I. REVIEW OF LITERATURE

A Introduction

Weeds are very plastic in nature in their ability to circumvent man's efforts to control them and have always resulted in suppression of crop yield. Thus weeds are plants that pose more harm than good. On this account, annual losses have been estimated to the tune of Rs. 420 crores (Joshi 1974). Under certain circumstances, any plant species whether useful or otherwise can be a weed if it grow in a wrong place. However, it hardly happens that useful plant grows without proper care, in this respect weed have tremendous capacity to grow any time at any place and face boldly "natures fury" and thereby they almost conquer the nature. This makes the weeds hardier than crop plants to withstand extreme environmental conditions. They are endowed with better survival capacity. They are better equipped in their reproductive capacity and their capacity to spread in a newer geographical area. Parthenium hysterophorus is one among the notorious famous weeds.

B. Distribution of Parthenium hysterophorus

Parthenium hysterophorus Linn. which entered

India in 50s, spread almost all over India like wild fire within quarter a century. As a species with high rate of fecundity, efficient dissemination tactices and with adverse allelopathic impact on many of the associated herbaceous plants, it has attained a prominant status in a short span of time in abandoned fields and wastelands and thus it has become notoriously popular in India.

Parthenium a native of West Indies, tropical South and North America (Fig. 3) now occurs as a naturalised weed in India, Pakistan, Bangla-Desh, Cuba and many other parts of the world (Joshi 1990). In India it was first described in Poona (Rao 1956) and in a span of two decades it had spread all over the country covering 5 million hectares of land (Gidwani 1975). It now occurs in almost all the states of India (Krishnamurthy et al. 1977) growing profusely in wasteland, on sides of railway tracks and roads where nitrogenous wastes of humans and other live stock are replenished regularly (Fig. 4). It grows best in areas with 600-1000mm rain fall and a diurnal temperature range of 5°C - 45°C. It also occurs in areas beyond this ecological range, however, in controllable

PLACES SHOWING DISTRIBUTION OF PARTHENIUM HYSTEROPHORUS IN WORLD.

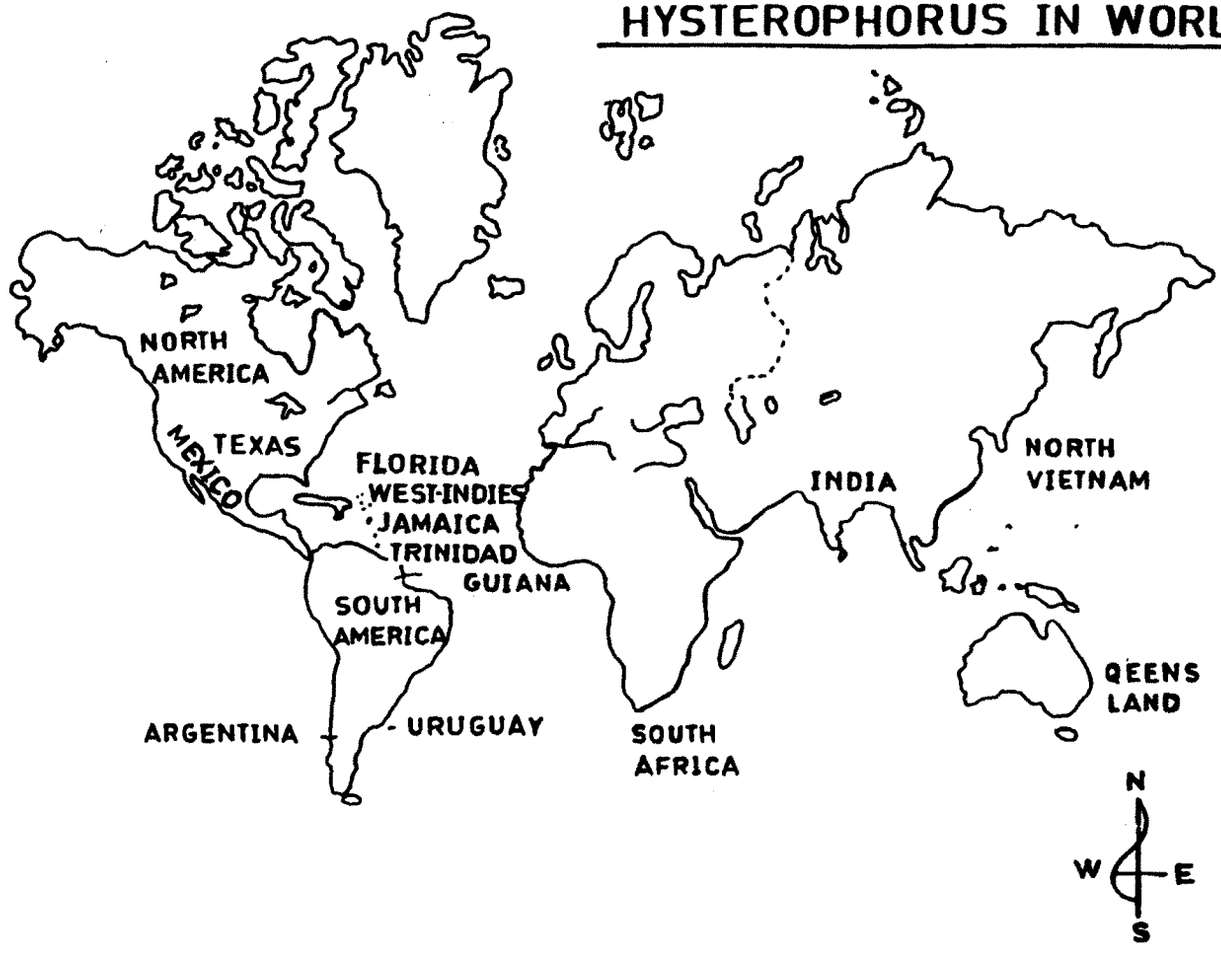

Fig-3

STATES SHOWING DISTRIBUTION OF PARTHENIUM HYSTEROPHORUS

IN INDIA & PAKISTAN

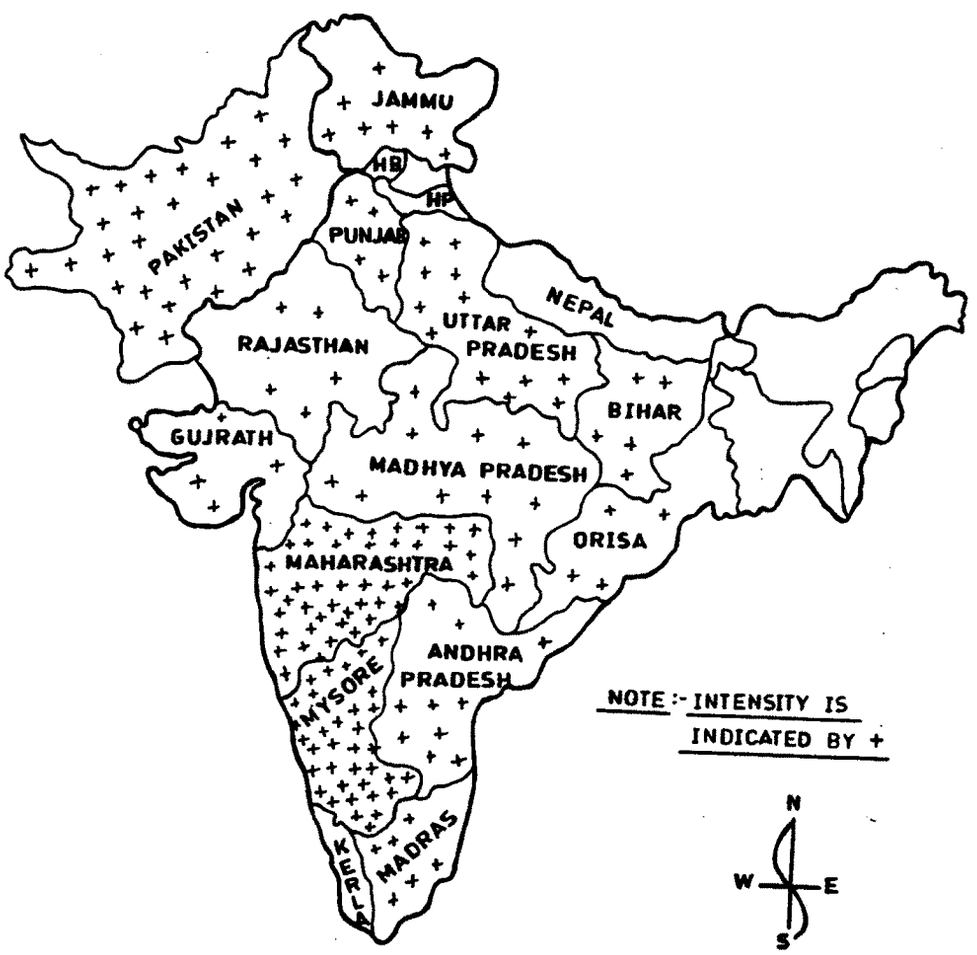

Fig-4

proportions. It also propagates parthenogenetically and appears to be nonphotoperiodic, which can be evidenced by its flowering throughout the year (Hegde and Patil 1976). The fact that this pernicious weed has spread from Jammu (Hakoo 1963) to South of Tamil-Nadu (Ellis and Swaminathan 1963), indicates that it possess greater ecological ad aptation.

C Menacing weed Parthenium hysterophorus

The weed has posed the greater problems and has thrown biggest challenge in front of scientists. It is not only known to cause a new kind of eczema to various parts of the body but also allergic diseases such as dermatitis, fever and asthma. The other nuiecence posed by Parthenium are :

i) It causes lesions in the buccal cavity and ulcers in the alimentary canal of the graizing animals (Sundara Rajulu et al. 1976, Narasimhan et al. 1977).

ii) It reduces yield of grass from pastures (Vartak 1968).

iii) It is a serious pest in short saturated cropping areas and orchards (Dube et al. 1979).

iv) It provides shelter to adult mosquitoes during day time and thereby protects them from their natural predators (Joshi 1990).

With the public awareness created about the economic losses and health hazzards caused by Parthenium (Lonkar et al. 1974, Krishnamurthy et al. 1977, Towers et al. 1977), incessant efforts are made to control this weed by manual, mechanical and chemical methods. At present the problem posed by Parthenium have variously been contemplated by scientific world, which is evidenced by various scientific and parascientific communications.

D Work done on Parthenium hysterophorus

Among the known 16 species of Parthenium, P. hysterophorus is the successful weed. Although P. argentatum is a econimically important plant, for it yields rubber, most others are weeds. The work on P. hysterophorus carried so far in India and abroad includes its biochemical analysis (Kanchan 1975, Shen et al. 1976, Rodriguez et al. 1978), effect of some

growth regulators on seed germination (Dagar et al. 1977) its allonomic and allelopathic effects (Sharma and Joshi 1977, Sharma et al. 1977, Kanchan and Jayachandra 1979, 1979 a, Patil and Hegde 1988), chemical control (Kasain 1971, Alden and Wilfred 1973, Patil et al. 1991), Biological control (Char et al. 1975, Hegde and Patil 1976, Vartak 1976, Vaidya and Vartak 1977, Dagar and Singh 1979, Hegde and Patil 1979) and its nutritive value as a source of food for ruminants and non-ruminants (Savangikar and Joshi 1978, Patil 1980).

Apart from these studies sizable work on physiology of P. hysterophorus so far as mechanism of growth, mode of adaptation to extreme environment and biochemistry of photosynthesis is concerned, has been reported. Patil and Hegde in their series of communications have reported the biochemistry of photosynthesis. The study revealed that the weed has C_3 - Path of photosynthesis with kranz anatomy (Hegde and Patil 1981). The starch distribution patern of the leaf and CO_2 compensation concentration of 27.5cm^{3}m^{-3} indicated the C_3-C_4 intermediary tendency of the plant (Patil and Hegde 1983). 13_C fractionation and photosynthetic enzyme in relation to plastochron index of P. hysterophorus has also been studied by Patil and Hegde (1983a) . The addaptation of this weed to salt stress and the carbon flow mechanism in this weed has been studied by Hegde and Patil (1982). P. hysterophorus has also been investigated for photorespiratory aspects (Patil and Hegde 1982), translocation of photosynthates under water stress

(Patil and Hegde 1983) and growth pattern under different ecological conditions (Hegde and Patil 1980). Francis and Radhakrishnan (1980) have studied polypeptides of P. hysterophorus chloroplast. According to them there are two groups of polypeptides, a higher molecular weight peptides enriched with photosystem I and low molecular weight peptides enriched with photosystem II. They have undertaken this study in order to emphasize the uncontrolled luxurious spread of this weed, probably because of the efficient photosystems present in the plant. Their study revealed that peptides resemble very closely with the spinach chloroplast and the lamellar and stromal peptides of maize bundle sheath chloroplasts. However, they are of the opinion that the efficiency of the weed for its uncontrolled spreading is not well understood.

The sesquiterpene lactone (SL) pseudoguaianolide - 'Parthenin' in the plant P. hysterophorus is the principle allergen which causes severe contact dermatitis, rhinitis and hay fever in sensitised humans and animals (French 1930, Lonkar et al. 1974, Rodriguez et al. 1977, Patil and Hegde 1988) has also been isolated and studied for its allelopathic effects.

From the foregoing literature survey it is vividly clear that the weed P. hysterophorus is highly plastic and sturdier having wide range of ecological addaptation to extreme environment. This sturdier weed which has shown cytotoxic, fungicidal property (Patil and Hegde 1988) and partially insecticidal property (Rodriguez 1983) has become a prey to a disease caused by MLO.

E Phyllody of Parthenium

During the summer of 1972 some plants growing as weed on both sides of a road in New Delhi were observed with diseased inflorescence. The plants showed typical symptoms of phyllody and the flowers had turned into leafy structures. The infected plants had excessive branching giving rise to witche's broom appearence. Sahambi (1970) observed P. hysterophorus plants spontaneously infected with the agent of Sesamum phyllody, which has now been reported to be due to a mycoplasma like organisms (Choopanya 1971, Cousin et al. 1970). Later on Ghosh and Raychaudhuri (1974) have casually listed P. hysterophorus phyllody among the various yellow diseases of plants occuring in India. Finally, with the help of electron

microscopy, Phatak et al. (1975) have confirmed the association of mycoplasma like bodies causing phyllody disease in P. hysterophorus.

F About MLOs

Though the study of plant mycoplasmas is in an infant stage as compared to other pathogenic organisms like fungi, bacteria and viruses, the increasing number of reports on the association of mycoplasma - like bodies (MLBs) in inducing diseases in plants of various types have strengthened the claim for establishing a separate decipline in the domain of plant pathology such as mycoplasmology or mycoplasmatology. The most exciting developments in mycoplasmatology took place during the past 20 years in the field of plant and insect mycoplasmas. For many years MLOs were known by the peculiar and very imprecise name pleuropneumonia like organisms (PPLOs). The first attempt of their systematic nomenclature and classification according to Linneaen principle was made by Sabin (1941). Subsequently the basis for universly addopted nomenclature and classification was laid by Edward and Freundt (1956). Later in 1967 the Sub-committee on the taxonomy of mycoplasmas

recommended that these organisms should be asigned to a separate new class and classified them according to the Linneaen system of classification. Now they are grouped under a new microbial class 'Mollicutes' refering to an outstanding property of this class viz., the absence of a true, rigid cell wall surrounding the triple layered cytoplasmic membrane. The class Mollicutes is further classfied by Tully and Razin (1977) and Razin (1978).

G Classification of MLOs

Class:Mollicutes.

Order - Mycolplasmatales

Family - I : Mycoplasmataceae

Genus. I - Mycoplasma (Number of species-50)

Characters - i) Sterol required for growth.

ii) Sensitive to digitonin.

iii) Genome size : 4.5 x 10^8 daltons

iv) Guanine + cytocine (G + C) ontent of DNA is 23 to 41%.

Genus II - Ureaplasma (Number of species-1)

Characters - i) Sterol required for growth.

ii) Sensitive to digitonin.

iii) Genome size : 4.5×10^8 daltons

iv) Guanine + Cytocine (G + C) content of DNA is 28%.

Family II : Acholeplasmataceae.

Genus I : Acholeplasma (Number of species - 6)

Characters - i) Sterol not required for growth

ii) Resistant to digitonin.

iii) Genome size - 1×10^9 daltons.

iv) Guanine+ Cytocine (G + C) content of DNA is 29 to 35%.

Family III : Spiroplasmataceae

Genus I : Spiroplasma (Number of species - 1)

Characters - i) Sterol required for growth

ii) Genome size - 1×10^9 daltons.

iii) Guanine + Cytocine (G + C) content of DNA is 26%.

Genera of uncertain taxonomic position :

a) Anaeroplasma (Number of species - 2)

Characters - i) Sterol required sometimes.

ii) Genome size not determined.

iii) Guanine + Cytocine (G + C) content of DNA is 29 to 34%.

b) Thermoplasma (Number of species-1)

Characters - i) Sterol not required for some time.

ii) Genome size - 1×10^9 daltons.

iii) Guanine + Cytocine (G + C) content of DNA is 46%.

Mycoplasmas are the smallest free living forms of life which can multiply in a cell free media. Their role as a etiological agent of diseases in plants, animals and man is now well established. They are of considerable economic importance in agriculture, biomedical research and human health (Gupta 1989). Hence it is essential to have a proper diagnosis of mycoplasma infection. It is observed that in many cases the symptoms of viral diseases and mineral deficiencies look similar to those of MLO diseases. Therefore in order to confirm the identity of causal organisms it's detection has become important.

H. Detection techniques of MLOs

Several indirect procedures that are evaluated for the diagnosis of infections and identification of MLOs includes -

1. Light microscopic method (Deeley et al. 1979, Kartha et. al. 1975)
2. Chemotherapeutic remission (Thirumalachar, 1975)
3. Fluorescence microscopy (Namba et al. 1981, Douglas 1986, Dale 1988)
4. Enzyme assay technique (Bionissol and Stoilkovic 1986)
5. Electron microscopy.
6. DNA Probes (Davis et al. 1987) etc.

The protocol for the above methods is as follows :

1. Light microscopy

The technique is based on the principle that, MLOs located in phloem tissue can be made visible under light microscope using different stains.

Stains

Diene's stain
(Widely used)

Composition

2.5 g methylene blue
1.25 g Azure II
10.0 g Maltose
0.25 g sodium carbonate

↓

Dissolve in 100 ml D.W. and filter.

↓

Use the stain in the range of 0.5-1% (v/v)

↓

Stain 10 μ thick sections

↓

Phloem tissue stains blue due to nuclear material and cytoplasm of MLOs

↓

Observe under light microscope.

Feulgen stain
(Not widely used)

Fix the tissue in Helley's fixative.

↓

Dehydrate the tissue with iodized ethanol

↓

Embed dehydrated tissue in paraffin wax.

↓

Cut the sections (10 μ) on microtome.

↓

Stain the sections with 1% aqueous feulgen stain

↓

Phloem stains due to neclear material of MLOs.

↓

Observe under light microscope.

2. Chemotherapeutic remission

It is a simple and basic method of diagnosis of mycoplasma infection. The principle used in the technique is that MLOs are sensitive to tetracycline group of antibiotics.

Select the plant showing symptoms
of MLOs infection at its early stage.

↓

Spray 500 ppm tetracycline

↓

If disease development inhibits

↓

Check the plant tissue for
the presence of bacteria.

↓

If bacterial test is -ve

↓

The infection is due to MLOs.

3. Fluorescence microscopy

The principle used in this method is that, the MLO-DNA produce detectable fluorescence upon staining with fluorescent dyes.

Fix MLOs infected sample in 2% glutaraldehyde + paraformaldehyde containing 0.05 M sodium cacodylate, 0.15 M sucrose and 2 mM $CaCl_2$.

↓

Cut free hand/freez microtome transections.

↓

Treat the sections with DNA bindig flurochrom 4' - 6 - diamidino - 2 - Phenylindole - HCL (1 µg/ml) and then with 0.05% aqueous aniline blue.

↓

Observe under Olympus BHA epifluorescence microscope

↓

Tiny bright green fluorescent organisms in chains or large aggregates in phloem region are MLOs.

4. Enzyme assay technique

The technique is based on detection of MLO specific enzyme from infected tissue.

The MLO specific enzymes are -

i) Adenosine phosphorylase and

ii) Pyrimidine nucleoside phosphorylase

Among these two enzymes adenosine phosphorylase is present in significant amount in MLOs

↓

Based on enzyme detection

MYCOTECT - R - KIT (GIBCO) has been marketed

Mycoplasmal preperation

↓

Adenosine phosphorylase

↓

Converts

6 methylpurine deoxyribose
(6 - MPDR) (Kit)

↓

in two toxic products which

↓ kills

↓

... contd

↓

3 T 6 indicator tissue culture present in kit

↓

subsequent staining with crystal violet shows

↓

weakly stained cells

↓

This indicate presence of MLOs

* Method is 98.8% accurate.

5. Isolation and detection of MLOs

MLO infected tissue (200 g)

↓

Homogenise in 200 ml cold isolation medium@ at low speed at 3 sec. interval in a mixer

↓

Filter through 3 layered muslin cloth

↓

Make the volume 800 ml with isolation medium.

↓

Centrifuge at 1500 g for 8 min (low speed)

↓

Collect supernatant and recentrifuge at 35000 g for 30 min (High speed)

↓

Discard supernatant

Collect pellete

↓

Suspend in suspending medium*

↓

Repeat low and high speed centrifugation as above

↓

Suspend the pelletes in chilled suspending medium

... Contd

Perform discontinuous gradient centrifugation using percoll gradient

MLOs fraction

Collect MLO fraction

Dilute with suspending medium immediately

Centrifuge at 100,000 g for 2 h to remove percoll.

Collect MLOs from percoll surface.

Suspend in phosphate buffer (pH 7.0)

Assay the activity of Adenosine phosphorylase

If more activity

MLOs confirmed.

@ Isolation Medium

0.3 M D-manitol
4mM $\mathcal{L}$- cysteine
30 mM EDTA
0.6% PVP
pH. 7.2

* Suspending Medium

0.3 M D-manitol containing 20 mM MOPS.

Hint : Treat healthy plant tissue as above and use as control.

I. Work done in our laboratory on MLOs.

A great deal of work has been carried out in this department on mycoplasma diseases in plant. grassy shoot disease (GSD) of sugarcane was extensively studied by Dhumal in 1983 in sugarcane cvs. Co 419 and Co 740. The study mainly deals with comparitive physiological aspects in healthy and GSD affected sugarcane which includes effect of GSD infection on mineral metabolism, carbohydrate content, photosynthesis and on enzymes of sucrose metabolism. Recently Patil (1992) has made extensive survey of MLO diseases of plants of Kolhapur and adjoining areas which includes phyllody of Verbena erinoides Lam. var. multifida Raiz. and Pav. (Verbenaceae), Tagetes patula Linn (Asteraceae), Gerbera jamesonii Hook. (Asteraceae), Eragrostis amabilis Wt. (Poaceae), Antirrhinum majus L. (Scrophulariaceae), Phlox drummondii Hook (Polemoniaceae), Verbena hybrid (Verbenaceae); Witches' broom Zizyphus rugosa Lamk. (Rhamnaceae) Leucas Stelligera (Labiatae), Evolvulus alsinoides L. (Convolvulaceae), Datura innoxa Mill. (Solanaceae) Dendrocalamus strictus Nees. (Poaceae), Blumea lacera DC. (Asteraceae), Blumea vightiana DC. (Astoraceae) Diacanthium annulatum stapf (Poaceae), Crotolaria retusa Linn.(Fabaceae), little leaf of

Justicia gendarussa Burm. (Acanthaceae), Solanum melongena L. (Solanaceae), proliferation of Catharanthus roseus (L.) G. Don. = Vinca rosea L. (Apocyanaceae) and white leaf of Cynodon dactylon Pers. (Poaceae). She has studied in detail healthy and MLO infected plants viz. Justicia gendarussa and catharanthus roseus, with reference to anatomy, mineral metabolism, organic constituents, hydrolytic and oxidative enzymes and composition of amino acids, sugars and organic acids.

J. Background and scope of the present investigation:

The abnoxious weed P. hysterophorus L. causes dermatitis and other forms of allergy. The ingredient responsible for allergy is well established as a 'sesquiterpene lactone' (SL) (Rodriguez 1977). The weed also supresses crops and plants around it by virtue of its rapid growth and allelopathic effects. To characterise the allelopathic principle an attempt has been made to extract and purify SL in our laboratory (Patil and Hegde 1988). Apart from this, extensive work has been carried out in this department to study growth pattern under different ecological conditions, biochemistry of photosynthesis particularly by studying rate of carbon fixation, activity of photosynthetic enzymes, photorespiratory metahbolism by studying light stimulated CO_2 evolution and the activity of glycolate oxidase. Efforts were also made to control the weed both by biological and chemical means. Patil (1980) has also observed and reported that the pernicious weed P. hysterophorus exhibit the symptoms of phyllody disease. Literature survey also revealed that the phyllody disease of Parthenium has been reported by many botanists. Phatak et al. (1975) have shown association of MLOs with phyllody of Parthenium. Since the possibility of biological control of Parthenium has

attracted the attention of some workers, Varma et al. (1974) have reported mycoplasmal etiology for this plant and proposed its utility for the biological control. However, the phyllody of Parthenium has not been looked in biochemical point of view. As such study of physiological changes in mycoplasmal diseases of plant is important, as the organism is mainly responsible for the induction of changes in leaf morphology, flowers leading to little leaf and phyllody respectively (Maharaj - Patil and Patil 1989).

In the present investigation therefore, an attempt has been made to study the effect of phyllody disease on the yield of sesquiterpene lactone and its allelopathy. In addition the other parameters such as polyphenols amino acids, chlorophylls, the activity of an enzymes polyphenol oxidase and IAA oxidase and characterisation of sesquiterpene lactone on NMR (Nuclear Magnetic Resonance) and HPLC (High Performance Liquid Chromatography) was thought to be worthwhile and hence attempted.