

Abstract

**EPIDEMIOLOGY, TRANSMISSION, BIOCHEMICAL
CHANGES AND MANAGEMENT OF PHYLLODY OF
SESAME (*Sesamum indicum L.*)**

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ABSTRACT

Sesame or Til (*Sesamum indicum L.*) belongs to family *Pedaliaceae* is one of the principal oilseeds in common use in India. Among the several diseases infecting sesame, phyllody seems much prevalent in Gujarat and especially in Saurashtra region. The incidence of phyllody during kharif and summer seasons ranged from 0 to 2.2 per cent and 0 to 4.7 per cent respectively, in scattered manner and spread of disease was very slow and first affected plant was observed at 50 days and maximum at 75 days after sowing.

The yield contributing characters of sesame (cv. Guj Til-2) plants i.e. plant height, number of capsules per plant, branches per plant, test weight were adversely affected due to phyllody. Losses in yield of sesame depend upon the infection time. Maximum reduction in yield was recorded in plants which were infected at an early stage rather than infected at advanced stage of growth.

The symptoms shown by infected plants include, yellowing, shortening of the internodes with stunted growth, smaller leaves, excessive proliferation of shoots resulting in a phyllody, virescence, sterile flowers, necrosis of the phloem tissues.

Phytoplasmas are phloem limited, therefore, only phloem feeding insects can potentially acquire and transmit the phytoplasmas. Leafhoppers have been confirmed as vectors of phytoplasmas.

Phytoplasmas enter the insect's body through the stylet, move through the intestine and are then absorbed in to the haemolymph. From here they proceed to colonise the salivary glands, a process that can take up to three weeks.

No mechanically sap as well as seed transmission was reported but some what 10 per cent transmission through grafting was reported.

Phyllody disease increased dry matter, phenol content, transpiration rate in infected parts of plants, while decreased moisture content, chlorophyll content, total nitrogen and crude protein.

Phyllody infection causes hormonal imbalance by altering the normal patterns in hormonal transport or translocation, ultimately resulting in such a drastic morphological, histopathological and anatomical changes.

No totally resistant cultivars found during study. Managed disease by check the vector population through systemic insecticide.

Epidemiology and survey study indicated considerable variation in the disease incidence between the seasons and from place to place. Incidence of phyllody correlated with its vector population, however, vector population build up dependent on temperature and relative humidity.

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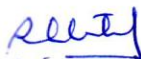
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This is to certify that the thesis entitled **“EPIDEMIOLOGY, TRANSMISSION, BIOCHEMICAL CHANGES AND MANAGEMENT OF PHYLLODY OF SESAME (*Sesamum indicum* L.)”** submitted by **Mr. PATHAK DIPAKKUMAR MAGANLAL** to Junagadh Agricultural University, Junagadh in partial fulfillment to the requirements for the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in the subject of **PLANT PATHOLOGY** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination was satisfactory; we therefore, recommended that the thesis be approved.



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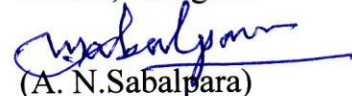
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CERTIFICATE

This is to certify that the thesis entitled "**EPIDEMIOLOGY, TRANSMISSION, BIOCHEMICAL CHANGES AND MANAGEMENT OF PHYLLODY OF SESAME (*Sesamum indicum L.*)**" submitted by **Mr. PATHAK DIPAKKUMAR MAGANLAL** in partial fulfillment of the requirements of the degree of **DOCTOR OF PHILOSOPHY** in the subject of **PLANT PATHOLOGY** of Junagadh Agricultural University, Gujarat state is a record of bonafide research carried out by him under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

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CHAPTER I

INTRODUCTION

Sesame (*Sesamum indicum* L.) belongs to family *Pedaliaceae* (Order: Tubiflorae) is native of India. It is commonly known as til (Hindi), tal (Gujarati), till (Punjabi), nuvvulu (Telugu), ellu (Tamil) and ragi (Oriya) in different parts of India. The plant has the basic chromosome number $2n = 26$. It is an annual, 1.0 to 1.5 meter tall, herbaceous plant maturing in about 70 to 140 days. The flowers are solitary, axillary, short pedicellate, zygomorphic and borne on the upper stem or branches. Self-pollination is the rule but cross-pollination due to visiting bees may usually be seen to extent of five per cent. The fruit is a capsule and contains numerous small ovate seeds (Vaughan, 1970).

Among the edible annual group of oilseed crops, sesame plays an important role in the oilseed economy throughout the world. It is considered as "Queen of Oilseeds" by users because of the quality of its oil and for its resistance to oxidation and rancidity even when it is stored at ambient air temperature.

Sesame is distributed mainly between 25°S to 33°S and 25°N to 40°N latitudes. It is a crop of both tropics and subtropics. The main sesame growing countries are India, China, Philippines, Korea, Thailand, Srilanka, Pakistan, Egypt, East Africa, Ethiopia, Sudan, Kenya, Tanzania,

Somalia, Uganda, Nigeria, Mexico, Venezuela, USA, and Australia (Balasubramanian and Palemiappan, 2000).

India ranks first, both in the area and production of sesame in the world. The annual area put under it in India is about 25 lakh ha (45 % of the world hectarage) and the total production is nearly 6.66 lakh tones. It is grown mostly in Gujarat, Rajasthan, Tamilnadu, Madhya Pradesh, Andhra Pradesh, Maharashtra and Orissa. In India, majority of sesame seeds are used for oil extraction (78%), 20% used for edible purposes and 2% for seed purpose. Sesame oil is mainly used for edible purposes (70%) *viz.* cooking and salad while for non edible purposes like domestic and toilet and margarine production accounts 14 and 16 per cent, respectively (Anon, 2008a).

In Gujarat, there are five districts *viz.* Amreli, Junagadh, Bhavnagar, Mehsana and Kaira under low runoff and medium yield gap region and three districts *viz.* Ahmedabad, Rajkot and Surendra Nagar under low runoff and high yield gap region accounts 3.72 lakh hectares area with an annual production of 1.70 lakh tonnes. The average yield of the state is 457kg/ha. In Gujarat, Surendranagar is leading with 0.90 lakh ha and 0.46 lakh tones production (Anon, 2008b).

Oil content of sesame seeds ranges from 46 to 52 per cent in different varieties. The oil is used as a

component of vegetable *ghee* and for anointing hair and skin. It is further used as a carrier for medicines and perfumes and as a synergist for pyrethrin based insecticides. The oil with 85 per cent unsaturated fatty acids is highly stable and has a reducing effect on cholesterol. It also prevents coronary heart diseases. It is highly rich in quality proteins and essential amino acids; especially methionine, is considered rejuvenative and anti-ageing for human body. It is also rich source of linoleic acid, vitamins-E, A, B₁, B₂ and niacin, minerals including calcium and phosphorus.

Seeds are used in preparation in baby foods, considered as the best substitutes for Mother's milk to compensate the breast feeding. After extraction of oil the remaining material i.e. sesame cake is used for cattle feeds and now a days as soil amendment for control of various diseases (Maiti *et al.*, 1988, Table – 1.1).

**Table – 1.1 : Nutritional value of edible portion per 100 g
of sesame seed**

Source : Sesame Seed (Til)

Energy Calorie(kg)	Protein(g)	Fat (g)	Calcium (mg)	Iron (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vit -A, Carotenoid(mg)
563	18.3	43	1450	10.5	1.01	0.34	4.4	60

The productivity level of 421 kg/ha achieved during the year 2001 – 02 is the record in the history of sesame cultivation and first time national average has surpassed the world average of 405 kg/ha, even though the yield potential of this crop is very low compared to major

oilseed crops. Major factors that limit its productivity besides narrow genetic base are extreme susceptibility to biotic and abiotic stresses. The phyllody an important disease of sesame is caused by mycoplasma – like organism (Phytoplasma) and transmitted by leaf hopper (Vasudeva and Sahambi, 1955).

In Saurashtra region of Gujarat state now a days phyllody become alarming problem especially in summer. No research work has been reported from this agro climatic zone. So looking to the importance of disease resulting in major loss, attempts were made to study following aspects of phyllody on sesame.

1. Transmission test.

2. Symptomatology.
3. Survey of sesame growing area for disease.
4. Occurrence of disease.
5. Epidemiological study.
6. Histopathological changes associated with phyllody.
7. Biochemical changes associated with phyllody.
8. Management of disease through vector control under field conditions.

CHAPTER II

REVIEW OF LITERATURE

2.1 INTRODUCTION AND GEOGRAPHICAL DISTRIBUTION

Plant diseases exhibiting symptoms like severe stunting, smalling of leaves, virescence and phyllody of flowers are collectively known as yellows type of diseases. Earlier, most of the yellows type of diseases were thought to be caused by viruses, until in 1967 when Doi and his co-workers reported presence of pleomorphic mycoplasma-like-organisms (now known as PLO's) in the phloem cells of plants infected with mulberry dwarf, paulownia witch's broom, aster yellows and potato witches broom diseases. Since then PLO's have been reported to be consistently associated with over 200 plant diseases causing serious losses to a variety of crops of economic importance (Agrios, 1988).

The occurrence of phyllody in sesame was first reported from Burma (Mc Gibbon, 1924) and later on for the first time from India by Pal and Pushkarnath (1935). The disease is now reported to occur in several countries viz., India (Vasudeva and Sahambi, 1955, Krantikumar *et al.*, 1962), Iran, Iraq and Israel (Klein, 1977), Sierra Leone (Deighton, 1932), Sudan (Mazzani and Malaguti, 1952), Tanzania (Deighton, 1932), Thailand (Choopanya, 1973), Turkey (Turkmenoglu and Ari, 1960) and Venezuela (Mazzani and Malaguti, 1952).

Choopanya (1973) reported for the first time that sesame phyllody is associated with mycoplasma like bodies. Purohit *et al.*, (1978a) detected mycoplasma like organism in the phyllody infected phloem cells of sesame.

2.2 ECONOMIC IMPORTANCE

Affected plants remain partially or completely sterile, resulting in total loss in yield. As much as 10 to 100 per cent incidence of the disease has been recorded in the sesame crop in India (Sahambi, 1970). The losses in plant yield, germination and oil content of sesame seeds may be as high as 93.66, 37.77 and 25.92 per cent respectively (Vyas, 1981). The disease is known to reduce plant yield, plant height and oil content of seeds (Verma and Daftari, 1985). It is estimated that one per cent increase in phyllody incidence decreases the sesame yield by 8.4 kg/ha under Coimbatore conditions in India (Anon, 1977., Murugesan *et al.*, 1978).

Prasad and Reddy (1997) reported up to 50% yield loss due to phyllody from Warangal and Karimnagar districts of Andhra Pradesh. Murugesan *et al.*, (1978), Krishnaswamy and Jayarajan (1983) reported 30 per cent loss in seed yield of sesame due to phyllody infection in Coimbatore(Tamilnadu). The age of plant at infected had a direct bearing on test weight of seeds. The reduction in test weight of seeds may be due to partial filling of the seeds at the time of maturity (Verma and

Daftari, 1985., Srinivasulu and Nayudh, 1983., Joshi and Bose, 1983).

The affected plants become stunted and the floral parts being modified in to leafy structures bearing no seeds causing heavy yield loss 26 – 33 per cent in Rajasthan and 15 per cent loss in Maharashtra (Abraham *et al.*, 1977b).

Generally the seeds from diseased plants are known to yield less oil than seeds from healthy plants. Verma and Daftari (1976 and 1985) and Kolte (1985) reported 18 per cent reduction in oil content in case of phyllody affected sesame plants. Verma and Daftari (1985) also noted that sesame phyllody reduced plant yield, test weight, germination percentage and oil content of seeds. The 25 percent transformation of the productive growth of plant into phyllody, it caused 39.73 per cent loss in seed yield of infected sesame plants(Krishnaswamy and Jayarajan, 1983).

Data pertaining to the field experiments carried out by Sarwar and Haq (2006) in Pakistan showed positive correlation between different morphological characters like days to maturity, plant height, branches per plant, number of capsules per plant, 1000 seed weight and seed yield per plant with sesame phyllody.

2.3 SYMPTOMATOLOGY AND HOST RANGE

Most of the PLO's – diseases are easily recognized by characteristic symptoms developed on the infected plants. The symptoms of various diseases can be grouped in to the following four categories (Varma and Rajamannar, 1989).

- (I) Yellows type with elongation of internodes, leaf yellowing and decline as in coconut lethal yellowing.
- (II) Stolbur type with reduced apical growth, stunting, smalling of leaves and virescence as in tomato stolbur.
- (III) Witches' broom type with proliferation of axillary shoots as in Potato witches' broom.
- (IV) Phyllody type with modification of floral parts into leafy structures, as in sesame phyllody.

The disease is characterized by the transformation of floral parts in to green leaf like structure followed by abundant vegetative growth. The calyx, which is gamosepalous alone, becomes polysepalous and has multicostate venation. The fine sepals become leaf like but are smaller in size. The veins of sepals and petals in the phylloid flowers are usually thick and prominent. The carpels get transformed in to leaf like out growths, which form a pseudosyncarpous ovary. The false ovary becomes very much enlarged and flattened with a soft texture and a wrinkled surface due to the thickening of the veins of the capillary wall. Small petiole like out growths which replace the ovules, it grown and burst through the wall

of the false ovary producing small shoots. These shoots continue to grow and produce more leaves and phylloid flowers. Thus, it seems as if the flower pedicel has grown through the flower and has an unlimited growth (Kolte, 1985).

Affected sesame plants express symptoms depending on the stage of the crops growth and time of infection. A plant infected in its early growth stage remains stunted to about two thirds of a normal plant and the entire plant may be affected. The entire inflorescence is replaced by a growth consisting of short, twisted leaves, closely arranged on a stem with every short internode. However, when infection takes place at later stages, normal capsules are formed on the lower portion of the plants and phylloid flowers are present on the tops of the main branches and on the new shoots that are produced from the lower portions (Vasudeva and Sahambi, 1955; 1957; Klein, 1977).

The phylloid flowers and new shoots produced are crowded together giving a bunchy top look to the infected plant (Vasudeva and Sahambi, 1955). Floral virescence, phylloid and proliferation, some times with yellowing, cracking of seed capsules, germination of seeds in capsules and formation of dark exudates on the foliage are the symptoms of phyllody observed by Salehi and Izadpanah, (1992) and Khalid Pervaiz *et al.*, (2009).

Franova *et al.*, (2007) have observed typical symptoms of phyllody like virescence, phyllody flowers, sterility of flowers, flat stem, proliferation of axillary buds and generalised stunting in cultivated as well as wild plants growing in Czech Republic and also identified Phytoplasma by electron microscopy.

Amongst the yellows type of disease reported so far in India, sesame phyllody is quite a serious disease as it spreads in nature through its leaf hopper vector to a large number of plant species, including several crop plants in addition to sesame (Vasudeva and Sahambi, 1955; Prasad, 1975). Varma *et al.*, (1969) reported the presence of mycoplasma – like organisms (PLO's) in the phloem cells of the leaf from little leaf infected brinjal plant.

Desmidts and Laboucheix (1974) described relationships between cotton phyllody and a similar disease of sesame that sesame may play an indirect role in the disease cycle as an alternate host for the vector, but is unlikely to be a direct natural source of inoculums for cotton. Rabindran *et al.*, (1988) showed phyllody symptoms on jute plants in Tamil Nadu. Prakasan and Vedamuthu (1988) also first time reported phyllody symptoms on *Trigonella foenum-graecum* (Fenugreek).

Sangeetha *et al.*, (1995) conducted serological tests suggested that the phytoplasma causing soyabean phyllody was serologically related to that causing sesame phyllody.

Paul Khurana *et al.*, (1981) reported that PLO's found in potato phyllody resemble those associated with other PLO's diseases but are distinct from that of electron – dense centered bodies of marginal flavescence. Phyllody symptoms of mung bean have reported by Lakshmanan *et al.*, (1988), Narendrasingh *et al.*, (2008) in India.

Prasad and Sahambi (1982) records new sesame phyllody host act as reservoirs of infection for sesame included some common perennial weeds viz. *Lathyrus odoratus*, *Pisum sativum*, *Portulaca trianthema*, *Eclipta alba*, *Lactuca sativa*, *Lamaea nudiculis*, *Vernoniq cinerea*, *Peristrophe bicalculata* and *Cannabinus sativa*. Phytoplasma are associated with plant disease and are known to cause more than 600 disease in several hundred plant species (McCoy *et al.*, 1989., Kirkpatrick, 1992).

2.4 CAUSAL ORGANISM

The causal agent of sesame phyllody is a phytoplasma (mycoplasma) like organism (PLO's). The mycoplasma like bodies are reported to be present in phloem sieve tubes of affected plants. Electron microscopy has revealed the big mycoplasma bodies ranging from 100 nm diameter to 625 nm diameter in sieve tubes. Bodies with beaded structure can

also be noticed. The PLO's are bounded by a single unit membrane and shows ribosome's – like structure and DNA – like strands (Klein, 1977).

Because of the difficulty in culturing phytoplasmas *in vitro*, the organisms remain one of the most poorly characterized groups of plant pathogens. In the last decade, however, molecular biological methods have made it possible to determine the phylogenetic and taxonomic relationships between phytoplasma strains and those between phytoplasmas and other prokaryotes. Currently, the classification of phytoplasmas is mainly based on the nucleotide sequence of the 16S rRNA gene (Gundersen *et al.*, 1994., Lim and Sears, 1989., Namba *et al.*, 1993., Seemuller *et al.*, 1994).

Phytoplasmas are prokaryotes lacking cell walls, Gram-Positive, low –G+C- content that are currently classified in the division Firmicutes, class Mollicutes, Order Acholeplasmatales, family Acholeplasmataceae, genus *Candidatus Phytoplasma* (Agrios, 2005., Bertaccini, 2007).

Association of PLO's with little leaf disease of brinjal was reported by Varma *et al.*, (1969), which was the first one regarding association of PLO's with plant diseases in India. Since then, a large number of PLO's – diseases have been reported from India (Varma and Rajmanner, 1989., Raychaudhuri, 1993).

At present, more than 300 distinct plant diseases affecting several hundred genera of plants have been reported to be caused by PLO's (Mc Coy *et al.*, 1989). Their name PLO's is due to the fact that, unlike mycoplasmas on animals, these plant pathogens can not be cultured artificially and can hardly be characterized taxonomically (Table – 2.2).

Table – 2.2 : Important Mycoplasma diseases of plants in India

Disease	Natural host	First report of the disease from India	First report of mycoplasma etiology
Acalypha witches' broom	<i>Acalypha indica</i>	Varma <i>et al.</i> , (1975)	-
Arecanut yellow leaf	<i>Areca catechu</i>	-	Nayar (1971)
Apple rubbery wood	<i>Malus domestics</i>	Bhargava and Bist (1960)	Beakbane <i>et al.</i> , (1971)
Bottlegourd phyllody	<i>Lagenaria siceraria</i>	-	Singh (1991)
Brinjal little leaf	<i>Solanum melongena</i>	Thomas and Krishnaswami (1939)	Anjaneyulu and Ramakrishnan (1969); Varma <i>et al.</i> , (1969)
Brinjal bushy stunt	<i>Solanum melongena</i>	Mitra and Chakraborty (1989)	-
Broombush witches' broom phyllody	<i>Parthenium hysterophorus</i>	Varma <i>et al.</i> , (1974)	Varma <i>et al.</i> , (1974) Phatak <i>et al.</i> ., (1975a)
Cannabis phyllody	<i>Cannabis sativa</i>	Phatak <i>et al.</i> , (1975b)	Phatak <i>et al.</i> , (1975b)

Citrus rubbery wood	<i>Citrus spp.</i>	Ahlawat and Chenulu (1985)	Ahlawat (1989)
Coconut (root) wilt	<i>Cocos nucifera</i>	Butler (1908)	Solomon <i>et al.</i> , (1983)
Cowpea witches' broom	<i>Vigna unguiculata</i>	Varma <i>et al.</i> , (1975)	Varma <i>et al.</i> , (1978)
Eucalyptus little leaf	<i>Eucalyptus tereticornis</i>	Sastry <i>et al.</i> , (1971)	Maramorosch <i>et al.</i> , (1982)
Grewia bushy stunt	<i>Grewia asiatica</i>	Rishi and Raychaudhuri (1981)	Rishi and Raychaudhuri (1981)
Marigold phyllody	<i>Tagetes erecta</i>	Varma <i>et al.</i> , (1975)	-
Mesta phyllody	<i>Hibiscus cannabinus</i>	-	Rangaswamy and Muniyappa (1987)
Mirabilis little leaf	<i>Mirabilis jalapa</i>	Ghosh <i>et al.</i> , (1975)	Ghosh <i>et al.</i> , (1975)
Mung (green gram) phyllody	<i>Vigna radiata</i>	Singh (1954)	-
Peach X	<i>Prunus spp.</i>	Ahlawat and Chenulu (1979)	Nasu <i>et al.</i> , (1979)
Peanut proliferation	<i>Arachis hypogaea</i>	Muthusami and Subramanian (1985)	Muthusami and Subramanian (1985)
Petunia little leaf	<i>Petunia spp.</i>	Varma <i>et al.</i> , (1975)	-

Phlox phyllody	<i>Phlox drunmondii</i>	-	Misra <i>et al.</i> , (1985)
Potato Phyllody	<i>Solanum tuberosum</i>	-	Khurana <i>et al.</i> , (1981)
Sesamum Phyllody	<i>Sesamum indicum</i>	Pal and Pushkarnath (1935)	Cousin <i>et al.</i> , (1970)
Sunnhemp phyllody	<i>Crotalaria juncea</i>	Bose and Misra (1938)	-
Sugarcane grassy shoot	<i>Saccharum officinarum</i>	Vasudeva (1956a)	Corbett <i>et al.</i> , (1972)
Tomato big bud	<i>Lycopersicon esculentum</i>	Vasudeva and Lal (1944)	Bowyer <i>et al.</i> , (1969)
Turnip phyllody	<i>Brassica campestris</i>	Varma <i>et al.</i> , (1975)	-
Urd (black gram) phyllody	<i>Vigna mungo</i>	Shyam and Bhatnagar (1965)	-
Zizyphus witches' broom	<i>Zizyphus jujube</i>	Pandey <i>et al.</i> , (1976)	Pandey <i>et al.</i> , (1976)

2.5 TRANSMISSION

Vasudeva and Sahambi (1955) indentified genus *Orosius albicinctus* Dist. as a vector of sesame phyllody and this disease was reported to be transmitted by the identified vector, while Choopanya (1972) carried out an attempt to transmit the pathogen through sap and seeds and have given negative results that no phyllody was observed on plants grown from seed from normal or phyllody infected plants.

In nature, the disease is transmitted by the leaf hopper vector, *Orosius albicinctus* Dist. (Vasudeva and Sahambi, 1955.,1957., 1958., Sahambi, 1958., Ghauri, 1966). Biology and bionomics (Bindra and Singh, 1970., Sundraraju and Jayaraj, 1977) and the vector – pathogen relationship (Sahambi, 1970) have also been studied in detail. The minimum acquisition feeding period, inoculation feeding period, incubation period in the vector and plants were found to be 4 hours and 30 minutes, 15 to 23 days and 13 to 25 days, respectively (Sahambi, 1970). Both male and female leaf hoppers were found to be equally effective in transmitting the disease. Natural transmission of PLO's is mainly through insect vectors. Out of the 300 PLO's diseases, more than 90 per cent are transmitted by leafhoppers (McCoy *et al.*, 1989).

The leaf hopper preferred diseased sesame plants, which were nutritionally favourable since they had higher moisture, N, Mg and Fe contents and lower carbohydrate and C : N ratio. The markedly low Ca content may allow easier penetration of the stylet and ovipositor.

2.5.1 PATHOGEN – VECTOR – HOST RELATIONSHIP

The plausible explanation for high incidence of phyllody in periods of low vector population could be that even a single leaf hopper vector carrying the inoculum from outside the field can cause infection in a number of plants. The pathogen

is acquired in persistent manner (Vasudeva, 1957) with acquisition feeding period of 8 hours (Bindra, 1973).

Hayashi *et al.*, (1996) conducted an ecological studies on sesame phyllody disease in Thailand. In the field survey, sesame phyllody occurred abundantly in the experimental field, which was transmitted by insect vector. The sesame phyllody became more prevalent in the field where sesame was cultivated previously in and nearby. Therefore, the sesame cultivation itself seems to accelerate the contamination of the environment with sesame phyllody – PLO's. This tendency seems to be related to the continuity of the leaf hopper vector and presence of sesame phyllody – PLO's in the field for next growing season. The population dynamics of *Orosius albicinctus* Dist. was the highest at the end of May and June. In other seasons it was rarely found in the field. Moreover, it lived only in sesame field and few was found in the field of other crops even in their population peak period. Srinivasan and Chellian (1978, 1981) reported that a single leaf hopper could transmit the PLO's within sesame field. They also observed relatively shorter incubation periods of 15 – 23 days and 23 – 67 days in the vector and host plant, respectively.

2.5.2 VECTOR POPULATION IN RELATION TO INCIDENCE OF DESEASE

Mathur and Verma (1973) reported that the incidence of disease increased with increase in vector population. The 20th June sown crop showed 26.74 per cent incidence of the disease with more *O. albicinctus* population while the 8th July sown crop showed 14.38 per cent disease with less vector population (4.36 per sweep), while controversial fact reported by Sellammal *et al.*, (1973) that the leaf hopper population recorded within the field has no significant influence on incidence of phyllody disease.

Observations on the biology of the leaf hopper, *Orosius albicinctus* Dist. have shown that the leaf hopper can over winter in all stages and is present in nature in smaller or greater number, all the year round in India. Consequently, the pathogen perpetuates through the leaf hopper vector from season to season with the help of the large variety of host plants (Sahambi, 1970., Bindra and Singh, 1970).

Khodeir (2006) in his ecological study, findout population density of the leafhopper *O. albicinctus* Dist. an identified vector of sesame phyllody on different field crops and weeds. Highest population observed per sweep was 11 to 14 individuals on cotton than other hosts.

2.6 BIOCHEMICAL CHANGES

2.6.1 Chlorophyll Content

Disease development causes impairment in the photosynthetic pigments which affects the utilization of light energy by the plants. This interferes with the biochemical events occurring in the diseased tissue and culminates in reduced photosynthesis and yield (Mahadevan and Sridhar, 1982).

Most of the yellows type of diseases caused by viruses and MLO's induce reduction in chlorophyll content. Mall and Seikh (1977) reported quantitative reduction of chlorophyll 'a' and 'b' in little leaf infected brinjal plants. Shukla *et al.*, (1984) have also reported significant reduction in chlorophyll 'a' and 'b' content in grassy shoot infected sugarcane leaves. Gupta and Chawla (1987) found a lower chlorophyll content in inoculated plants than in healthy ones in *Phaseolus vulgaris* infected with bean common mosaic virus.

Purohit *et al.*, (1978) have reported that chlorophyll content (total, a and b) of leaf tissue decreased with increasing disease severity although the ratio between chlorophylls 'a' and 'b' remained constant while Purohit *et al.*, (1983) have also observed in diseased tissues that ascorbic acid content increased and availability of sugars in diseased tissues may enhance ascorbic acid synthesis, these factors leading to proliferation of the tissues.

2.6.2 Nitrogen Content

With the exception of carbon, hydrogen and oxygen, nitrogen is the most prevalent element in the living organism and is found in such essential compounds as proteins, nucleic acids, some of the plant growth regulators and in many of the vitamins. Nitrogen is involved in most of the biochemical reactions which are vital for life processes (Rao,1996).

Regupathy and Jayraj (1973) reported that phyllody infected sesame plants contained higher nitrogen than the healthy plants. Shukla et al; (1988) also reported significant increase in total nitrogen in grassy shoot disease infected sugarcane plants compared to healthy ones. Total nitrogen content was also reported to be decreased in *Vinca rosea* due to the proliferation disease caused by PLO's (Patil and Patil, 1989).

2.7 HISTOPATHOLOGICAL STUDIES

Histopathology occupies an important place in the field of plant pathology today as it provides an insight to the interaction of both host and pathogen during the infection process. To understand the disease, pathological, anatomical and histological relationship between the affected organ or part of the plant and the parasite are essential to study. Mitra *et al.*, (1976) reported increase in respiration rate in little leaf

infected plants whereas enzymes like peroxidase, polyphenol oxidase and catalase showed decreased activity.

2.7.1 Anatomical Studies

Kothari *et al.*, (1982) carried out investigation on ultrastructural changes in phloem cells of little leaf infected brinjal plants. Mycoplasma infection resulted in the production of wall in growth, dilation of endoplasmic reticulum and deformation and destruction of mitochondria, plastids and tonoplasts.

Joshi and Bose (1983) tried to correlate histopathology with yield attributes characters in sesame due to transformation into leaf structures and failure of pollen grain and ovule productions. Singh and Mitra (1990) also observed several changes including transformed stigma into deformed swollen mass of shriveled cells. Ovules were aborted ultimately the gynoecium was transformed into green expanded leafy structure. Shukla *et al.*, (1988) studied histopathological changes in grassy shoot diseased sugarcane plant. The investigation showed structural changes in the chloroplast, mitochondria and nuclei in the diseased leaf tissues. The number and size of the chloroplast was reduced in the diseased plant and the grana and stroma were indistinguishable.

Nalini *et al.*, (1996) also noticed histological and biochemical changes like shrinkage of anther sacs with sterile pollen grains, hypertrophied anther walls, saccate pistil with phylloid ovules, and transformation of ground tissue to mesophyll in the calyx and corolla in affected plants, together with increases in polysaccharides and protein content in phyllody affected chickpea plant.

The modification observed in calyx, corolla and reproductive parts of the phyllody affected flowers are

(i) **Calyx** : In normal flowers the calyx was green, gamosepalous and s-partite. The segments in the calyx were linear with apices. However, in phylloid flower a complete division of the calyx tube occurred resulting in polysepalous condition. The sepals were leaking in pedicels and were leaf like in appearance. They were smaller and simpler in configuration than foliage leaves.

(ii) **Corolla** : The corolla turned green in colour in the phyllody affected flowers. The surface of corolla was rough. Gamopetalous condition of normal flowers changed to a polypetalous one. Petal apics were rounded.

(iii) **Androecium** : Unlike the four epipetalous stamens in normal flowers a fifth stamen was also observed in phylloid flowers. Further, the stamens were not epipetalous and they were green in colour.

(iv) **Gynoeceium** : The ovary was enlarged in size and the stigma was transformed into two flattened greenish structures. The carpellary wall too was transformed in to leaf like structures.

2.7.2 Detection of PLO's

In the absence of *in vitro* cultivation of PLO's, only electron microscopy is the only method by which the detection and characteristic ultrastructure of PLO's can be observed, however it is time consuming and expensive. Ultra-thin sections of phyllody sesame phloem tissue revealed the presence of pleomorphic membrane bounded bodies resembling rickettsia bodies (Sahambi and Prasad, 1977). Salehi and Izad Panah (1992) and Kersting (1993) observed blue areas in the phloem region when hand sections of stuns treated with Dienes strain and PLO's in the sieve cells of thin sections of infected plants seen under electron microscope.

Monoclonal gold-labeled antibodies have been developed for immunosorbent electron microscopy (ISEM) for detection of PLO's including maize bushy stunt (Chen and Jiang, 1988) Peach eastern X disease (Jiang *et al.*, 1989), aster yellows (Lin and Chen, 1985).

Recently, with the production of nucleic acid probes and discovery of polymerase chain reaction (PCR), a new dimension in detection of PLO's has been added. Nucleic acid

hybridization based on DNA probes with high specificity has been used to detect several PLO's including western X (Kirkpatrick *et al.*, 1987), Clover proliferation (Deng and Hiruki, 1991), faba bean phyllody (Saeed *et al.*, 1994) and Periwinkle little leaf (Davis *et al.*, 1990).

The PCR has been used to amplify a DNA segment specific for PLO's in nucleic acid extracts from plants infected with these pathogens. Namba *et al.*, (1993) demonstrated the utility of PCR in diagnosing PLO's diseased and the development of a phylogenetically based PLO's taxonomy.

2.8 MONITORING THE DISEASE ON WEATHER

PARAMETER

2.8.1 Correlation with weather parameter

Sesame phyllody is more destructive in summer period rather than kharif period as well as rabi summer period.

Mathur and Verma (1973) carried out a field plot experiment to find out relation between sowing date (from 6th July to 6th August) on incidence of phyllody and abundance of its vector *Orosius albicinctus* Dist. As the sowing date become later, the percentage of infected plants and the population size of the cicadellid vector decreased vector indexing for phyllody disease in sesame was carried out by collecting the leaf hopper *Orosius albicinctus* Dist. at 15 days interval during experiment

period. The study revealed that leaf hoppers collected on 15th February were found to be highly inoculative. The inoculative insects ranged from 16 to 60 per cent for the entire period of sampling with the maximum (60 per cent) on 15th February. There was a highly significant positive correlation between the percentage of leaf hoppers and phyllody incidence at 45 days after sampling. Srinivasulu and Narayanasamy (1995) also noted that sesame crops sown in January, February and March recorded a high incidence of phyllody disease with a maximum and minimum being in the crop sown on 1st March and 1st October, respectively.

Mathur and Verma (1973) observed that lowest percentage phyllody was recorded in the crop sown on 6th August followed by that on 26th July. Delayed Kharif sowing may control the incidence of Phyllody. Incubation period observed by Sahambi (1970) during summer was 13 – 25 days, while during winter it was 27 – 55 days.

Significantly higher percentage of phyllody has been observed in all the varieties in the mid July and lowest in the crop sown in late July and mid August months (Vachhani, 1945, Mathur and Verma, 1973, Baskaran *et al.*, 1991, Paramjit *et al.*, 1993).

2.9 MANAGEMENT

2.9.1 Cultural Control

An appropriate sowing date may be useful in avoiding severe occurrence of the disease. The incidence of the disease reduces considerably by sowing the crop in early August under Indian condition. The reduced population of the vector during growth period of sesame crop is perhaps important in keeping the disease under check (Mathur and Verma, 1973).

2.9.2 Chemical Control

Attempts were made to check sesame phyllody by controlling its vector with some insecticides (Sahambi, 1958; Muheet and Chauhan, 1975; Abraham *et al.*,1977a; Tandon and Banerjee, 1988).

At the time of sowing, soil may be treated with Thimet 10 g @ 10 kg / ha (Muheet and Chauhan, 1975) or with phorate 10 g @ 11 kg / ha (Tandon and Banerjee, 1988) or with Temik 10 g @ 25 kg / ha (Brar and Sandhu, 1976) to get the control of the disease. Effective degree of disease control is obtained when the above treatment is combined with spraying of the crop with methyle-o-demeton (0.1 %) or with any other effective systemic insecticide (Muheet and Chauhan, 1975, Tandon and Banerjee, 1988).

Tetracycline sprays at 500 ppm concentration at the initiation of flowering stage have been found effective against phyllody but recovery is temporary. A possibility of

biochemical control by spraying manganese chloride has been indicated (Purohit and Arya, 1980). It appears that manganese chloride oxidizes the phenol and protects or inhibits the enzymes, bringing the auxin level to normal. Once hyper auxin is oxidized, the plant can grow to its normal condition.

Ali (2003) managed the phyllody in sesame by two spraying of endosulfan 0.7 % through vector control.

Misra (2003) conducted a field experiment to evaluate efficacy of insecticides against sesame phyllody through vector control and reported that ethion and cypermethrin, cypermethrin and quinalphos, deltamethrin and triazophos combinations resulted in the lowest phyllody (3.9 to 8.7 per cent) than control (12 per cent) .

2.10 SCREENING

Dandnaik *et al.*, (2002) carried out a field experiment in Maharashtra to determine the susceptibility of 50 sesame lines to phyllody disease. Out of the 50 lines, 15, 3, 9 and 23 lines were found immune, resistant, moderately resistant and highly susceptible, respectively.

A total 42 advanced sesame genotypes were assessed by Gopal *et al.*, (2005) for their reaction to phyllody at Mallepally in Andhra Pradesh, results revealed that none of the genotypes were free from phyllody.

CHAPTER III

MATERIALS AND METHODS

The present investigation on "Epidemiology, Transmission, Biochemical changes and Management of sesame phyllody" was conducted at Department of Plant Pathology, Junagadh Agricultural University, Junagadh. To carry out the present investigation following materials and methods were adopted.

3.1 MATERIALS

3.1.1 Seeds

Genetically pure seeds of different varieties were obtained from Model Farm, Junagadh Agricultural University, Amreli.

3.1.2 Miscellaneous

All the required glasswares used in the experimental work were of Borosil grade. Chemicals were from BDH or E. Merck and Biorads (USA). Cleaned glasswares were sterilized in hot air oven at 180°C for 60 minutes.

3.2 RAISING OF SEEDLING

The present investigations were carried out in an insect proof cage house conditions.

A commercially important sesame variety was selected as test plant and raised from seeds in earthen pots of suitable size, which were filled with sterilized mixture of sand, medium black soil and well decomposed FYM. Seeds were treated with

appropriate seed dressing chemical before sowing to prevent from seed and soil borne pathogens.

3.2.1 Isolates of sesame phyllody

In order to study the prevalence and possible existence of phytoplasma like organism's causing sesame phyllody, samples of sesame phyllody disease, each consisting of young symptomatic leaves and young shoots were collected from the fields of Department of Plant Pathology, Junagadh Agricultural University, Junagadh. These samples were collected separately in polythene bags and were duly labelled and transported immediately in ice box to working laboratory for further studies.

3.2.2 Isolation and maintenance of isolates

Isolation of sesame phyllody disease collected from the field of Department of Plant Pathology, Junagadh Agricultural University, Junagadh, were established and maintained on single host sesame (*Sesamum indicum*) by mechanical sap inoculation and by vector transmission in an insect proof cage house. Isolation of sesame phyllody disease maintained on this host served as sources of PLO's for all the subsequent tests. Test plant sesame (*Sesamum indicum*) was raised from healthy seeds in earthen pots (15 cm diameter) containing steam sterilized soil, compost and sand (2:1:1) mixture.

3.3 TRANSMISSION STUDIES

3.3.1 Mechanical transmission

Standard inoculum of different sesame phyllody disease isolate was prepared in cold 0.05 M potassium phosphate (KH_2PO_4) buffer (pH 7.0) + 0.02 M 2-mercapto ethanol, by titrating young symptomatic leaves in a chilled mortar and pestle. The resultant inocula were filtered separately through double covered muslin cloth, before being used for mechanical inoculation.

Twenty one days old plants of sesame were inoculated by conventional leaf rub method using cotton swab described by Holmes(1929). Carborundum (800 mesh) was used as an abrasive. Immediately after inoculation, plants were rinsed with water. Healthy seedlings of sesame were inoculated similarly with distilled water and were used as controls. All the inoculated plants were maintained in an insect proof cage house and were regularly observed for development of symptoms. Observations with respect to the incubation period, sequence of symptom development and level of PLO's transmission were recorded.

3.3.2 Transmission by grafting

The diseased material was grafted on to healthy sesame plant cv.Guj. Til-2 maintained in an insect proof cage house for

further use. When the plants had 4 to 5 leaf stage, they were grafted with the material from naturally infected sesame plants.

Ten 4 week old sesame plants were graft inoculated using phytoplasma inoculum under insect proof cage house conditions. For grafting, a sliced cut was made on the stem 2 cm below the tip. A 12 cm long sesame branch exhibiting typical phyllody symptoms was detached from an infected plant and a similar cut (as on the test plant) was made on this branch. The corresponding cut surfaces were tied together with parafilm. Grafted plants were observed periodically for symptom development.

3.3.3 Seed Transmission

3.3.3.1 Transmission through seed

Seed transmission provides an effective means of introduction of pathogens especially virus and fungi into crop at an early stage, giving randomized foci of infection throughout the field. To study the spread of pathogens within the growing crop, seed transmission is important. Especially distribution of seed borne virus over long distance may occur due to long or high persistence of viruses of seed.

For seed transmission, the seeds were collected from PLO's infected sesame plants. Seeds were sown in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture and maintained in an insect proof cage house. For

determining the level of seed transmission, number of infected plants by seed transmission were counted in total population.

3.3.3.2 Infected seed and PLO's transmission

In the initial stages the possibility of PLO's transmission through seed was determined by sowing 100 seeds in 10 pots (10 seeds per pot) filled with sterilized soil. The growing seedlings were observed daily for symptoms expression.

3.3.3.3 Seed grades and PLO'S transmission

Completely matured seeds were divided into three grades on the basis of deformity and coloration as described below

Grade – 1 Normal, healthy looking(Smooth Bold Seeds)

Grade – 2 Deformed Sunken Seeds(Small and Shriveled)

Grade – 3 Discoloured Seeds

These were grown separately and observed periodically for transmission of the PLO's in seedlings.

3.3.4 Vector transmission

3.3.4.1 Maintenance of pure culture of leaf hopper's

Orosius albicinctus Dist. species used in transmission tests, were collected from sesame. For all the vector

transmission tests, hopper's raised from single hopper's colony, the healthy leaves of feed plant was placed in Petridishes on slightly wet filter paper, finally hopper's were transferred separately with camel hair brush to the test leaves. Healthy plants were kept in muslin cloth in insect proof cage house.

For vector transmission, adult and nymph form of hopper's were given a pre-acquisition fasting period of 24 hrs. by transferring them to clean Petridishes. This was followed by an acquisition feeding of 24 hrs to three weeks on phyllody infected detached leaves of the source plants. Each hopper's during the PLO's acquisition probe was watched with microscope and timed with a watch. Hopper's still in probing position were picked up with camel hair brush and transferred in batches of 5 to test plants for different inoculation feeding periods. Later, hopper's were killed by spraying with 0.025 % methyle-o-demeton insecticides and plants were maintained in an insect proof cage house for 6 weeks and observations were recorded for the type of symptoms, incubation period and the level of transmission. The healthy seedlings receiving non-PLO's hopper's served as control.

3.3.4.2 Determination Of hopper's transmission

For determining hopper's transmission efficiency, PLO's source (*S. indicum*) and test plant (*S. indicum*) were used. A varying number of hopper's viz, 1, 5, 10, 15, 20, 25 and 30 of

Orosius albicinctus Dist. were used in these tests. After a pre-acquisition starvation period of 24 hours and acquisition feeding of 24 hrs to three weeks on the leaves of test source host, different number of hopper's were transferred separately to sets of twenty healthy test plants, and allowed for an inoculation feeding of 21 days. Later, hopper's were killed by spraying with 0.025 % methyle-o-demeton insecticide. Test plants were maintained in an insect proof cage house. The efficiency of each hopper's treatment was assessed by computing per cent infectivity based on twenty test plants.

All the transmission tests were carried out preferably on 4 to 5 leaves of 21 days old seedlings. All the inoculated plants were maintained in an insect proof cage house.

3.4 SYMPTOMATOLOGY

The symptomatology of phyllody disease on naturally infected plants was studied. The symptoms in mechanically inoculated plants of sesame cv. Gujarat Til – 2 under insect proof conditions were also observed and reported in detail.

3.5 BIOCHEMICAL CHANGES

For studying biochemical changes in phyllody infected leaves of sesame, diseased leaves were collected and care was taken to select only such leaves, which were identified in age

and well developed symptoms. Healthy leaves collected from healthy plants of same age served as check. Analysis for the estimation of protein (Lowry *et al.*, 1951), estimation of Phenol (Bray and Thorpe, 1954), determination of chlorophyll content by colorimeter method (Witham *et al.*, 1971) and moisture as well as dry matter content on weight basis.

The glasswares were scrubbed and washed thoroughly with detergent, washed with tap water followed by distilled water. Finally, the glasswares were dried in an oven before use. All polywares were thoroughly cleaned as stated above and air-dried before use.

All the stated chemicals and reagents used were of analytical grade purity and obtained from standard company.

3.5.1 Estimation of chlorophyll content

Chlorophyll content in the leaves was measured by following the colorimeter method. 2 g of finely chopped from leaf tissues were thoroughly homogenized with 80 % acetone. The supernatant was decanted and filtered through whatman no. 4. into a 100 ml volumetric flask. The extraction process was repeated 3 times, all the extract pooled and the final volume was made up with 80 % acetone of this, 5 ml was taken and volume made up to 25 ml with 80 % acetone. Absorbance was measured in a spectronic 20 spectrophotometer at 645 nm and 663 nm for chlorophyll 'a'

and 'b' contents. Chlorophyll 'a' and 'b' were calculated according to the following formula and total chlorophyll content was expressed in mg g^{-1} f.w. of the leaf tissue.

C 'a' =	$12.7 (A\ 663) - 2.69 (A\ 645) \times \text{Volume of sample}$
	$1000 \times \text{Weight of sample (g)}$

C 'b' =	$22.9 (A\ 645) - 4.68 (A\ 663) \times \text{Volume of sample}$
	$1000 \times \text{Weight of sample (g)}$

Chlorophyll total =	$20.2 (A\ 645) - 8.02 (A\ 663) \times \text{Volume of sample}$
	$1000 \times \text{Weight of sample (g)}$

Where,

C'a' = Chlorophyll a (mg g^{-1} f.w.)

C'b' = Chlorophyll b (mg g^{-1} f.w.)

3.5.2 Estimation of protein

Protein in crude extracts was estimated following Lowry *et al.*, (1951) standard curve was prepared using Bovine Serum Albumin (BSA) as the reference protein in the range of 40 to 400 μg per ml at 610 nm.

The different reagents used were as follows

- (a) Protein working solutions : A series of Bovine Serum Albumin (BSA) working solutions ranging from 40 to 400 $\mu\text{g protein ml}^{-1}$.
- (b) Anhydrous Na_2CO_3 2 % (v/v) in 0.1 N NaOH.
- (c) Cupric sulphate ($\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$) solution : 1 % (w/v).
- (d) Sodium Potassium tartarate solution : 2 % (w/v).
- (e) Alkaline copper reagent : Prepared by mixing 1.0 ml Sodium potassium tartarate, 48 ml anhydrous Na_2CO_3 and 1.0 ml Cupric sulphate solutions in that given order.
- (f) Folin – Ciocaltean Phenol reagent :

Commercially available reagent was diluted with distilled water to 1:1 ratio to make 1 N.

1 g of leaf tissue was homogenized with 3.0 ml of 0.05 M Tris-HCl buffer (pH 7.6). The homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and fractions of 0.02 ml was taken out in separate tubes. The volume in each tube was made up to 1.0 ml with double distilled water. Colour was then developed by adding 5.0 ml of alkaline CuSO_4 and 0.5 ml of Folin's reagent. After 30 min. absorbance was measured at 610 nm. The amount of protein in the sample was determined from the standard curve prepared with BSA. The protein content was expressed in mg of protein g^{-1} fresh weight.

3.5.3 Determination of moisture content and dry matter content

Immediately after collection, the plant samples were weighed to determine the fresh weight. The samples were dried at 75 °c in hot air oven till constant weights were obtained (Each time the materials were brought to room temperature and then weighed). Dry weights were then recorded and from the difference between fresh and dry weights, moisture content was determined.

3.5.4 Estimation of phenol

Phenol estimation was carried out by following the methods of Bray and Thorpe (1954)

Five gram of fresh tissue was taken in 50 ml of 80 % methanol and heated in a boiling water bath for 10 minutes. The tissue was ground in an electric mixer and filtered 0.1 to 0.5 ml of the supernatant was taken in tube and volume made up to 1 ml by adding distilled water. 0.5 ml of folin – ciocalteu reagent was added followed by 1 ml of Na_2CO_3 solution. The tube was shaken and heated in a boiling water bath for exactly 1 minute. After cooling under a running tap water, the blue

solution was diluted to 25 ml with distilled water and its absorbance was measured at 650 nm in a spectrophotometer. Total phenol was calculated with the help of reference curve using catechol (100 mg / 100 ml) as standard and expressed as mg/g.

3.5.5 Determination of rate of transpiration

Rate of transpiration of healthy and severely infected leaves were measured using a battery operated portable photosynthetic system, reading were taken on a bright sunny day. The leaves were inserted into the chamber. The chamber was properly sealed taking care that there was no leakage and the leaf was not injured. Care was also taken that no shadow fell in the chamber while taking the reading.

3.6 HISTOPATHOLOGICAL STUDIES

3.6.1 Sectioning and staining

Samples were collected from phyllody infected plants showing pronounced symptoms. Similarly, samples were also collected from disease free plants of same age and grown under identical conditions. Hand sections were cut from different parts of the plant e.g. stem, petiole, leaf and flower. The sections were stained with methylene blue. Some of the

sections were stained with safranin and fast green combination (Johansen, 1940).

Methylene blue preparation :

Methylene blue 0.3 g.

Ethyl alcohol (95%) 30 ml.

Safranin preparation :

Safranin 1g

Distilled water 100 ml

Fast green preparation :

Fast green 0.5 g

Alcohol (95%) 100 ml

The stained sections were dehydrated and brought to pure Xylene, through graded alcohol series (40 %, 60 %, 75 %, 95 %) and the sections were mounted and slides prepared this way were then observed under light microscope for histopathology.

3.6.2 Purification and electron microscopy

The partial purification of the sesame phyllody(PLO's) was carried out following the methods described by Gibbs *et al.*, (1963).

Gibbs Method :

For purification, the PLO's was extracted from leaves of young sesame plants, 21 days after PLO's inoculation. The leaves (120 g) were homogenized in a waring blender in an equal volume of buffer(W/V), pH 7.0 (made by mixing equal volumes of 0.02 disodium hydrogen phosphate and 0.1 M ascorbic acid). Chilled chloroform (half the volume of the buffer) was then added and the mixture was homogenized further to form an emulsion. The emulsion was centrifuged at 14000 rpm for 15 minutes. The aqueous phase was removed and stored over night at 4 °C and was again centrifuged at 14000 rpm for 5 minutes. The supernatant was centrifuged at 36000 rpm for 90 minutes. The PLO's containing pellet was suspended in 0.01M ammonium acetate, pH 7.0 and finally centrifuged at 4000 rpm for 5 minutes.

The supernatant was used as partially purified PLO's preparation in infectivity test, electron microscopy. The electron microscopy of the PLO's under investigation was accomplished at sophisticated instrumentation centre for applied research and testing, Vallabh Vidyanagar using partially purified PLO's preparations. For electron microscopy, a drop of partially purified preparation was transferred to 300 mesh copper grids coated with carbon. After 5 minutes, excess liquid was absorbed carefully with the help of blotter paper and the grids were stained with 2 per cent phosphotungstic

acid (PTA), pH 6.5 . The grids were then, air dried and examined in a electron microscope. The actual magnification (at 80 KV)was used for computing the size of the PLO's particles.

3.7 EPIDEMIOLOGY

3.7.1 Survey of phyllody disease in saurashtra areas.

A roving survey was conducted in leading sesame growing districts viz., Junagadh, Porbandar, Amreli, Rajkot, Surendranagar and Jamnagar during Kharif – 2008-09 and Summer – 2008-09 to assess the present level of incidence of phyllody disease in sesame. To assess the percentage incidence of phyllody disease, two fields were selected at random in a village or place where sesame was grown in more than 10 ha. In each field, hundred plants from four different locations were chosen at random and the number of plants showing the symptoms were counted and the percentage incidence was recorded. The crop was visited at the age of 7 to 8 weeks. Information was also gathered on the incidence of the disease in the popular varieties grown in the respective areas.

3.7.2 Assessment of losses due to sesame phyllody

The effect of sesame phyllody on growth characteristics and seed yield in sesame (cv.Guj Til-2) was studied under field conditions on a crop raised at a distance of 45 x 15 cm during two seasons viz, kharif 2008-09 and summer 2008-09. Plant tagging was initiated as soon as the first diseased plants were seen and tagging continued until 50 per cent flowering with an interval of a week. At harvesting, observations like height, number of branches, capsules, number of grains/plants, and grain weight/plant were recorded on 20 fields in randomly selected infected plants and that were tagged at different periods after sowing. Similar observations were recorded on equal number of randomly selected healthy plants. Reduction in growth parameters and grain yield over control was worked using the following formula.

Reduction over control(per cent) =

Mean of healthy plant – Mean of diseased plant	X 100
Mean of healthy plant	

3.7.3 Hopper's population dynamics and incidence pattern of sesame phyllody disease

Sesame (cv. Guj Til-2) was sown in a plot measuring 15 X 15 m² in kharif and summer seasons for studying

hopper's population dynamics and disease incidence pattern. For hopper's population dynamics, the number of hopper's from five cm shoot length and top five cm length of all branches were counted from 20 randomly selected plants. Hopper's count was made early in the morning usually before 9 o'clock on the day of observation. The first observation on hopper's count was recorded immediately after the appearance of the phyllody symptoms in the field and subsequent observations were taken at weekly interval until 50 per cent flowering. The average number of hopper's per five cm shoot length and top five cm length of all branches of plants were worked out. For the incidence pattern of sesame phyllody disease, number of infected plants were recorded from crop emergence to 50 per cent flowering of the crop at weekly interval and the percentage of infected plants was worked out. In order to study the effect of hopper's population on the incidence of sesame phyllody disease, simple correlation between hopper's population and disease incidence was worked out.

3.8 MONITORING OF DISEASE

3.8.1 Disease incidence in relation to the vector population and weather parameters.

During kharif – 2008-09 and summer – 2008-09 season, the incidence of phyllody disease in the field of Junagadh

Agricultural University, was recorded at fortnightly interval from the day of sowing along with the population of leaf hopper vector (*Orosius albicinctus* Dist.).

The disease incidence was recorded on per cent basis

% disease incidence	=	Number of plants with symptoms	X
		Total number of plants counted	100

The insect vector count was noted after every 5 sweeps with an insect net over the plants and sweepings were done at 10 different places in the field. The total number of leaf hopper from 5 sweeps at each place was recorded. Vector population was represented by the total of 10 places in a field. Vector population counts were taken at fortnightly interval after sowing.

The weather parameters (temperature and humidity – maximum, minimum and mean) were recorded.

The relationship, if any between the disease incidence vector population and weather factors was studied. A simple linear correlation between disease incidence and vector population was studied by putting up a regression line.

$$y = a + bx$$

Where,

$$y = \% \text{ disease incidence}$$

a = constant

b = regression coefficient and

x = No. of insects (Vector population)

A similar relationship between vector population and weather factors viz., temperature (°C) and R.H. (%) were also studied. Wherever, a significant correlation was observed with more than one factor, a multiple linear correlation analysis was also done putting up a regression line.

$$y = a + b_1x_1 + b_2x_2$$

Where,

y = Insect vector population

a = Constant

b₁ and b₂ = Partial regression coefficient

x₁ = Maximum temperature (°C)

x₂ = Mean R.H. (%)

3.9 SCREENING

3.9.1 Screening of sesame varieties / germplasm lines under natural field condition.

A field experiment was conducted in Kharif season of 2008 at Department of Plant Pathology Farm, Junagadh Agricultural University, Junagadh with a view to screening sesame varieties for resistance to phyllody disease under natural field conditions. Hundred plants from each varieties /

lines were grown in a line for experimental observations. Similar observations were recorded from 21 DAS.

3.10 CONTROL

3.10.1 Management through vector control

A field trial was laid out during kharif 2008-09 and summer 2008-09 to evaluate efficacy of different insecticides separately for the control of vector(*Orosius albicinctus* Dist.) of phyllody on sesame cv Guj Til-2. The insecticides were applied at 35 and 50 days after sowing. Observation on vector population were recorded 1 day before spraying and at 1 and 7 days after spraying.

The trial was laid out in the field of university farm. The field was ploughed well and fertilizers were applied as per recommendation. Weeding was done regularly and irrigation was given as and when necessary. The trial included the following 6 treatments replicated thrice in a randomized block design with accommodating 150 plants of plot size 4.5 m x 2.25 m.

1.	Control	No treatment
2.	Dimethoate	0.025% a.i. sprayed fortnightly
3.	Methyle-o-demeton	0.025% a.i. sprayed fortnightly
4.	Phosphamidon	0.03% a.i. sprayed fortnightly
5.	Imidacloprid	0.009% a.i. sprayed fortnightly
6.	Monocrotophos	0.05% a.i. sprayed fortnightly

The spray fluid was adjusted according to crop stage between 300 to 500 liters per hectare and same trial was repeated during summer season.

CHAPTER IV

EXPERIMENTAL RESULTS

4.1 OCCURRENCE OF DISEASE

During Kharif (2008) occurrence of phyllody was noted in the range of 0-3.79 per cent in different cultivated varieties and germplasm lines under field conditions in scattered manner and spread of disease was very slow. The first affected plant was observed at 50 days and maximum affected plants at 75 days after sowing in field conditions (Table 4.1).

In sesame (cv Guj Til-2) phyllody significantly and adversely affected the yield contributing characters of plants. There was reduction in number of capsules, branches per plant, plant height and grain yield of phyllody affected plants, which was range from 11.5 to 47.5, 2.3 to 3.8, 69.4 to 113.4 cm and 0.23 to 3.90 g, respectively in comparison to 55.8, 4.2, 118.76 cm and 4.30 g, respectively in healthy plant (Table 4.2 to 4.5, Fig. 1,3,4,5).

Test weight (1000 grains weight) was ranged from 0.476 to 1.644 g in diseased plant in comparison to 2.784 g in healthy plant and thereby 40.94 to 82.90 per cent reduction in yield over healthy plant. It could be attributed due to phyllody (Table 4.6, Fig. 2).

Table 4.1 : Incidence of phyllody in different cultivated varieties and germplasms in field conditions.

Cultivar/line	Total no. of plants	No. of plants affected at different age					Total no. of affected plants	Incidence (%)
		40	50	60	70	75		
Guj-Til-1	112	-	2	1	-	-	3	2.68
Guj-Til-2	158	-	3	2	1	-	6	3.79
Guj-Til-10	96	-	1	-	-	-	1	1.04
V-76-21	36	-	-	-	-	-	-	0.00
CST-781	42	-	1	-	-	-	1	2.38
JR-15	41	-	-	-	-	-	-	0.00
MT-67-13	34	-	-	-	-	-	-	0.00
Guj.SCC.38	48	-	1	-	-	-	1	2.08

K.71-10	32	-	-	-	-	-	-	0.00
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Conti..

ES-3	36	-	-	-	-	-	-	0.00
JGL-127	32	-	-	-	-	-	-	0.00
V-6-3	43	-	1	-	-	-	1	2.32
CHAPRI-5	32	-	-	-	-	-	-	0.00
KATARI-1	22	-	-	-	-	-	-	0.00
TADA-2	42	-	1	-	-	-	1	2.38
DARFD-3	38	-	-	-	-	-	-	0.00
AT-107	39	-	-	-	-	-	-	0.00
RT-44	26	-	-	-	-	-	-	0.00
TAP 1	34	-	-	-	-	-	-	0.00

DHORAJI-1	42	-	1	-	-	-	1	2.38
IC-54039	43	-	-	-	-	-	-	0.00

Conti..

IC-96127	32	-	-	-	-	-	-	0.00
IC-205791	33	-	-	-	-	-	-	0.00
NIC-7800	35	-	-	-	-	-	-	0.00
L-45	35	-	1	-	-	-	1	2.94
RT-127	34	-	-	-	-	-	-	0.00
TMV-1	33	-	-	-	-	-	-	0.00
JT-71-1-1	32	-	-	-	-	-	-	0.00

Table 4.2: Effect of phyllody appearance at different growth stages on number of capsules per plant

Age at symptoms appear (days)	Number of capsules(*)	Reduction in No. of capsule over healthy (%)
50	11.5	79.39
55	22.2	60.21
60	35.4	36.55
65	47.5	14.87
Healthy	55.8	0.00

(*) Average of five plants

Table 4.3: Effect of phyllody appearance at different growth stages on seed yield per plant

Age at symptoms appear (days)	Seed yield (g)	Reduction in seed yield over healthy (%)
50	0.23	94.65
55	1.10	74.41
60	2.30	46.51
65	3.90	9.30
Healthy	4.30	0.00

Table 4.4: Effect of phyllody appearance at different growth stages on number of branches produced per plant

Age at symptoms appear (days)	Number of branches (*)	No. of branches decrease or increase in over healthy (%)
50	2.3	-34.29
55	3.3	-5.71
60	3.5	8.57
65	3.8	20.0
Healthy	4.2	0.00

(*) Average of five plants

Table 4.5: Effect of phyllody appearance at different growth stages on plant height

Age at symptoms appear (days)	Plant height (*) (Cm)	Reduction in height over healthy (%)
50	69.4	41.56
55	90.72	23.61
60	104.4	12.09
65	113.4	4.51
Healthy	118.76	0.00

(*) Average of five plants

Table 4.6: Effect of phyllody appearance at different growth stages on test weight of seeds

Age at symptoms appear (days)	Test weight (g)(*)	Reduction in test weight over healthy (%)
50	0.476	82.90
55	0.667	75.32
60	1.139	59.08
65	1.644	40.94
Healthy	2.784	0.00

(*) Average of five plants

Figure-1 Effect of phyllody appearance at different crop growth stages on number of capsules per plant

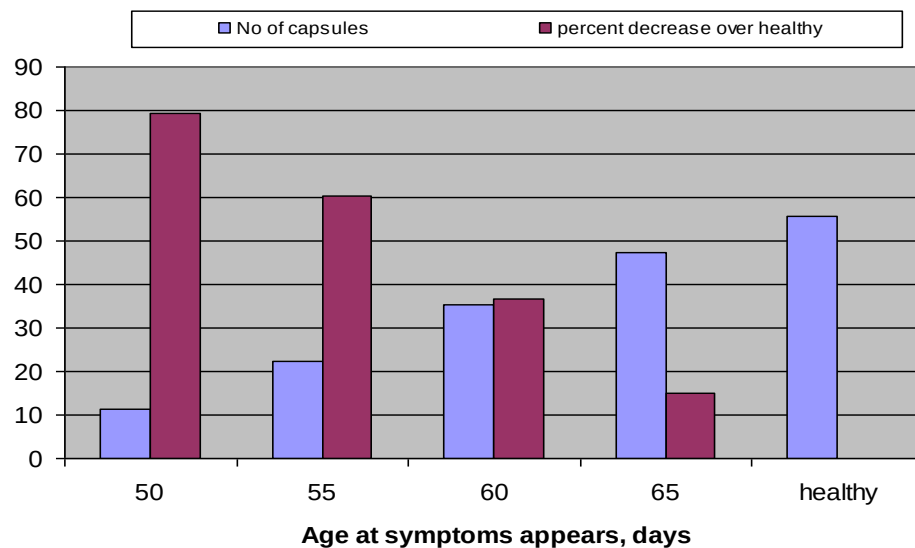


Figure:2 Effect of phyllody appearance at different crop growth stages on seed yield per plant

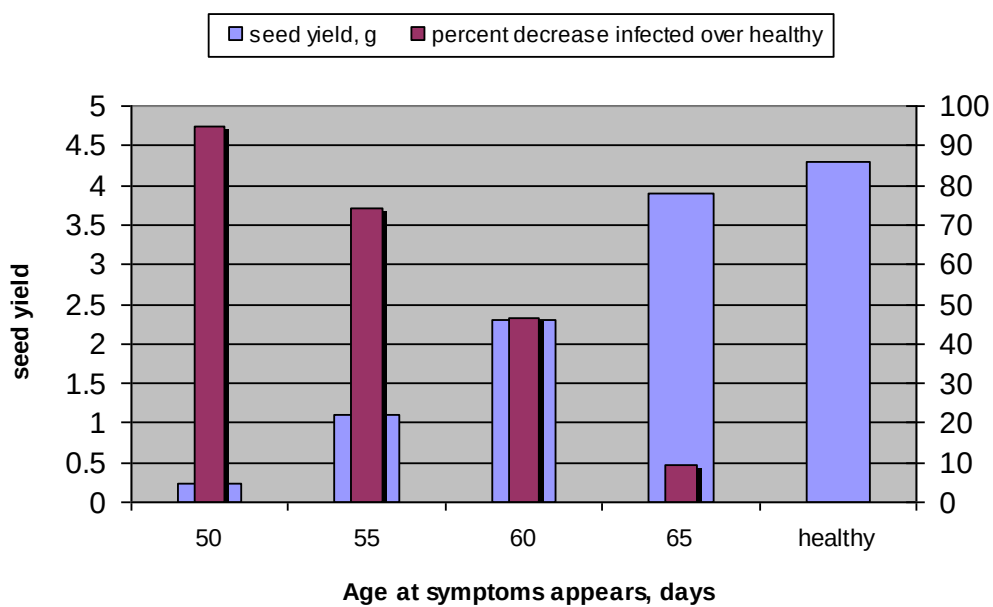


Figure:3 Effect of phyllody appearance at different crop growth stages on branches produced per plant

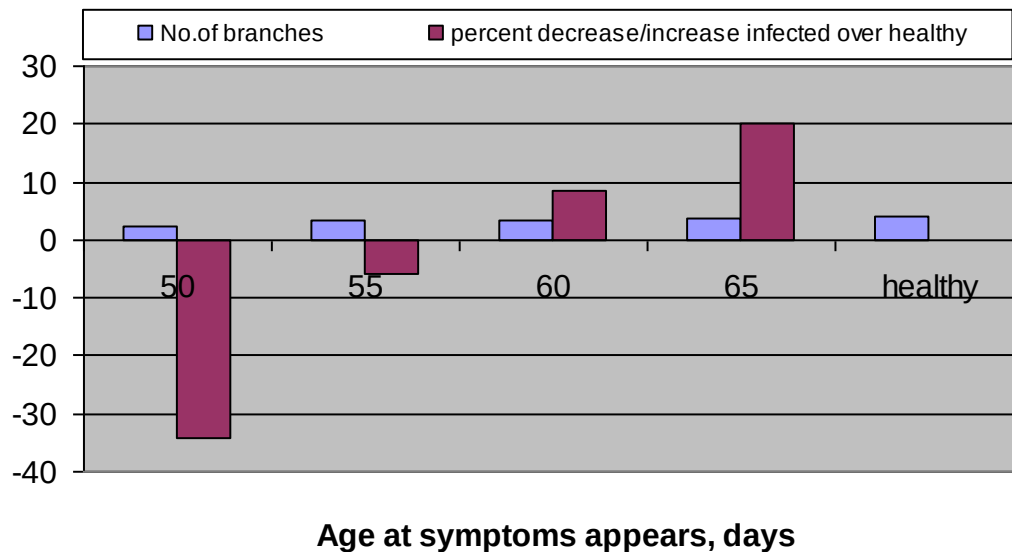
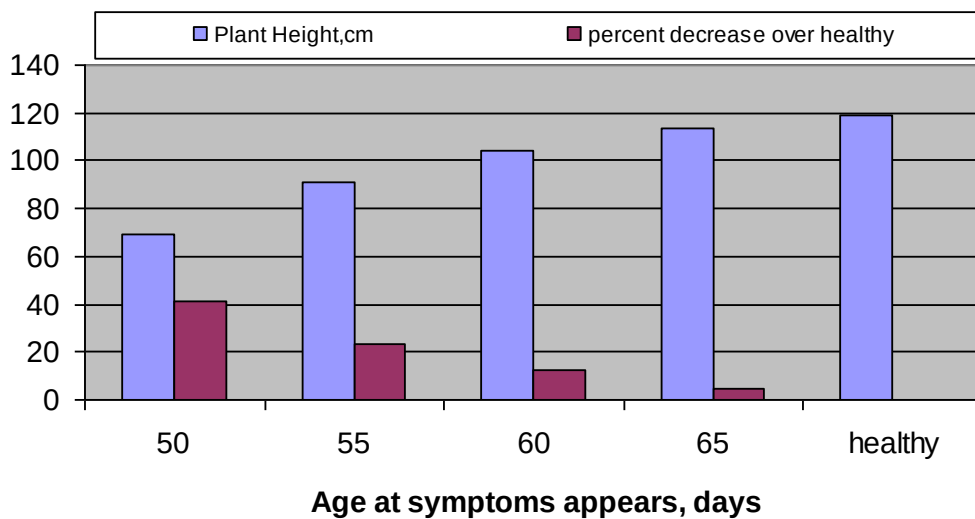
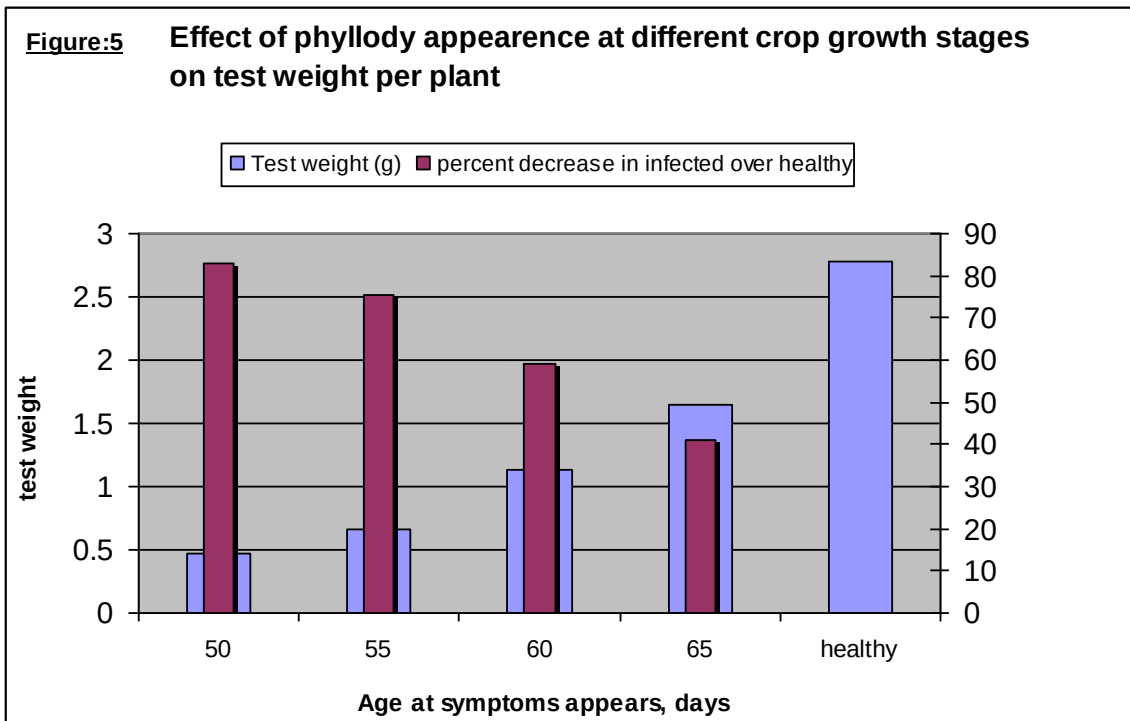


Figure:4 Effect of phyllody appearance at different crop growth stages on plant height





4.2 SYMPTOMATOLOGY

Different types of phyllody symptoms were observed on sesame plants grown under field and control conditions. The major disease symptoms were floral virescence (plate 1A, 2A), proliferation (plate 1CD) and yellowing sometimes accompanied the disease. Shoot apex fasciation (plate 2C) was also observed on occasion. Phyllody infected sesame plants exhibited symptoms that varied according to growth stage and time of infection. Infection at an early stage of growth resulted in cessation of internodes elongation, reduction in leaf size. The entire inflorescence was converted into twisted reduced leaves closely arranged on the top of the stem, with very short internodes (plate 1B).

The severity of the transformation of floral parts into green leaf like structures was associated with the time of infection. Plant infected before flowering had severe symptoms on the entire plant, while plants infected during flowering had severe symptoms on the upper part of the plant. Leaves on the lower parts of infected plants, stems and roots did not exhibit any visible symptoms. If only the top portion was affected, the lower part may beared normal capsules, but seeds within such parts were observed light and of low quality (plate 2A).

4.3 TRANSMISSION

4.3.1 Transmission by mechanical inoculation

Mechanical inoculation of the sap into the young plants of sesame (cv Guj Til-2) at 3-5 leaf stage induced no transmission of the phytoplasma. The inoculated plants didn't produce any typical phyllody symptoms during the observation period up to the maturity. The result clearly indicated no mechanical transmissibility of phytoplasma(Plate 3B).

4.3.2 Transmission by grafting

Graft transmission was attempted on sesame cv Guj Til-2, initially 10 sesame seedling were grafted with naturally infected sesame plants. Symptoms started appearing from 30 to 40th day after grafting. Out of 10, only 1 plants developed typical phyllody symptoms indicating only 10 per cent transmission(Plate 3D and Table 4.7).

Table 4.7 : Transmission percentage of phytoplasma by grafting

No. of healthy plants grafted by naturally affected plant	No. of plants infected	Transmission (%)
10	1	10

SYMPTOMATOLOGY



A. FLORAL VIRESCENCE



B. PHYLLODY SYMPTOMS



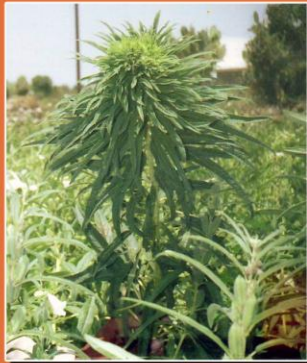
C. FLORAL PROLIFERATION



D. FLORAL PROLIFERATION

PLATE - 1

SYMPTOMATOLOGY



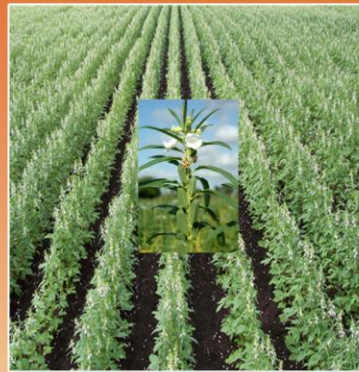
A. FLORAL PROLIFERATION



B. PHYLLODY FLOWERS



C. SHOOT APEX FASCIATION



D. HEALTHY

TRANSMISSION TEST



A. NATURALLY INFECTED PLANT



B. MECHANICAL TRANSMISSION



C. VECTOR TRANSMISSION



D. GRAFT TRANSMISSION

PLATE 3

4.3.3 Transmission through seeds

4.3.3.1 Seed grades and phytoplasma transmission

The seeds collected from the capsules of severely phyllody affected sesame (cv Guj Til-2) plants were categorized into three grades viz., smooth bold seed, shriveled sunken and discoloured seeds. These were sown in earthen pots filled with sterilized soil. Twenty seeds were sown in each pot didn't produced any typical phyllody symptoms through out the observation period. The results also clearly indicated that no seed transmissibility of phytoplasma (Table 4.8).

Table 4.8 : Seed transmission of phytoplasma in different categories of sesame (cv Guj Til-2) seeds

Seed grade	No. of seeds sown	No. of seeds germinated	No. of plants showing typical phyllody symptoms	Transmission (%)
Bold smooth	20	20	0.00	0.00
Shrivelled sunken	20	17	0.00	0.00
Discoloured	20	15	0.00	0.00

4.3.3.2 Infected seed and phytoplasma transmission

The sesame seeds sown in plots, which were collected from the capsules of transmission test plants. Seed transmission of the infectious agent could not be achieved, which indicated that sesame phyllody is not seed transmissible (Table 4.9).

Table 4.9: Percentage transmission of phytoplasma in seeds of sesame (cv Guj Til-2) after sowing of phyllody infected seeds

Sr no	Seed lots collected from plants infected after days of sowing	No of seeds germinated / No of seeds sown	Germination (%)	Reduction in germination (%)	Seedlings with phyllody symptoms (%)
1	Seeds collected from plants infected after 30 days of sowing	80/100	80	20	0
2	Seeds collected from plants infected after 40 days of sowing	76/100	76	24	0
3	Seeds collected from plants infected after 50 days of sowing	81/100	81	19	0
4	Seeds collected from plants infected after 60 days of sowing	78/100	78	22	0
5	Seeds collected from plants infected after 70 days of sowing	80/100	81	20	0
6	Seeds collected from healthy plants	90/100	90	10	0

4.3.3.3 Transmission by insect vectors

The plant to plant spread of phytopathogenic mollicutes in nature is mediated by phloem feeding insects, leaf hopper. *Orosius albicinctus* Dist. are the phloem-feeding insects and vector of sesame phyllody. For this reason, *Orosius albicinctus* Dist. were released on phyllody infected plants for 24 hr's to three weeks acquisition feeding period and they were released separately (5 number per plant) on to healthy sesame (cv Guj Til-2) plant at 3 to 5 leaf stage for up to 6 week of inoculation feeding period. The leaf hopper *Orosius albicinctus* Dist. successfully transmitted the phytoplasma from infected sesame plants up to 50 per cent of healthy plant (Plate 3, C4 and Table 4.10).

Table 4.10 : Percentage transmission of phytoplasma by *Orosius albicinctus* Dist. after various incubation periods

Incubation period (days)	No. of plants infected per No. of plants inoculated	Transmission (%)
20	0/10	0.00
30	5/10	50
40	3/10	30
50	0/10	0.00
60	0/10	0.00
up to maturity	0/10	0.00

4.4 BIOCHEMICAL STUDIES

4.4.1 Dry matter content and moisture content

Dry matter content and moisture content were determined from the difference between fresh and dry weight (constant weights obtained after drying at 75°C) and the data obtained are presented in Table 4.11.

Table 4.11: Effect of phyllody disease on the dry matter content and moisture content in the infected leaves of sesame*

Sample	Dry matter (%)	Increase (+) or decrease (-) %	Moisture (%)	Increase (+) or decrease (-) %
Healthy	16.39	0	83.61	0.00
Mild Symptoms	18.64	+ 13.72	81.36	- 2.69
Severe Symptoms	19.93	+ 21.59	80.07	- 4.23

From the Table 4.11, it is evident that phyllody disease considerably increased the dry matter content in the infected plant. Increase in dry matter content was 13.72 per cent and 21.59 per cent in mildly infected and severely infected plants, respectively. However, no considerable difference in moisture content was observed in both mildly and severely infected plants compared to the healthy ones.

4.4.2 Chlorophyll content

From Table 4.12, it is evident that total chlorophyll content in diseased plants was reduced. The reduction was more pronounced in severely infected plants. Chlorophyll a and b were reduced to the tune of 25 – 40 per cent in different samples.

Total chlorophyll content was reduced by 39.92 per cent in severely infected plants, while in mildly infected plant it was reduced by 25.24 per cent. Data also showed that the ratio between chlorophyll 'a' and chlorophyll 'b' progressively reduced as a result of infection. The ratio was 1.04 in healthy plants, while it was 0.99 and 0.94 in case of mild infection and severe infection, respectively.

Table 4.12 : Changes in chlorophyll content sesame leaves as a result phyllody infection*

Sample	Chlorophyll content (mg. g ⁻¹ , f.w.)			Total chlorophyll	Reduction (%)
	Chl. 'a'	Chl. 'b'	Ratio Chl. 'a' / Chl. 'b'		
Healthy	0.742	0.716	1.04	1.458	0.00
Mild Symptoms	0.544	0.546	0.99	1.090	25.24
Severe Symptoms	0.424	0.452	0.94	0.876	39.92

* Average of five replicates

4.4.3 Nitrogen and crude protein

Total nitrogen and crude protein content results are presented in table 4.13.

Table 4.13 : Effect of phyllody disease on total nitrogen and crude protein content*

Sample	N content (% dry weight)	Protein content (% dry weight)	Reduction in protein (%)
Healthy	3.142	19.637	0.00
Mild infection	2.832	17.700	9.86
Severe infection	2.594	16.212	17.44

* Average of five plants

The results indicate that phyllody disease decreased the total nitrogen as well as protein content. The reduction in protein content was 9.86 per cent in mildly infected leaves and 17.44 per cent in severely infected leaves.

4.4.4 Phenol

The total phenol content in healthy and diseased leaves of phyllody affected sesame are presented in Table 4.14.

Table 4.14 : Phenol content in leaves of phyllody affected sesame

Sample	Phenol mg / g
Healthy	1.68
Mild symptoms	2.02
Severe symptoms	2.42

It seems from Table 4.14 that total phenol content was increased as disease advance from milder symptoms stage to severely symptoms stage from 2.02 mg / g to 2.42 mg / g respectively as compared to healthy leaves 1.68 mg / g.

4.4.5 Rate of transpiration

Effect of phyllody on transpiration in healthy and diseased leaves are presented in Table 4.15

Table 4.15: Rate of Transpiration in leaves of phyllody affected sesame

Sample	Transpiration (mg of H₂O. mm⁻². s⁻¹)
A.Healthy	
1.	104.50
2.	122.90
3.	108.00
4.	120.00
Average	113.85
A.Diseased	
1.	142.50
2.	153.30
3.	173.40
4.	165.48
Average	158.67

% increased over healthy	44.82
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From the above table it is evident that in diseased plants transpiration rate increased considerably. Rate of transpiration increased to the extent of 44.82 per cent.

4.5 HISTOPATHOLOGICAL STUDIES

Anatomical changes in leaf tissues, stem, petiole and flowers of sesame due to phyllody were studied by staining hard cut transverse sections (T.S.) with methylene blue, safranin and fast green. Observations were made under light microscope and recorded (plate 6 & 7).

4.5.1 Stem

The transverse sections from healthy as well as severely infected plants were showed most extensive changes as a result of infection occurred in the vascular bundle region. The

phloem region showed proliferation and hypertrophy. Another very interesting observation was considerable thickening of the lumen of the internal phloem tissues.

4.5.2 Petiole

The anatomical differences were more pronounced in vascular bundle region. The arrangement of the xylem and phloem tissues in the infected petiole was irregular and distorted.

4.5.3 Leaf

Disintegration of tissues was observed in both mesophyll and the vascular region of infected leaves, destruction and deformation of the phloem tissues were also observed.

4.5.4 Floral parts

The abnormal flowers were crowded together. The leaf like structures which were produced in the floral region were often lighter in colour. Normal flowers were known to lack glandular hair on filaments and connectives of stamens, style,

stigma and ovules however, such hairs were found in phyllody flowers.

The following modifications were observed in calyx, corolla and reproductive parts of the phyllody affected flowers of sesame.

4.5.4.1 Calyx

In normal flowers, the calyx was green gamosepalous and 5-partite. The segments in the calyx were linear with acute apices. However, in phyllody flowers a complete division of the calyx tube occurred resulting in polysepalous condition. The sepals were lacking in pedicels and were leaf like in appearance. They were smaller and simpler in configuration than foliage leaves.

4.5.4.2 Corolla

The corolla turned green in colour in the phyllody affected flowers. The surface of corolla was rough. Gamopetalous condition of normal flowers changed to a polypetalous one. Petal apics were rounded.

4.5.4.3 Androecium

Unlike the four epipetalous stamens in normal flowers a fifth stamen was also observed in phyllody flowers. Further,

the stamens were not epipatalous and they were green in colour.

4.5.4.4 Gynoecium

The ovary was enlarged in size and the stigma was transformed in to two flattened greenish structures. The carpellary wall too was transformed into leaf – like structures.

4.5.5 Variation in cell number

Cells of the different tissues from stem, petiole and leaf were counted (at 40 x magnification). Data obtained were presented in Table 4.16. It was evident from the results in table that at different zones of the stem tissues, cell number increased due to infection.

In infected petiole compared to the healthy one, cell number decreased at vascular bundle and cortical region. In the leaf, the number of spongy parenchymatous cells decreased in diseased tissue.

4.5.6 Variation in trichome morphology

Trichomes are the hair like projections from a plant epidermal cell. These structures are found in both abaxial and adaxial leaf surfaces.

While studying the leaf surface, variation in structure and shape of trichomes was observed. While the healthy leaves had large number of well developed multicellular stellate trichomes, those from phyllody infected plants were very few in number and were generally unbranched in to unequal arms.

Table 4.16 : Variation in cell number in sesame tissues as a result of phyllody*

Sample	Cell number from tissue			
	VB	CR	PR	MR
A. Healthy				
1. Stem	38.3	37.9	15.1	0.00
2. Petioles	35.0	48.6	0.00	0.00
3. Leaf	0.00	0.00	0.00	23.3
B. Diseased				
1. Stem	54.3	42.9	19.7	0.00
2. Petiole	29.2	35.0	0.00	0.00
3. Leaf	0.00	0.00	0.00	19.3

* Each value is the mean of 25 observations

Index :

VB : Vascular bundle; CR : Cortical region; PR : Pith region and MR : Mesophyll region.

The stellate trichomes on diseased leaves were asymmetrical as compared to those on healthy leaf. They lacked the characteristic circular groove at the bases and the branches were relatively shorter. The radiating branches were also reduced in length and number.

4.5.7 MICROSCOPY

PLO's were observed only in the sieve elements of phloem of diseased leaves and phylloid floral parts of sesame cv Guj-Til-2. No PLO's were found to be present in the healthy tissues of same cultivar. Most of the PLO's ranged between 150-350 nm in diameter and those resemble with other diseases caused by PLO's (Plate 7).

4.6 EPIDEMIOLOGY

4.6.1 Survey

The incidence of sesame phyllody disease in Porbandar, Junagadh, Jamnagar, Surendranagar, Rajkot and Amreli districts surveyed during Kharif and Summer seasons of 2008 and 2009 are given in Table 4.17 (plate 8 & 9).

Table 4.17 : The incidence of phyllody disease of sesame in Saurashtra region

Districts	Season	No. of fields surveyed	Incidence of phyllody of sesame (per cent)
Porbandar	Kharif – 2008	10	0 to 0.8
	Kharif – 2009	10	0 to 1.5
	Summer – 2008	10	0 to 4.1
	Summer – 2009	10	0 to 4.7
Junagadh	Kharif – 2008	10	0 to 1.0
	Kharif – 2009	10	0 to 1.7
	Summer – 2008	10	0 to 4.4
	Summer – 2009	10	0 to 4.6
Rajkot	Kharif – 2008	10	0 to 1.1
	Kharif – 2009	10	0 to 1.5
	Summer – 2008	10	0 to 2.8

	Summer – 2009	10	0 to 3.9
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Conti....

Jamnagar	Kharif – 2008	10	0 to 1.1
	Kharif – 2009	10	0 to 1.9
	Summer – 2008	10	0 to 3.1
	Summer – 2009	10	0 to 4.4
Surendranagar	Kharif – 2008	10	0 to 1.2
	Kharif – 2009	10	0 to 1.4
	Summer – 2008	10	0 to 3.9
	Summer – 2009	10	0 to 4.1
Amreli	Kharif – 2008	10	0 to 1.6
	Kharif – 2009	10	0 to 2.2
	Summer – 2008	10	0 to 4.0
	Summer – 2009	10	0 to 4.3

The disease incidence ranged from 0 to 2.2 in Kharif season, while it was ranged from 0 to 4.7 in summer season.

The data (Table 4.17) showed that the disease incidence was low during Kharif 2008 as compare to Kharif – 2009. Further, there was variation in the disease incidence from place to place which was particularly obvious in summer seasons of both the year as the incidence was relatively more.

Sesame is grown extensively almost throughout Saurashtra. Phyllody disease appears every year because sesame is cultivated in both Kharif as well as summer season in Saurashtra region. It has been observed during survey that sesame crop sown earlier, i.e., May end to mid- June, always shown higher incidence than that sown during end of June or early July.

In addition, survey results indicated that incidence of the disease was greater in the summer season as compared to crop grown in Kharif season.

4.6.2 MONITORING

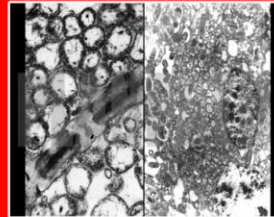
4.6.2.1 Factors influencing disease incidence

Epidemiology of phyllody of sesame was studied during the Kharif and summer seasons of 2008 and 2009. In both the seasons of both the year disease incidence has a simple positive correlation with the population of leaf hopper vector

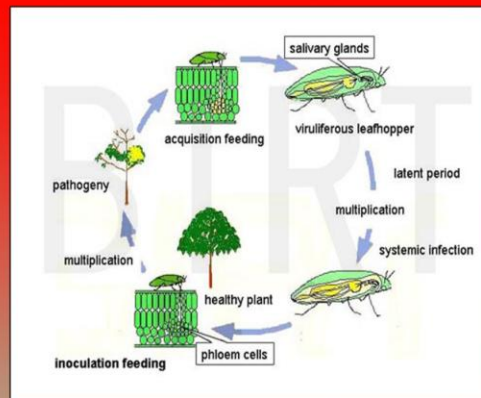
INSECT VECTOR



A. *Orosius albicinctus*



B. PHYTOPLASMA IN PLANT CELL (LEFT)
AND INSECT CELL (RIGHT)



C. INSECT VECTOR RELATION SHIP



D. Feeding bottle & Aspirator for aquisition
inoculation feeding of leafhoppers

PLATE - 4

HISTOPATHOLOGY



A. PLANT WITH HEALTHY
FLOWERS & CAPSULES



B. PLANT WITH PHYTOID FLOWERS



C. AHE PLANT



D. HEALTHY CAPSULES



E. INFECTED CAPSULES

PLATE - 6

HISTOPATHOLOGY



A. FRUITING AND
FLOWERING PLANT



B. INFECTION AT DIFFERENT STAGE



1. FLOWERING BRANCH
2. OPENED COROLLA
3. CROSS SECTION OF OVARY
4. CAPSULE
5. SEEDS



C. LONGITUDINAL AND
CROSS SECTION OF
CAPSULES



D. CRACKING OF CAPSULES



E. CLOSE UP OF CAPSULES

PLATE-7

SURVEY DURING SUMMER



A. SEVERE INFECTION



B. General View of field



C. TYPICAL SYMPTOMS



D. INFECTED FIELDS

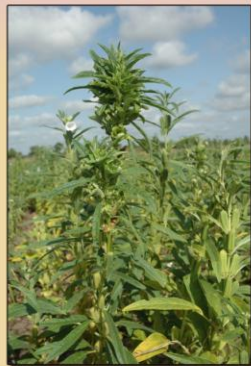


PLATE - 8

SURVEY DURING KHARIF



A. General View of field



B. TYPICAL SYMPTOMS



D. INFECTED FIELDS



PLATE - 9

(*O. albicinctus* Dist.). It has been observed that the females were generally bigger in size and more in number than the males. To the naked eye, the leaf hoppers were pale-brownish grey in colour which, however varied considerably in the field some were dark greyish brown or even greyish – black while others were light brownish – grey in colour. An attempt was made to correlate the percentage of vector population with the phyllody disease incidence at 50 days after sowing. This indicates that the extent of phyllody incidence under field conditions may possibly be predicted 50 days earlier by vector indexing.

The simple linear equation was worked out by regression line to predict the phyllody incidence at 50 days after sowing with the data on vector indexing(Fig. 6 to 9 &Table 4.18).

$$Y = 0.333 + 1.701 X (R^2 = 0.973) \text{ Summer} - 2008$$

$$Y = 0.660 + 5.260 X (R^2 = 0.907) \text{ Kharif} - 2008$$

$$Y = 0.429 + 1.627 X (R^2 = 0.973) \text{ Summer} - 2009$$

$$Y = 1.009 + 3.193 X (R^2 = 0.847) \text{ Kharif} - 2009$$

Where, Y = Phyllody incidence

x = Vector population

Table 4.18 : Linear correlations($Y = a + bX$) of leaf hopper populations(X) with incidence of phyllody(Y) during kharif & summer seasons.

Year & Season	a	b	R²
2008 Summer	0.333	1.701	0.973
Kharif	0.660	5.260	0.907
2009 Summer	0.429	1.627	0.973
Kharif	1.009	3.193	0.847

However, it is difficult to correlate high vector population with high disease incidence since the phytoplasma vector relationship is of persistent nature.

Linear correlation of leafhopper population with weather factors (Kharif and summer seasons of 2008 and 2009) indicated that the *O. albicinctus* Dist. population is influenced by both temperature and humidity. It was also observed that vector population is dependant on temperature and RH for its build up.

Figure : 6 : Relationship between incidence of *Orosius albicinctus* and phyllody disease during kharif seasons

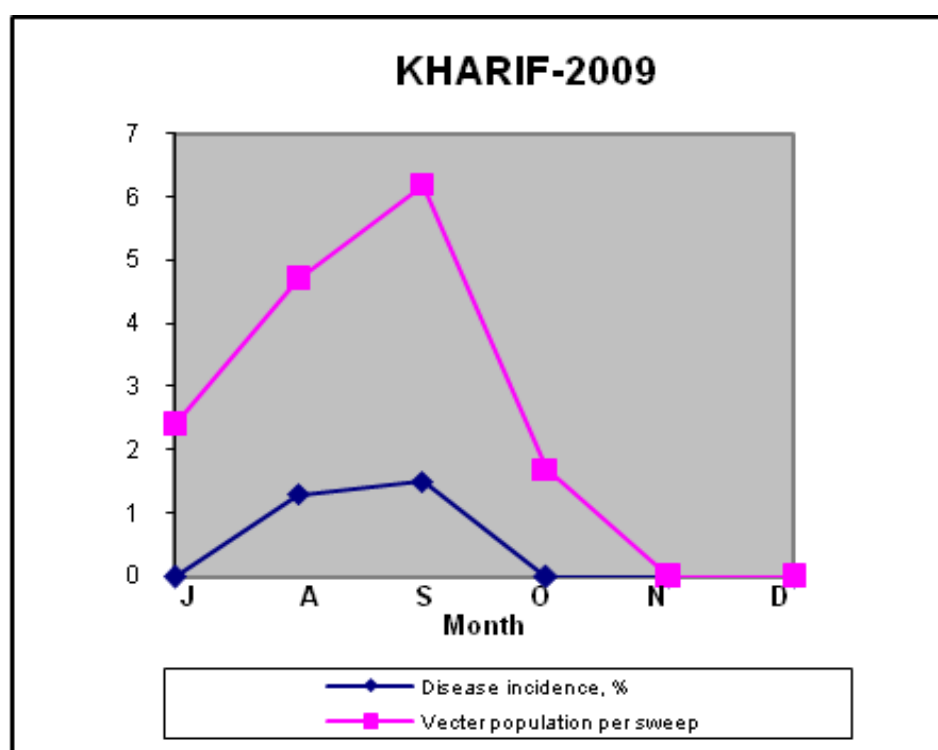
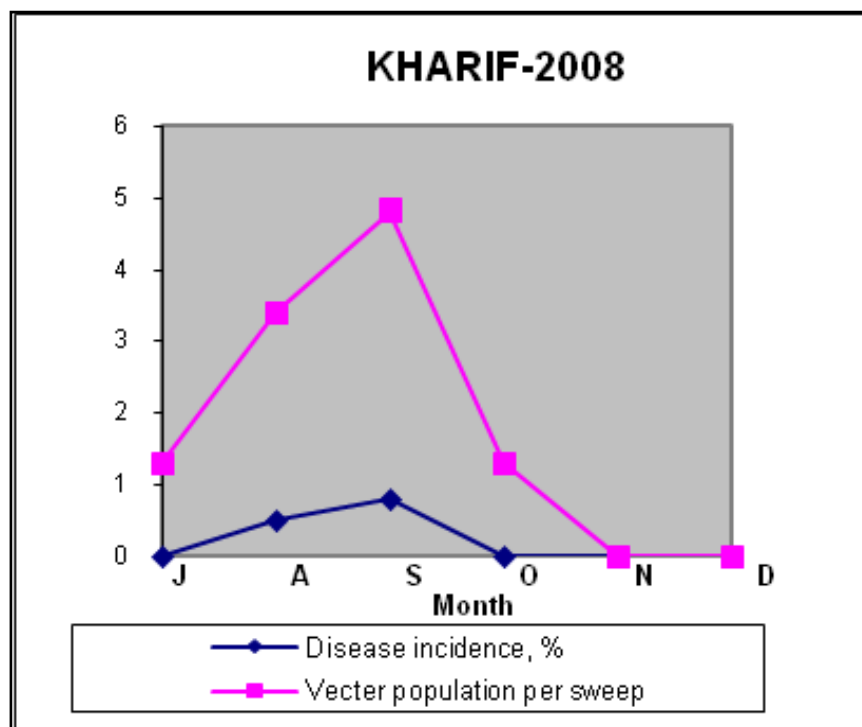


Figure : 7 : Relationship between incidence of *Orosius albicinctus* and phyllody disease during Summer seasons

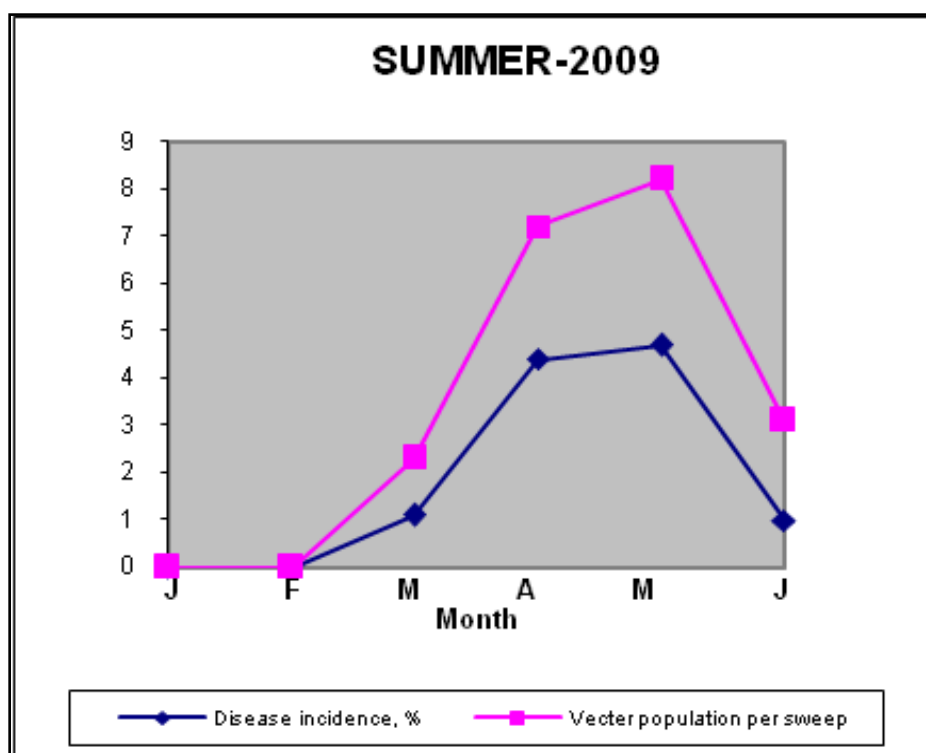
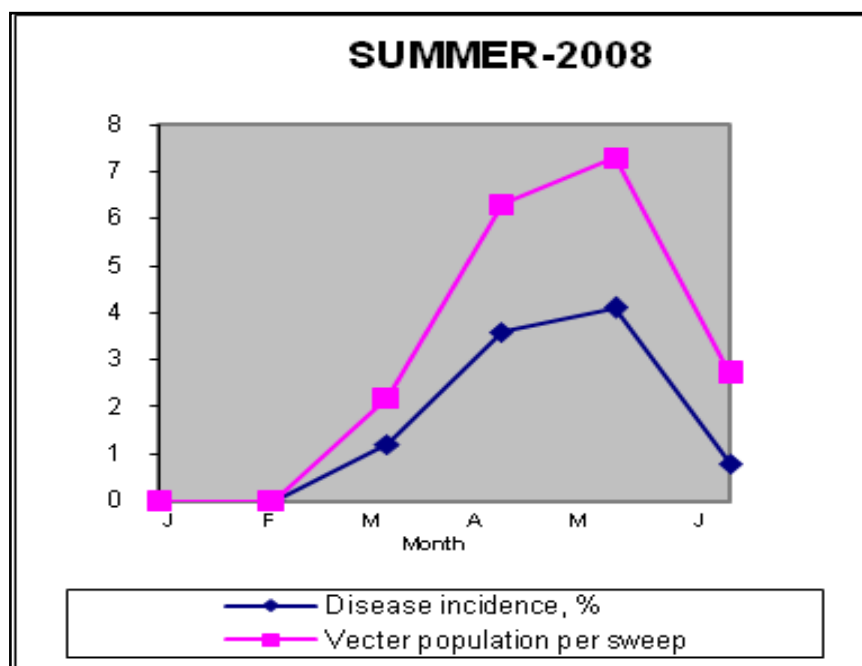


Figure : 8 : Standard curve for vector relationship with incidence of disease during kharif seasons

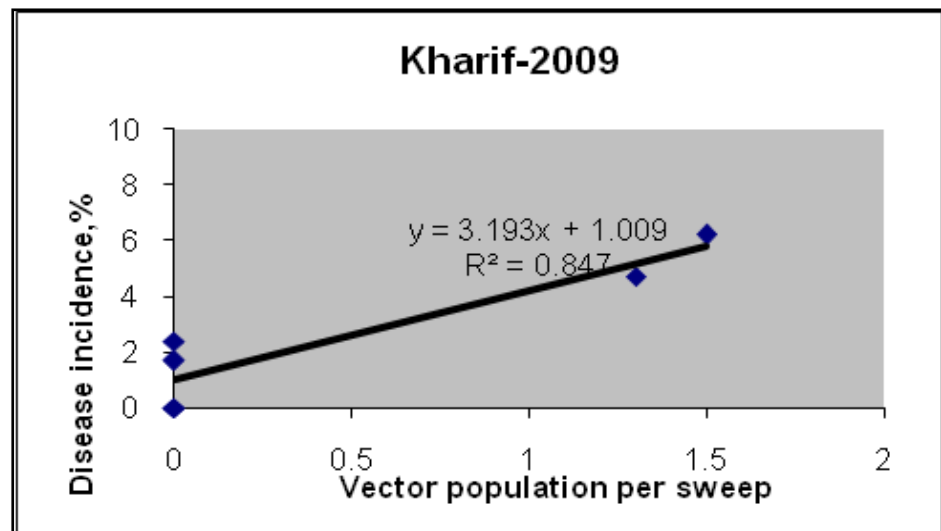
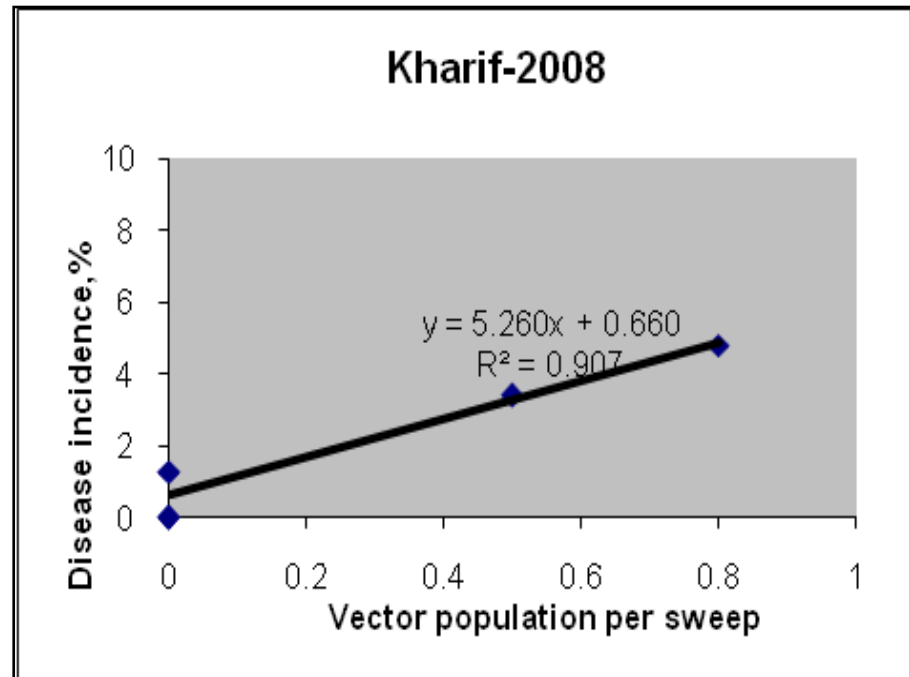
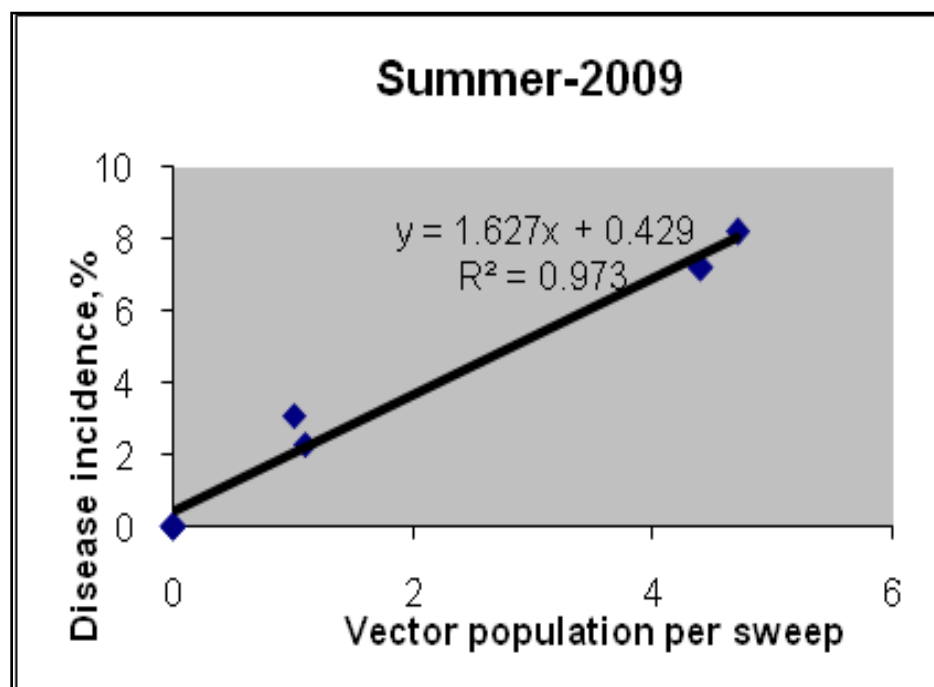
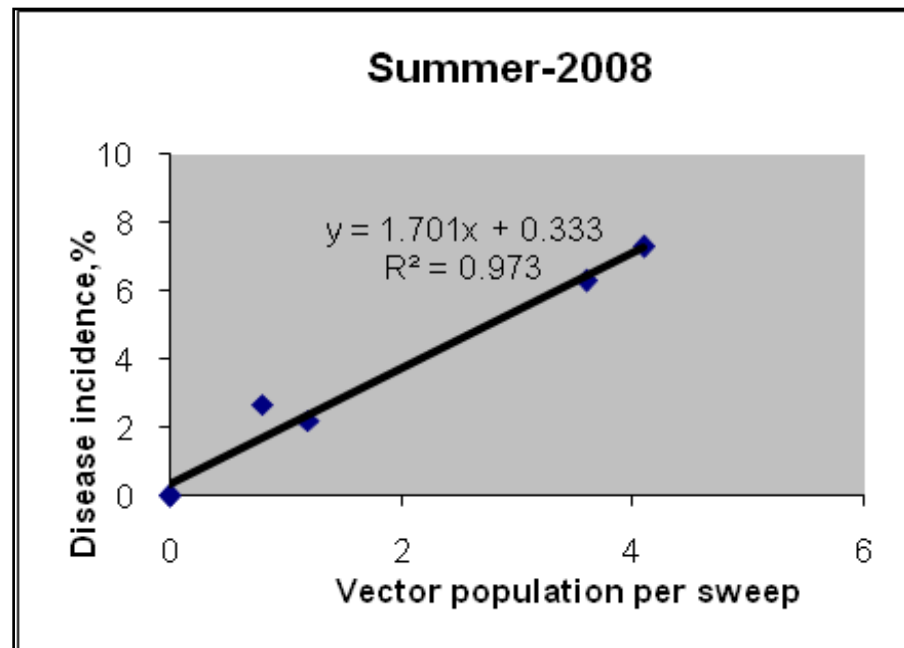


Figure : 9 : Standard curve for vector relationship with incidence of disease during summer seasons



Since, *O. albicinctus* Dist. population is dependent on both temperature and relative humidity, for that a multiple linear correlation equation should worked out.

The incidence of phyllody disease of sesame was recorded in sesame cv. Guj. Til – 2 under surveyed during kharif and summer, 2008 and 2009.

In 2008 and 2009, there was not much difference in the average temperature and relative humidity although 2009 was slightly warmer as compared to 2008.

Vector population and incidence of phyllody were influenced by both temperature and relative humidity, during kharif season disease incidence and vector population were less as compared to summer season of both the year during the period of survey.

$$Y = 54.6883 - 29.8201 + 1.0831X_1 - 0.0126X_2 \text{ (Summer-08)}$$

$$Y = 14.3226 - 11.0702 + 0.2605X_1 + 0.0874X_2 \text{ (Kharif-08)}$$

$$Y = 79.938 - 33.3902 + 1.0300X_1 + 0.0747X_2 \text{ (Summer-09)}$$

$$Y = 121.929 - 60.1132 + 0.1163X_1 + 0.0937X_2 \text{ (Kharif-09)}$$

Where,

Figure-10: Comparison of weather parameter during kharif seasons

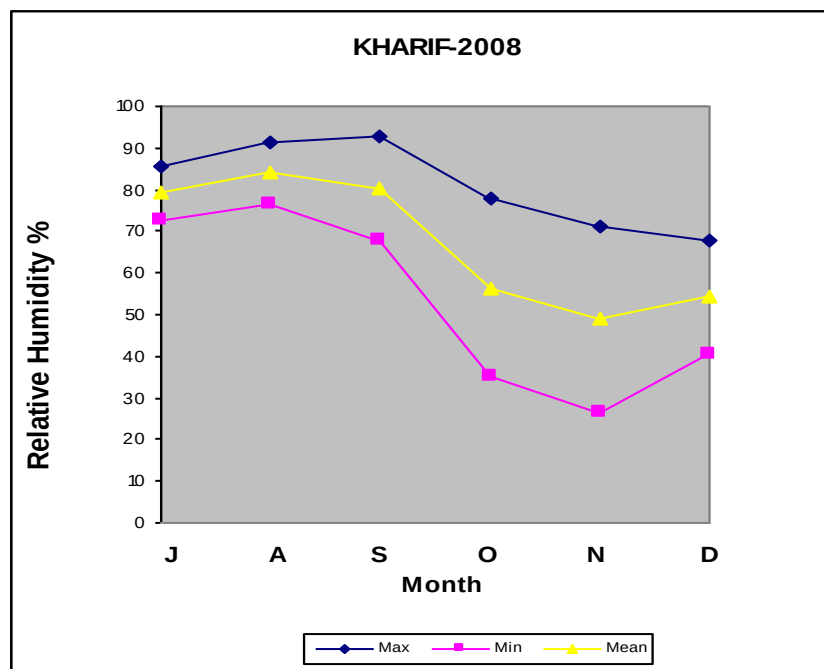
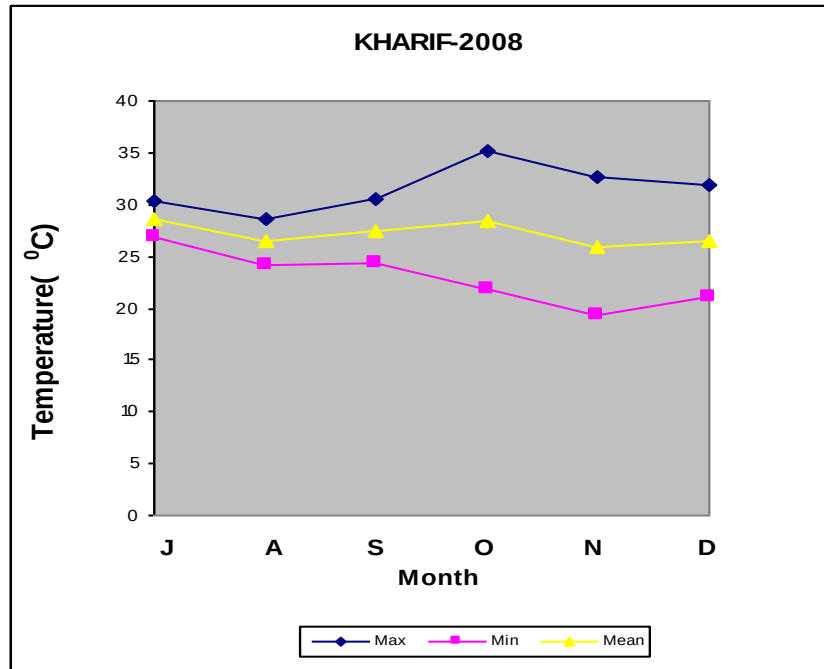


Figure-11: Comparison of weather parameter during summer seasons

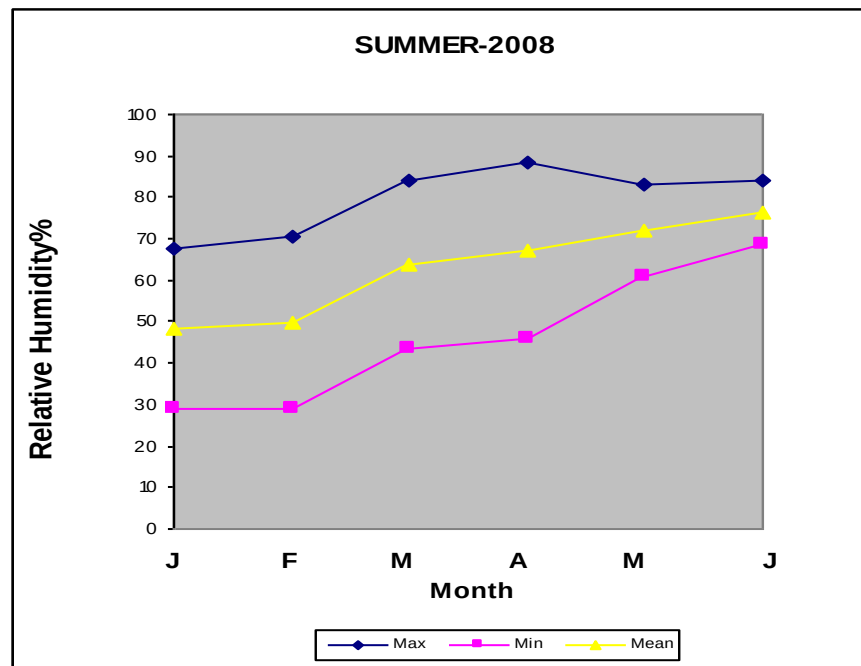
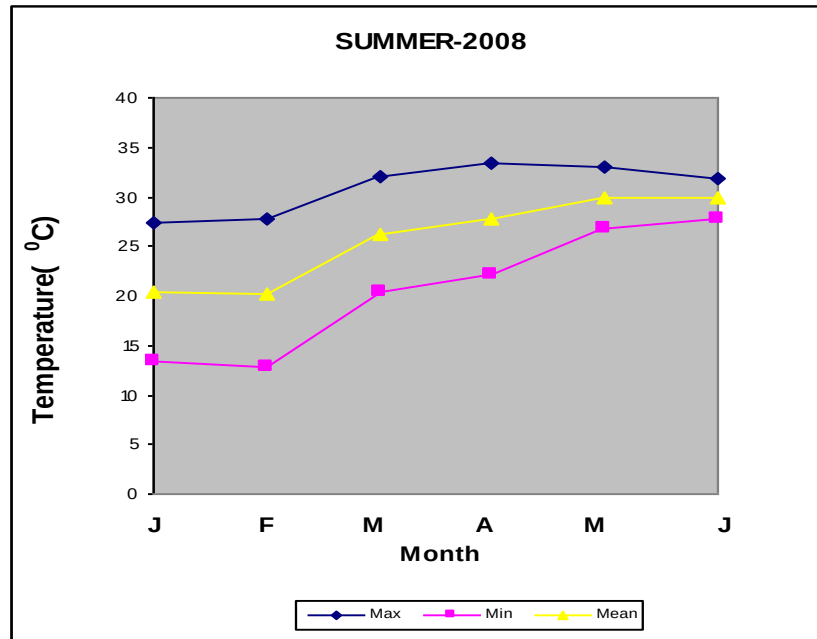


Figure-12: Comparison of weather parameter during kharif seasons

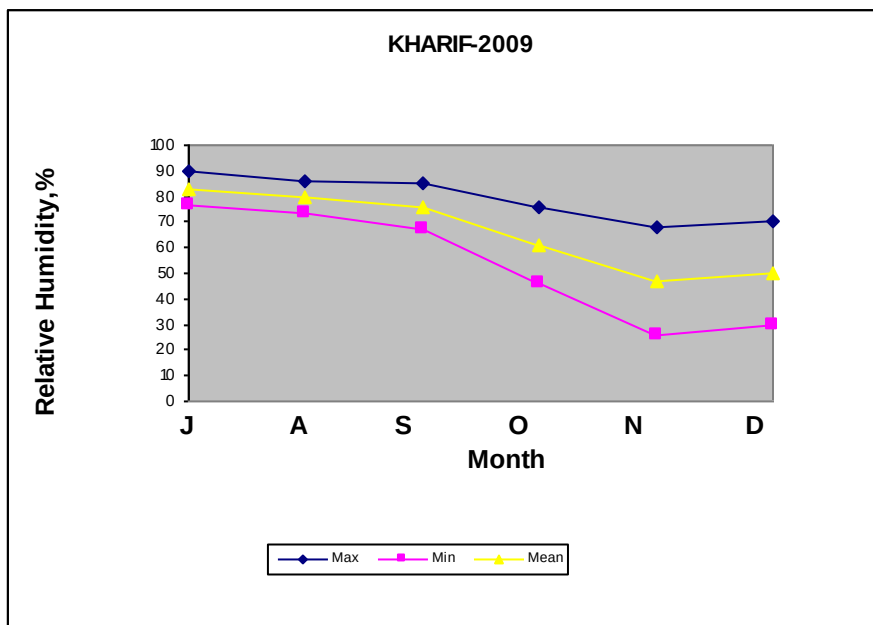
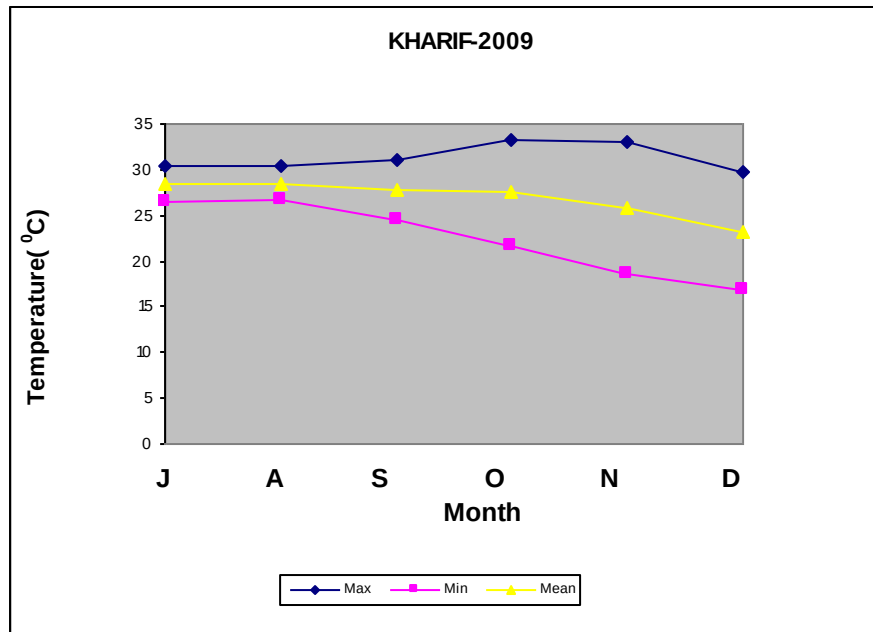
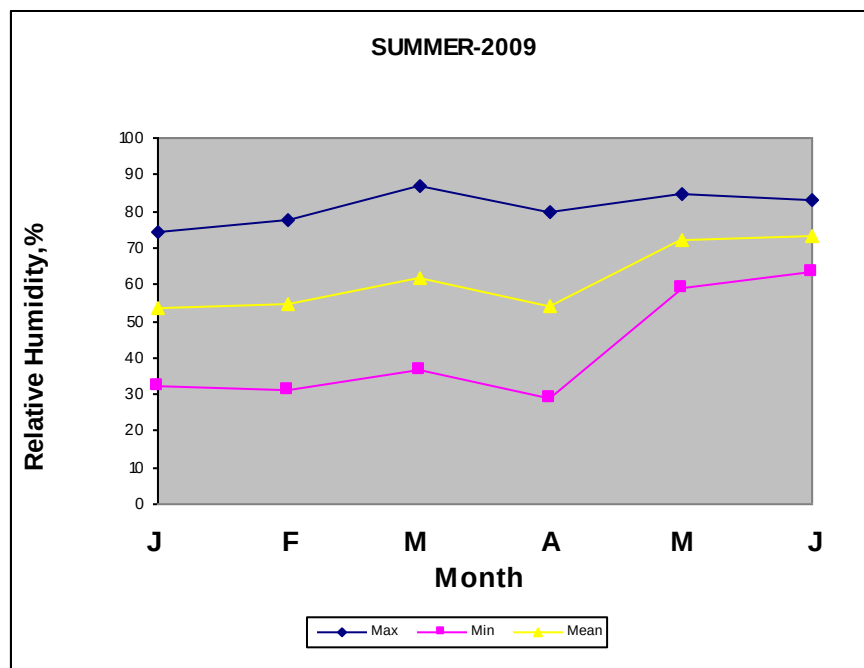
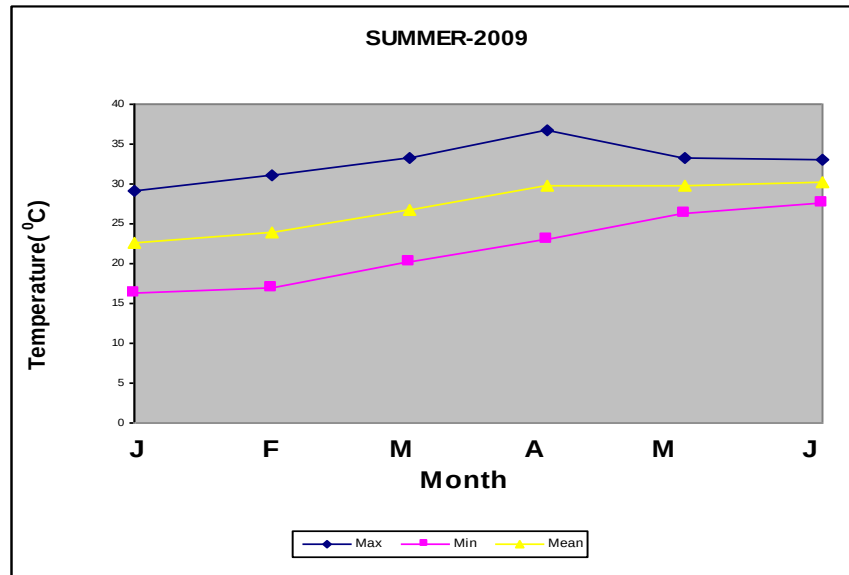


Figure-13: Comparison of weather parameter during summer seasons



$Y = O. albicinctus$ population

X_1 = Max. temperature ($^{\circ}\text{C}$)

X_2 = Mean RH (%)

The above regression equations indicate that a combination of temperature and relative humidity favoured the leaf hopper population (Fig. 10 to 13).

4.7 MANAGEMENT OF PHYLLODY

Since it is persistent type of relationship, protection against insect vectors during the growth of the crop is one of the important strategies to manage the disease.

Result of field trial indicated that out of five insecticides, application of methyle-o-demeton @ 0.025 % significantly reduced the vector population in both the seasons. The percentage of phyllody was lower in methyle-o-demeton treated plants (1.2 and 1.6 %) than in the control (2.6 and 2.9 %) during kharif and summer season, respectively. Same treatments also check the phyllody incidence for further spread in both the seasons. Application of methyle-o-demeton also increased seed yield (380 and 330 kg/ha) as compared to check (210 and 180 kg/ha) during kharif and summer season, respectively (Table 4.19 & 4.20).

Table 4.19: Effect of systemic insecticides on vector population and incidence of phyllody(Kharif).

Treatments	Vector population (per sweep)*		Incidence of phyllody (per cent)	seed yield kg/he
	1(DAS)	7(DAS)		
Methyl-o-demeton	0.0	1.3	1.2	380
Dimethoate	0.8	2.2	2.1	340
Phosphamidon	0.5	1.2	2.2	350
Imidacloprid	0.8	1.3	1.6	360
Monocrotophos	1.2	2.2	2.2	350
Control	3.6	4.2	2.6	330

Table 4.20: Effect of systemic insecticides on vector population and incidence of phyllody (Summer).

Treatments	Vector population (per sweep) *		Incidence of phyllody (per cent)	seed yied kg/he
	1(DAS)	7(DAS)		
Methyl-o-demeton	0.0	1.6	1.6	210
Dimethoate	1.2	2.5	2.3	180
Phosphamidon	1.5	2.2	2.1	200
Imidacloprid	1.8	2.3	1.9	205
Monocrotophos	1.6	2.5	2.5	190
Control	4.3	6.2	2.9	180

* Average of five sweep

4.8 SCREENING AND HOST RANGE, DAMAGE BY PHYLLODY DURING SURVEY

Survey work indicated that out of two cultivated varieties viz., Guj. Til -2 and Guj. Til - 10, cv. Guj. Til - 10 was found best variety against phyllody incidence and recorded 0.0 to 1.2 and 0.0 to 2.8 per cent in all over Saurashtra region as compared to 0.0 to 2.2 and 0.0 to 4.7 per cent in cultivar Guj. Til-2 in kharif as well as summer season of both the year, respectively (Table 4.21 and 4.22, Fig. 14, Plate 8, 9,10,11).

Table 4.21 : The incidence of phyllody disease in two cultivated promising varieties of sesame in Saurashtra region (kharif).

Districts	Varieties	No. of fields surveyed	Incidence of phyllody of sesame (per cent)	
			2008	2009
Porbandar	cv Guj Til-2	10	0 to 0.8	0 to 1.5
	cv Guj Til-10	10	0 to 0.5	0 to 0.8

Junagadh	cv Guj Til-2	10	0 to 1.0	0 to 1.7
	cv Guj Til-10	10	0 to 0.5	0 to 1.2
Jamnagar	cv Guj Til-2	10	0 to 1.1	0 to 1.9
	cv Guj Til-10	10	0 to 0.8	0 to 1.2
Surendranagar	cv Guj Til-2	10	0 to 1.2	0 to 1.4
	cv Guj Til-10	10	0 to 0.2	0 to 0.5
Amreli	cv Guj Til-2	10	0 to 1.6	0 to 2.2
	cv Guj Til-10	10	0 to 0.5	0 to 0.8
Rajkot	cv Guj Til-2	10	0 to 1.1	0 to 1.3
	cv Guj Til-10	10	0 to 0.3	0 to 0.4

Table 4.22 : The incidence of phyllody disease in two cultivated promising varieties of sesame in Saurashtra region (summer).

Districts	Varieties	No. of fields surveyed	Incidence of phyllody of sesame (per cent)	
			2008	2009
Porbandar	cv Guj Til-2	10	0 to 4.1	0 to 4.7
	cv Guj Til-10	10	0 to 1.5	0 to 1.8
Junagadh	cv Guj Til-2	10	0 to 4.4	0 to 4.6
	cv Guj Til-10	10	0 to 1.2	0 to 1.5

Jamnagar	cv Guj Til-2	10	0 to 3.1	0 to 4.4
	cv Guj Til-10	10	0 to 2.1	0 to 2.3

Surendranagar	cv Guj Til-2	10	0 to 3.9	0 to 4.1
	cv Guj Til-10	10	0 to 1.2	0 to 1.5

Amreli	cv Guj Til-2	10	0 to 4.0	0 to 4.3
	cv Guj Til-10	10	0 to 2.5	0 to 2.8

Rajkot	cv Guj Til-2	10	0 to 3.2	0 to 3.8
	cv Guj Til-10	10	0 to 1.1	0 to 1.3

PHYLLODY DAMAGE



A. INFECTED SEEDS



B. HEALTHY SEEDS



C. INFECTION AT LATER STAGE



D. INFECTED PLANTS



E. HEALTHY PLANTS

PLATE - 10

HOST RANGE DURING SURVEY



A. WEED



B. MUNG (*Vigna Radiata*)



C. PEA NUT (*Arachia hypogea*)



D. *Parthenium* spp (Weed)

CHAPTER V

DISCUSSION

Sesame or Til (*Sesamum indicum* L.) belonging to family pedaliaceae is one of the principal oilseeds in common use in India where it is grown in India since ancient times. There are several biotic and abiotic stresses, which are responsible for the low production of sesame. Among the various biotic stresses, diseases caused by fungi and bacteria are the major limiting factor in the cultivation of sesame. Several diseases infecting sesame, phyllody caused by PLO's seems much prevalent and new alarming disease. The first evidence of association of phytoplasma like organism with the disease was reported by Cousin *et al.*, in 1970 and this has been further confirmed by several workers (Choopanya, 1972; Klein, 1977; Purohit and Arya, 1980; Matlapudi, 1989).

The incidence of phyllody was observed during Kharif season which ranged from 0 to 2.2 per cent and during summer season which ranged from 0 to 4.7 per cent in different cultivated varieties and germplasm lines in scattered manner. As much as 10 to 100 per cent incidence of the disease has been recorded in the sesame crop in India (Sahambi, 1970). The first affected plant was observed at 50 days and maximum affected plant at 75 days after sowing in control as well as field conditions.

Sesame is grown extensively almost throughout India. Phyllody disease appears every years wherever sesame is cultivated in the country and incidence of phyllody in India reported earlier by Pal and Pushkarnath, (1935); Krantikumar *et al.*, (1962) and Vasudeva and Sahambi, (1955).

Under the symptomatology study, the initial symptoms of infected by PLO's on sesame are reduction in leaf size and proliferation of a few axillary buds. However, the most conspicuous symptoms are seen when inflorescence is formed.

The inflorescence may be either compact and small with increase in number of flowers which are sterile or a small inflorescence with a few virescent flowers. The symptoms are more pronounced and changes are drastic, where the leaves are greatly reduced in size with altered shape and more yellowing. There is profuse proliferation of axillary growth. The inflorescence has been completely transformed bearing virescent and phylloid flowers with small leaves and many branches (Vasudeva and Sahambi, 1955). The disease was characterized by the transformation of floral parts in to green leaf like structures followed by abundant vegetative growth and another typical symptoms was produced phylloid flowers. The size of the leave in the phylloid shoots gets very much reduced and the internodes are also shortened. The phylloid flowers and new shoots produced are crowded together giving a bunchy top look to the infected plant has been also observed by Vasudeva and Sahambi, (1955).

Floral virescence, proliferation, some times with yellowing, cracking of seed capsules and rarely found shoot apex fasciation symptoms were also recorded during present study. These findings are in agreement with those of Salehi and Izadpanah, (1992).

This disease experimentally transmitted to the healthy sesame through grafting and leaf hoppers as *Orosius albicinctus* Dist. identified vector of this disease (Vasudeva and Sahambi, 1955). Sesame phyllody agent is persistent in the leaf hopper vector. The leaf hopper, *O. albicinctus* Dist. is not a pest of the sesame crop as its population is far below the pest level. That is why this leaf – hopper never attracted the attention of the entomologist. The importance of the leaf – hopper was only realized when it was reported to be the vector of sesame phyllody (Vasudeva and Sahami, 1955; Ghauri, 1966; Sahambi, 1966 and 1970). On infecting as insect, phytoplasmas traverse the wall of the intestinal tract, multiply in the hemolymph, pass through the salivary glands and multiply further. When the insect feeds on a new host plant, phytoplasmas are inserted into the phloem tissue along with salivary fluids and infect phloem cells. Once the leaf-hopper becomes infective, it remains so throughout the remaining part of its life. Thus, a single leaf-hopper can cause infection in a number of plants that it happens to feed upon till its death. Increase in the percentage of infective leaf-hoppers in sesame is bound to result in appreciably higher incidence of

phyllody. In present study vector population per sweep and incidence of phyllody (per cent) recorded were 0 to 6.2 and 0 to 1.5 during kharif season and 0 to 8.2 and 0 to 4.7 during summer season, respectively. If there are overlapping crops of sesame, previous crops would serve as immediate source of the disease for the latter. Almost 100 per cent incidence of phyllody disease in sesame has been reported from Raichur, Karnataka (Sahambi, 1966).

In the present study phyllody disease was successfully transmitted from diseased to healthy sesame plants using grafting and the leaf hopper *Orosius albicinctus* Dist. The disease was previously observed to be vectored by *O. albicinctus* Dist. in India (Kolte, 1985; Srinivasulu and Narayanasamy, 1995) and Iran (Esmailzadeh – Hosseini *et al.*, 2007), by *O.cellulosus* Lindberg in Upper Volta (Desmits and Laboucheix, 1974) and by *Neoliturus haematoceps forma opacipennis* in Iran (Salehi and Izadpahan, 1992). Phyllody has also been transmitted from diseased sesame to *Cathoranthus roseus* L. by *Circulifer haematoceps* in Turkey (Kersting, 1993).

Attempts were failed to transmit the pathogen through seeds, Chopanya in 1972 have also got the negative results regarding attempts to transmit the pathogen through sap and seeds.

Reduction in height of phyllody infected plants may be due to inhibition of auxin production or inactivation of auxin during translocation to the growing points as reported in case of Gingelly by Krishnaswamy and Jayarajan(1983) and in case of Brinjal by Choudhuri and Garg(1955) when plant infected by phyllody and little leaf disease, respectively. It may also be due to low production of growth promoting substances which ultimately results in dwarfing of the plants as reported by Diener (1963) in TMV infected Tobacco plants.

Irrespective of the sesame variety phyllody infection resulted in reduced number of branches per plant during the earlier stages of plant growth and increased number of branches during the later stages (Klein, 1977).

The reduction in number of capsules per plant may be accounted for by pollen sterility induced by phyllody infection and / or by the effects of PLO on floral parts of the infected sesame.

Investigations on the biochemical changes brought about by the sesame phyllody agent revealed that the phyllody affected leaves of sesame contained more dry matter, higher phenol contents and lower chlorophylls and nitrogen contents.

With regard to pathophysiology, pronounced difference was observed between the healthy and infected plant. Phyllody disease increased the dry matter content in the infected plant and slightly decreased the moisture content.

These findings are in agreement with those of Chakrabarti and Chaudhary (1974). Increase in dry matter content in stems and roots and its decrease in the leaves following infection has been reported by Choudhuri and Garg (1955). Similar observations were also made in case of purple top roll infected potato plants (Kaley and Nagaich, 1977).

In case of disease, including PLO disease, photosynthetic activity has been reported to be generally decreased (Tschen, 1976; Mitra and Sengupta, 1980; Doi and Asuyama, 1981; Purohit *et al.*, 1978). Photosynthetic activity and dry matter content are positively correlated. However, during the present investigation, the dry matter content was increased whereas photosynthetic activity (Chlorophyll content) was reported to decrease considerably (Mitra and Sengupta, 1980). It appears that severe blockage of translocation of photosynthates might have caused increase in the dry matter content. However, it needs further investigation.

Apparently phyllody infection resulted in reduced the uptake of water by plant from the soil and consequently the plants were subnormal (Chakrabarti and Chaudhary, 1974). The observed changes in moisture content following infection reflect upon the changes in the permeability of the leaf tissues resulting in water loss. During investigation no considerable increase or decrease in moisture content was observed, these findings are agreement with several workers (Regapathy and

Jayraj, 1973; Srinivasan and Chellian, 1978; Patil and Patil, 1989).

Total chlorophyll content in diseased plant was reduced(25.24 to 39.92 per cent). Reduction in chlorophyll content in the phyllody infected sesame plants has been observed to be positively correlated with the severity of infection. Several reports are available regarding the decrease in chlorophyll content due to mycoplasmal disease (Kaminska *et al.*, 1971; Carling and Milliken, 1977; Nagaich, 1978).

According to Patil and Patil (1989) decrease in chlorophyll content may be attributed to lower rate of synthesis and its accelerated breakdown. The chloroplasts may be considerably damaged which might be responsible for the reduction in chlorophyll content (Shulka *et al.*, 1988). It appears that phyllody infection definitely inhibits biosynthesis of chlorophyll, thereby affecting the photosynthetic activity.

Reduction in total nitrogen and crude protein(9.86 to 17.44 per cent) content has been observed in case of phyllody infection. Similar results have been obtained in case of PLO's diseases (Carling and Milliken, 1977; Patil and Patil, 1989; Prasad and Sahambi, 1980). However, increase in nitrogen content was also observed by several workers (Ragupathy and Jayraj, 1973).

It is difficult to attribute the exact reasons for reduction in nitrogen and protein content in infected leaves. It is

probable that either diseased tissues synthesized the protein at a slow rate compared to the actively metabolizing healthy tissues (Uritani, 1971) or pathogen's protein is synthesized at the direct expense of normal host protein as suggested by Subba Rao *et al.*, (1985), in case of sterility mosaic of pigeon pea.

It may be possible that due to mycoplasmal infection, protein synthesis gets disturbed. The decrease in soluble protein content may be also due to disturbed translocation and dilution effect of accumulating carbohydrates as suggested by Singh *et al.*, (1982), or destruction of photosynthetic centers (Orlab and Arny, 1961), or reduced photosynthetic area, a condition known to exist in phyllody infected sesame leaves.

Rate of transpiration increased (44.82 per cent) with increasing severity of infection, while stomatal resistance decreased. Changes in transpiration as a result of pathogenic infection have been reported by several workers (Mignucie and Boyer, 1979; Ellis *et al.*,1981).

Phenols have been implicated in the defense mechanism of plants to infection by many disease causing agents. In higher plants, phenols are synthesized from carbohydrates. The depleted carbohydrate reserve in the present study due to sesame phyllody explains the excess accumulation of phenols(Mitra and Mazumdar,1977).

Anatomical changes in host plants as a result of PLO's infection have been reported by several workers (Patel and Shah, 1972; Kothari *et al.*, 1982; Singh and Mitra, 1990). It may be possible that hormonal changes triggered off by phyllody infection might have resulted in different types of abnormalities like hypertrophies, hyperplasia and other anatomical changes as has been observed by the above workers.

Thus, it may be concluded from the present investigation that the altered auxin – cytokinin level could be the cause of development of typical phyllody syndrome. However, more elaborate works in needed to postulate what exactly triggers hormonal imbalance thereby, resulting in such drastic morphological changes in the host it remains to be seen whether it is a toxin as speculated by Mara morosch (1976).

Histopathological changes in floral parts, stem, leaf and petiole due to phyllody were studied through light microscopy.

Anatomical changes in the stem due to phyllody infection included phloem proliferation and increase in cell number (hyperplasia). Xylem vessels were also observed to be hypertrophied. In the cortical region, some bigger than normal cells were noticed.

Microscopic examination revealed abnormal thickening of the lumen of the internal phloem cells. This thickening may be due to callous deposition which have been reported in case

of several PLO diseases (Hiruki and Dijkstra, 1973; Braun and Sinclari, 1978; 1979). Samuel *et al.*, (1933) reported similar histopathological changes in internal phloem tissues of big bud infected tomato plants. Morelli *et al.*, (1987) also reported similar histopathological abnormalities in phloem tissues of chrysanthemum infected with chrysanthemum stunt viroid.

Both xylem vessel and phloem cells were observed to be hypertrophied. According to Esau (1948) and Girdlomi (1955), this kind of hypertrophy could be the result of deranged metabolism of normally differentiated cells leading to hypertrophy.

The anatomy of healthy and diseased leaf petioles was examined. In the infected petiole, arrangement of xylem and phloem tissues of the vascular bundle was some what irregular and distorted. This is probably due to hypertrophy of the xylem vessels and phloem cells as observed in case of stem.

Necrosis was observed in mesophyll and vascular tissues. Necrosis in mesophyll tissues resulted in disintegration in cells and their eventual collapse. It may be possible that some translocatable substance is produced as a result of infection, which diffuses out causing necrosis and disintegration of cells in area where direct vascular supply is not present. Similar observation were also made by Schneider (1977) who reported induction of metabolically active intermediary phloem cells in

fine minor veins which produce some translocatable metabolites, as a result of PLO infection. Necrosis may also be responsible for blockage of translocation in photosynthates in little leaf infected brinjal plants (Mitra and Bhattacharya, 1984).

According to Esau (1967), toxic metabolites are produced by pathogen itself or intermediary cells which could move basipetally on phloem and along the way diffuse laterally from sieve tubes into bundle sheath and adjacent tissues causing necrosis. Kothari *et al.*, (1982), while working on ultrastructural changes in the little leaf infected brinjal tissues, observed some histological abnormalities e.g., wall ingrowth, dilated endoplasmic reticulum and ruptured cristae, deformed outer mitochondrial membrane and tonoplast. According to them some diffusible toxin is produced as a result of infection which affects the cell membrane system in brinjal. It would be interesting if any such toxin could be isolated from diseased plants and characterized.

Cells of the different tissues from stem, petiole and leaf were counted and measured for observing variation, if any, in cell size and number as a result of phyllody infection.

In stem, substantial increase in cell number has been observed while the size of the cortical cells was considerably reduced. It is probable that the increased cell number at the initial stages might have resulted in increase in the number of

lateral branches while the reduced cell size might have been responsible for their thinness. In infected petiole, cell number considerably decreased in the vascular bundle and cortical region. However, no difference in cell size was observed. In the leaf, number of spongy parenchymatous cells decreased in diseased tissue, but cell size remained comparable. It may be possible that due to necrosis in mesophyll region, the number of parenchymatous cells have been less resulting in reduction in the leaf lamina.

No report is available regarding effect of PLO's disease on the cell number and dimension of the host tissues. Such a drastic change in cell number and size in the infected tissues of sesame plant can be explained from hormonal point of view. Imbalance of plant growth regulators could result conceivably from several modes. Impairment of phloem function is expected to slow translocation of growth hormones. Alteration of the turnover rate of certain hormones (mediated by PLO's) may be another cause of hormonal imbalance (Davis and Lee, 1982).

PLO's may cause hormonal imbalance in the vicinity of infected areas by altering the normal patterns in hormonal transport or translocation.

Petru *et al.*, (1971) noted that growth of callus tissue derived from diseased plants was more vigorous than that of tissue derived from healthy plants and the more vigorous

growth of the diseased callus tissue continued after several subcultivations in the same medium. One possible explanation for this phenomenon is that PLO's might actually synthesize certain hormones or hormone-like toxin *in vivo*.

Generally growth hormones like auxins and cytokinins are responsible for stimulation of growth and development of plant tissues. So any imbalance in these hormones may result in different types of abnormalities like hypertrophy and hyperplasia. Even plant growth inhibitor like Absciscic acid (AbA) may disrupt the normal growth and development of cells. It may be possible that these type of hormonal changes triggered off by phyllody infection might have resulted in increase in cell number and reduction in cell size.

PLO's infection has already been reported to influence hormonal balance in host plant (Parthasarathi, 1979). However, more elaborate work on the quantitative estimation of hormonal imbalances, if any, as a result of phyllody infection would properly explain the observations made during the present investigation.

During light microscopic investigations, variations in structures and shape of trichomes were observed in phyllody infected leaves. The trichomes on diseased leaves were unbranched or oddly branched into unequal arms. The stellate trichomes were not symmetrical in its architecture as the healthy ones. The vertical branch of a trichome was

relatively shorter as compared to healthy ones. The number of radiating branches was also less; these branches were reduced in length and did not overlap with branches of adjacent trichomes. The angular grooves where trichome branches joined were more prominent in healthy trichomes than in those from a diseased plant. Such change in microstructures of plant organs due to phyllody infection was in consonance with the observations made by Singh and Mitra (1990).

Such drastic changes in trichome morphology as a result of phyllody infection is significant and worth further investigation. According to Regupathy and Jayraj (1973), low calcium content is responsible for reduction in cell wall strength of phyllody infected sesame plants leading to microstructural aberrations in infected plants. Whether some such nutritional deficiencies are also responsible for such changes in trichome structures as a result of phyllody infection, needs further investigation.

Trichomes have been observed on the anthers for the first time. However, normal stelate trichomes similar to those on healthy leaf surface were observed on anthers from healthy as well as infected plant. This observation is significant because while the trichomes on infected leaves were entirely morphologically different from those on healthy leaves, no such difference was observed between those on anthers from healthy and infected flowers.

Epidemiology of phyllody of sesame was studied during the kharif and summer seasons of 2008 and 2009. In both the seasons of both the year, disease incidence has a simple positive correlation with the population of leaf hopper (*O. albicinctus* Dist.).

The highly positive correlation between incidence of little leaf disease and leaf hopper population in brinjal was reported by Mayee and Kaur (1975) and Rajamannar (1989). The incidence of disease was also reported to be positively correlated with the vector population in case of rice yellow dwarf and parthenium phyllody disease. It was also observed that vector population is dependent on temperature and RH for its build up (Myniyappa and Ramakrishnan, 1976; Mathur and Muniyappa, 1993).

Disease prediction by vector indexing is one of the reliable methods for monitoring a vector borne disease under field conditions (Srinivasulu and Narayanasamy, 1995).

Higher incidence of phyllody in the early sown sesame crop has also been recorded by several workers including Pal and Pushkarnath (1935); Vachhani (1945). In addition, incidence of phyllody is more in summer sown crop, during summer months incubation period of the disease agent in the host plant as well as in the leaf hopper vector is obviously shortened during summer months due to the comparatively

higher temperature prevailing them. Prevailing temperature during different seasons have a pronounced affect on the incubation period of the sesame phyllody agent both in the insect vector and the plant host. During summer months, the disease agent under goes and incubation period of 15 to 23 days (Sahambi, 1970; Bindra and Singh,1970; Sundararaju and Jayaraj,1977;Choudhary and Prasad,2007).

Variations in the incidence of the sesame phyllody disease during different seasons have been reported (Sellammal *et al.*, 1973).

Incidence of phyllody in a particular crop may be attributed to the percentage of infective leaf hopper present in the crop and also to the prevailing environmental conditions, particularly the temperature. Sesame phyllody agent is persistent in the leaf hopper vector. Once the leaf hopper becomes infective, it remains so throughout the remaining part of its life. Thus, even a single leaf hopper can cause infection in a number of plants that it happens to feed upon till its death. If there are overlapping crops of sesame, previous crop would serve as immediate source of the disease for the latter. As even a low population (0.6 per cent) of infective leaf hoppers can cause 10 per cent incidence of phyllody. Almost 100 per cent incidence of phyllody disease in sesame has been reported from Raichur, Karnataka (Sahambi, 1967).

Sellammal *et al.*, (1973) reported a significant positive correlation between phyllody incidence and maximum temperature and no correlation between phyllody incidence and leaf hopper population was observed. It is likely that activity of a single inoculative vector was highly influenced by the weather parameter.

Despite wide interest in chemotherapy of plant phytoplasma disease, no effective mycoplasmacical agent that can cure the disease completely has yet been found (Raychaudhuri *et al.*, 1988). In practice, control of the disease by prophylactic treatments rather than cure of affected plants. Also, a schedule needs to be worked out in relation to the appearance of vectors along with timely application of systemic insecticides for keeping the plants free from the disease. Under these circumstance development of resistant or tolerant variety would be best. But no any cultivars found 100 per cent resist or tolerant to phyllody.

CHAPTER VI

SUMMARY AND CONCLUSION

Sesame or Til (*Sesamum indicum* L.) belongs to family *Pedaliaceae* is one of the principal oilseeds in common use in India. Among the several diseases infecting sesame, phyllody seems much prevalent in Gujarat and especially in Saurashtra region. The incidence of phyllody during kharif and summer seasons ranged from 0 to 2.2 per cent and 0 to 4.7 per cent respectively, in scattered manner and spread of disease was very slow. The first infected plant was observed at 50 days and maximum at 75 days after sowing.

The yield contributing characters of sesame (cv. Guj Til – 2) plants i.e. plant height, number of capsule per plant, branches per plant, test weight were adversely affected due to phyllody. There was reduction in number of capsules in phyllody infected plant range up to 11.5 and 47.5 in comparison to 55.8 in healthy plants. Plant height in phyllody infected plant range up to 69.4 and 113.4 cm in comparison to 118.76 cm in healthy plants, while test weight basis (1000 seeds weight), the yield in phyllody infected plant range up to 0.23 gm and 3.90 gm in comparison to 4.30 gm in healthy plant and there by 40.94 to 82.90 per cent reduction in seed yield occurred due to the disease.

The present investigation indicated that the losses in yield of sesame due to phyllody depend upon the infection time. Maximum reduction in seed yield was recorded in plants which were infected at an early stage. The loss become progressively lesser when the plants were infected at advanced stages of growth. The symptoms shown by infected plants include, yellowing, shortening of the internodes with stunted growth, smaller leaves, excessive proliferation of shoots resulting in a phyllody, virescence, sterile flowers, necrosis of the phloem tissues.

Phytoplasmas are phloem limited pathogen, therefore, only phloem feeding insects can potentially acquire and transmit the phytoplasmas. However, among phloem feeding insects, leafhoppers have been confirmed as vector of phytoplasmas, which feed on the phloem tissues of infected plants, picking up the phytoplasmas and transmitting them to the next plant they feed on. For this reason the host range of phytoplasmas is strongly dependent upon its insect vector.

Phytoplasmas enter the insect's body through the stylet, move through the intestine and are then absorbed in to the haemolymph. From here they proceed to colonise the salivary glands, a process that can take up to three weeks.

Phytoplasmas contain a major antigenic protein that makes up the majority of their cell surface proteins. This protein has been shown to interact with insect microfilament

complexes and is believed to be the determining factor in insect phytoplasma interaction. Phytoplasmas may overwinter in insect vectors or perennial plants.

No mechanically sap as well as seed transmission was reported but some what 10 per cent transmission through grafting was reported.

Histopathological changes in host plants are generally brought about by pathogenic infection and mycoplasmal diseases are no exception. However, although studies in this regard have been carried out in great detail particularly in case of viral diseases, not much work has been done with regard to mycoplasmal diseases of plants in general and phyllody of sesame in particular, especially on biochemical and histopathological aspects, which would generate some pertinent information on host pathogen interaction, resulting in such drastic changes in host morphology.

The present investigation was therefore, undertaken with a view to obtaining pertinent information on chlorophyll content, protein content and phenol content. Some physical parameters like transpiration rate, dry matter and moisture content. Anatomical changes like cell number, trichome morphology, which would throw some light on the drastic morphological changes in the infected plant. Following is a resume of the results obtained.

Phyllody disease increased the dry matter(13.72 to 21.59 per cent) content while a very little decrease in moisture content was observed. Increase in dry matter might be due to severe blockage of translocation of photosynthates in the diseased leaf tissues.

Total nitrogen and crude protein(9.86 to 17.44 per cent) content were observed to be reduced due to infection. It appears that either diseased tissues synthesized the protein at a slow rate compared to healthy one.

phenol content(2.02 to 2.42 mg/g) was increased in infected part of the plant.

Reduction in chlorophyll content(25.24 to 39.92 per cent) was also observed. It appears that Phyllody infection inhibited biosynthesis of chlorophyll thereby affecting the photosynthetic activity.

Rate of transpiration increased(44.82 per cent) on severely infected plant. It is apparent that higher transpiration rate was influenced by lower stomatal resistance.

During the course of these investigations, several anatomical changes were observed in the infected plant. The modification observed were, phloem cells as well as xylem vessels, thickening of the wall of phloem cells, irregular and distorted arrangement of xylem and phloem tissues in the infected parts of plants. Variation in trichomes in infected plants.

It may be possible that phyllody infection causes hormonal imbalance in the vicinity of infected areas by altering the normal patterns in hormonal transport or translocation, which leads to increase in cell number and reduction in cell size ultimately resulting in such a drastic morphological change.

Among the two popular cultivars of Saurashtra region viz., Guj Til -2 and Guj Til - 10 screened for resistance against phyllody during survey, none was found absolutely resistant. However, Guj Til - 10 (black seeded) cultivar showed negligible infection as compared to cultivar Guj Til-2.

Epidemiology and survey study indicated considerable variation in the disease incidence between the seasons and from place to place. Incidence of phyllody correlated with its vector population, however, population build up dependent on temperature and relative humidity. Disease prediction by vector indexing is one of the reliable methods for monitoring a vector borne disease under field conditions.

Management of plant disease caused by phytoplasma has often focused on controlling the insect vector populations by applications of insecticides, but mitigation of disease is slowly shifting toward habitat management and the use of genetic methods to produce naturally disease resistant plants.

In conclusion, although none of the PLO's has so far been cultured on synthetic media and persistent type of insect

vector relationship, it is very difficult to distinguish naturally occurring variants of this important group of pathogens and understand their epidemiology and interactions with the host. However, the use of molecular biology during the past decade has greatly enhanced knowledge on plant pathogenic mollicutes. Different approaches can now be used to investigate interactions of mollicutes with their plant and insect hosts and new avenues are provided through transgenesis for the control of mollicutes induced disease.

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* Original not seen

