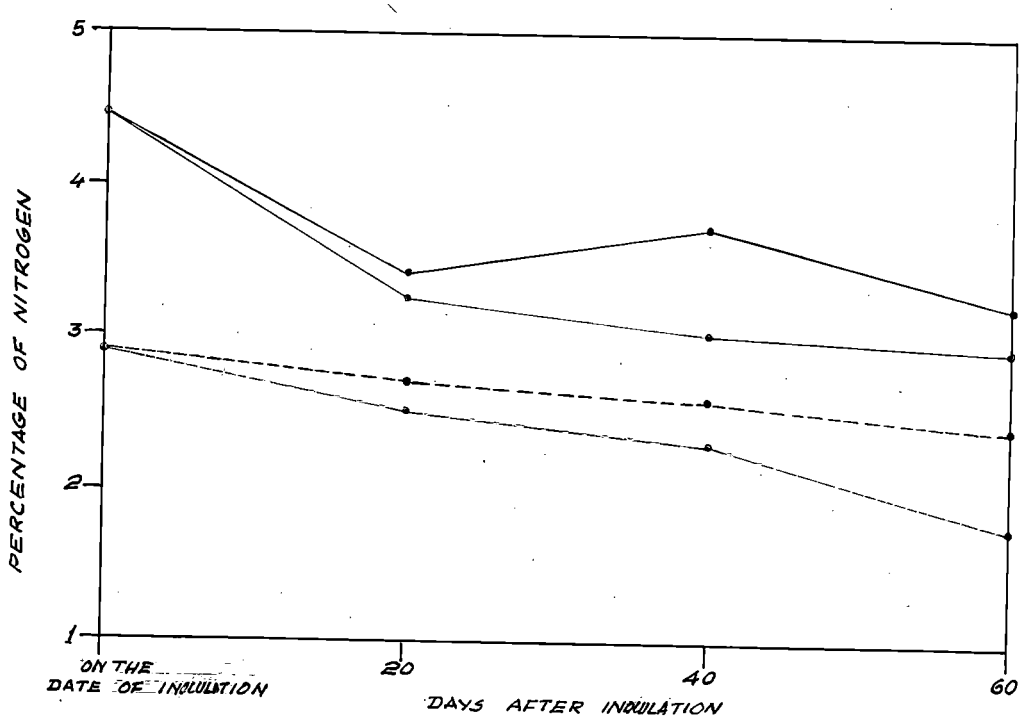
FIG 8



TOTAL CARBOHYDRATES
(FRESH BASIS)

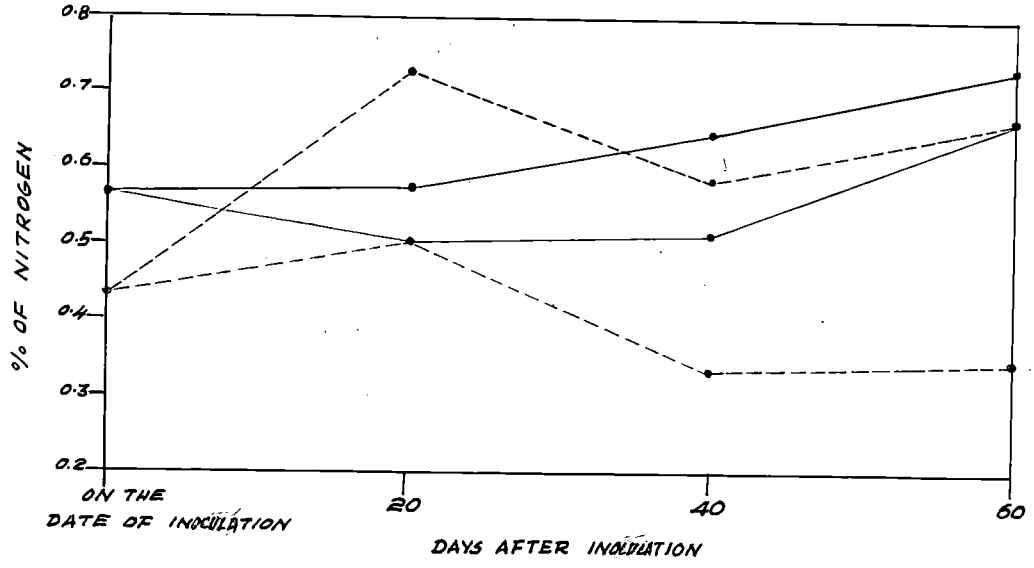
FIG 9

——HEALTHY SHOOT.----HEALTHY ROOT.——INFECTED SHOOT.------INFECTED ROOT.



NITROGEN
(DRY Wt BASIS)

FIG 10



NITROGEN
(FRESH BASIS)

FIG 11

——— HEALTHY SHOOT
——— INFECTED SHOOT. - - - - - INFECTED ROOT. —·—·— HEALTHY ROOT.

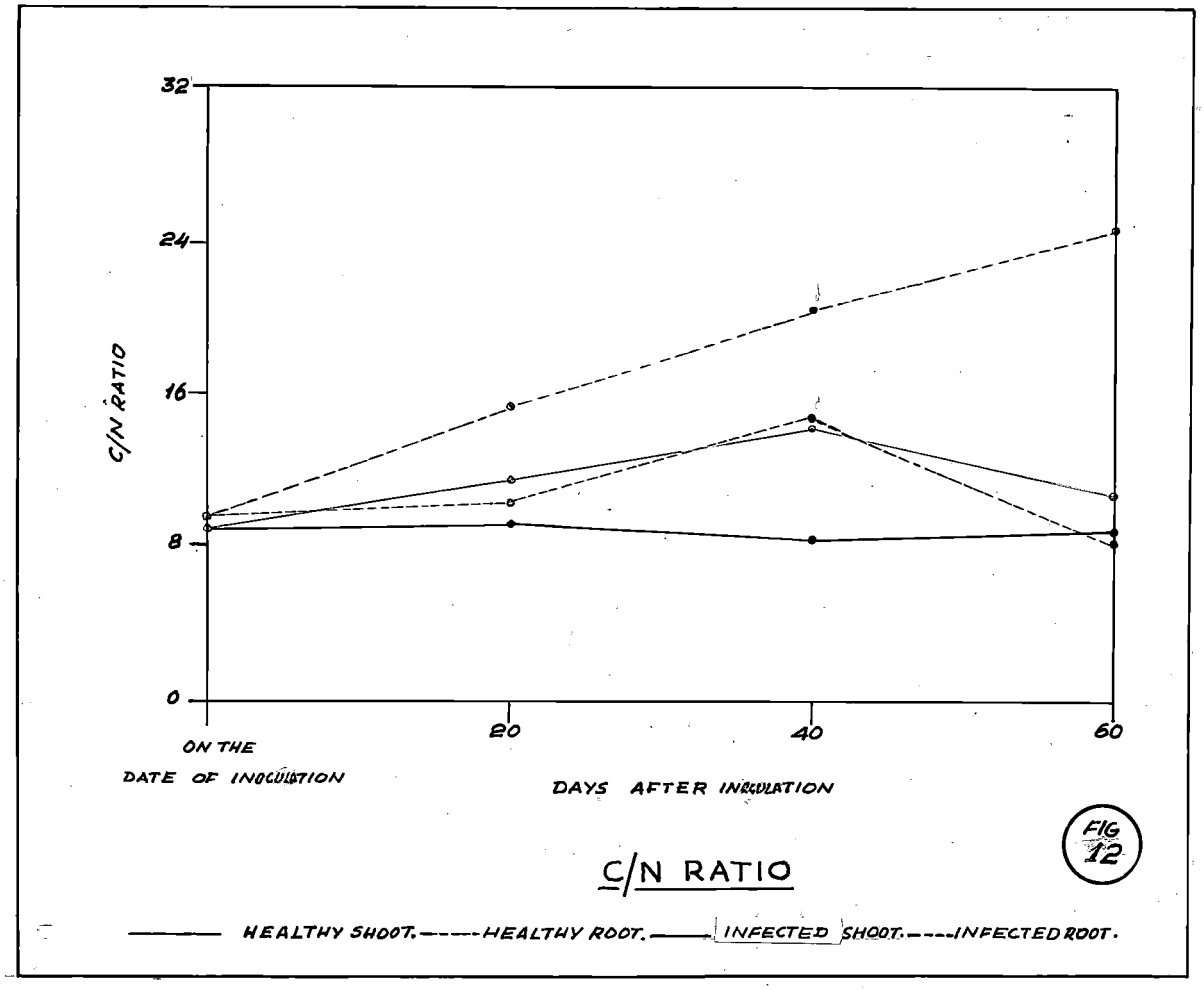


FIG 12

C/N RATIO

——— HEALTHY SHOOT. ----- HEALTHY ROOT. ——— INFECTED SHOOT. ---- INFECTED ROOT.

DISCUSSION

D I S C U S S I O N

The virus causing mosaic of Dolichos biflorus was found to be transmissible by inarching and also by the agency of white-flies. The disease could not be transmitted by sap inoculation or through seeds.

Though the vector could acquire the virus with a minimum feeding period of 15 minutes, it was observed that an acquisition feeding of two hours or more was necessary to obtain a higher percentage of infected plants. This observation is in accordence with that of Varma (1961) who reported a similar phenomenon in the case of yellow vein mosaic of bhindi.

The failure to get infection with single white-flies is not in keeping with the observations of earlier authors. Varma (1962) found that single white-flies were able to transmit the virus causing yellow vein mosaic of bhindi, but greater transmission occurred only by using a large number. This aspect therefore needs more elaborate study.

It was observed that the disease was not transmissible through seeds. Out of 884 seeds collected

from early infected plants none gave rise to diseased plants. Uppal (1931) got 25 per cent seed transmission in the case of the mosaic disease of D.biflorus which he reported from Bombay. Capoor and Varma (1948) who considered that the above disease is caused by the double bean yellow mosaic virus could not, however, get any seed transmission.

Among the twentysix plant species tested for host range, the disease could be transmitted only to Phaseolus vulgaris. In their studies on double bean yellow mosaic, Capoor and Varma (1948) observed that in addition to D.biflorus the disease could be transmitted to Phaseolus limensis, P.vulgaris, P.aureus and Canavalia ensiformis. The virus under study seems to resemble the double bean yellow mosaic virus in its symptoms on horsegram and modes of transmission, but it differs in that it was not transmissible to Phaseolus aureus and Canavalia ensiformis which are known hosts of double bean yellow mosaic virus. The present virus is also distinct from the one causing yellow mosaic of Phaseolus aureus reported by Nariani (1960) in symptoms as well as in host range. Based on the symptomatology and modes of transmission as well as the transmissibility

to P.vulgaris, it is proposed to consider the virus causing mosaic of Dolichos biflorus reported herein, as a strain of double bean yellow mosaic virus of Capoor and Varma.

It was found that virus infection did not in any way effect the moisture content of the shoots. But the root system of diseased plants had a comparatively lower moisture content.

The total carbohydrate in the diseased plant was lower than that in the healthy plant. This reduction in the carbohydrate content was noticeable in the shoot as well as in the root systems. Similar reduction in the carbohydrate content of infected plants was reported by earlier workers like Matsmoto (1922), Brewer (1926), Cordingly et al (1934) Dimoffe (1942), Richkov (1943) and John (1960). Sadasivan (1963) has pointed out that reduction in carbohydrates is characteristic of many virus diseases though there are also cases where infected plants have a higher carbohydrate content. Bawden (1963) has observed that a decreased rate of photosynthesis eventually leads to a lower rate of carbohydrate synthesis. The green area of the leaf is considerably reduced by the horsegram mosaic and naturally this is

expected to adversely affect the photosynthetic activity of the plant.

The nitrogen content of the infected plant was found to be higher than that of the healthy plant both in the shoot and root systems. Such increases in the nitrogen content of infected plants have been reported by a number of earlier workers like Campbell (1925), Spencer (1930), Cordingly et al (1934), Commoner et al (1953), Best and Gallus (1953), Watson and Watson (1953) and John (1963).

There was a narrowing of the C/N ratio of the infected plant as a result of the decrease in the carbohydrate content and the increase in nitrogen content of the plant. Mosaic type of Viruses are generally believed to bring about a narrowing of the C/N ratio. This narrowing of the C/N ratio was more pronounced in the root system of infected plants than in the shoot system, since the decrease in the carbohydrate and the increase in the nitrogen contents in the infected plant was greater in the root system as compared to the shoot system.

The root system of infected plants was found to be poorly developed with poor nodulation.

Further the infected plants recorded lesser pod formation with decreased weight of pods and seeds and a consequent reduction in the yield. Such reductions in yield, as a result of Virus infection have been reported by earlier workers like Kendrick and Gardner (1924) in Soybean mosaic; Racicot (1928) and Boning (1928) in bean mosaic; Snyder (1934) in pea mosaic; Harter (1936) in mosaic of lima beans and Chenulu et al (1936) in mosaic affected groundnut. The poor root development and the fall in yield may be attributable to the weakening of the plant brought about by virus infection.

SUMMARY

S U M M A R Y

A mosaic disease of Dolichos biflorus L., was studied.

The virus was readily transmitted by inarching, but was neither sap transmissible nor carried in the seed of horsegram.

The white-fly Bemisia tabaci Gen; was found to be the vector of the virus. The insect acquired the virus with a minimum feeding time of 15 minutes. Infection was obtained when six or more insects were used per plant.

Out of twentysix plant species tested, the virus was transmitted only to french bean. The symptoms produced on the french bean plants were different from those on horsegram.

The percentage of moisture in healthy and infected shoots were found to be more or less similar. However, root system of infected plants showed a lower percentage of moisture as compared to the healthy plants.

Total carbohydrate content was found to be lower in the shoot and root systems of infected plants.

The nitrogen contents of shoot and root systems of infected plants were found to be higher than that of healthy plants.

A narrow C/N ratio was noted in the infected plants both in the shoot and root systems.

The roots of diseased plants were found to be shorter with poor nodulation.

Diseased plants recorded a lower yield.

The virus resemble the double bean yellow mosaic virus of Phaseolus lunatus reported by other authors in symptomatology and modes of transmission eventhough it could not be transmitted to certain known hosts of the double bean yellow mosaic virus. Hence it is considered possible that the virus under study is a strain of the yellow mosaic virus of Phaseolus lunatus.

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