III. RESULTS AND DISSCUSSION

A. Density of the disease

The density of the phyllody disease was studied by laying the quadrats in densely populated areas of Parthenium hysterophorus L. (Fig. 5) The frequency, abundance and density of P. hysterophorus studied in the area is given in Table - 1. It is clear from the table that the weed is affected more by the phyllody disease as evidenced by 73.33% frequency of the diseased plants as against 93.33% frequency of healthy ones. However, the abundance and density observed for diseased plants was comparitively less than that of healthy plants. Careful examination of the infected plant showed the symptoms of phyllody in which the flowers turned into leafy structure (Fig. 6). The infected plants exhibited excessive branching giving rise to broom like appearence. Generally there are five ray florets situeted peripherally in the inflorescence of P. hysterophorus. Each one of the ray floret is enclasped by two lateral disc florets (Fig. 7). However, in the infected plants both ray and disc florets eventually turn into leafy outgrowths (Fig.8) and finally appears like a small seedlings (Fig. 9). Thus the entire inflorescence has an appearance of a witche's broom and the plant fail to produce any seeds.

Fig. 5 : A field showing healthy and MLO infected plants of P. hysterophorus.

Fig. 5

Table 1 : The frequency, abundance and density of MLO infected and healthy plants of Parthenium hysterophorus L. from densely populated areas.

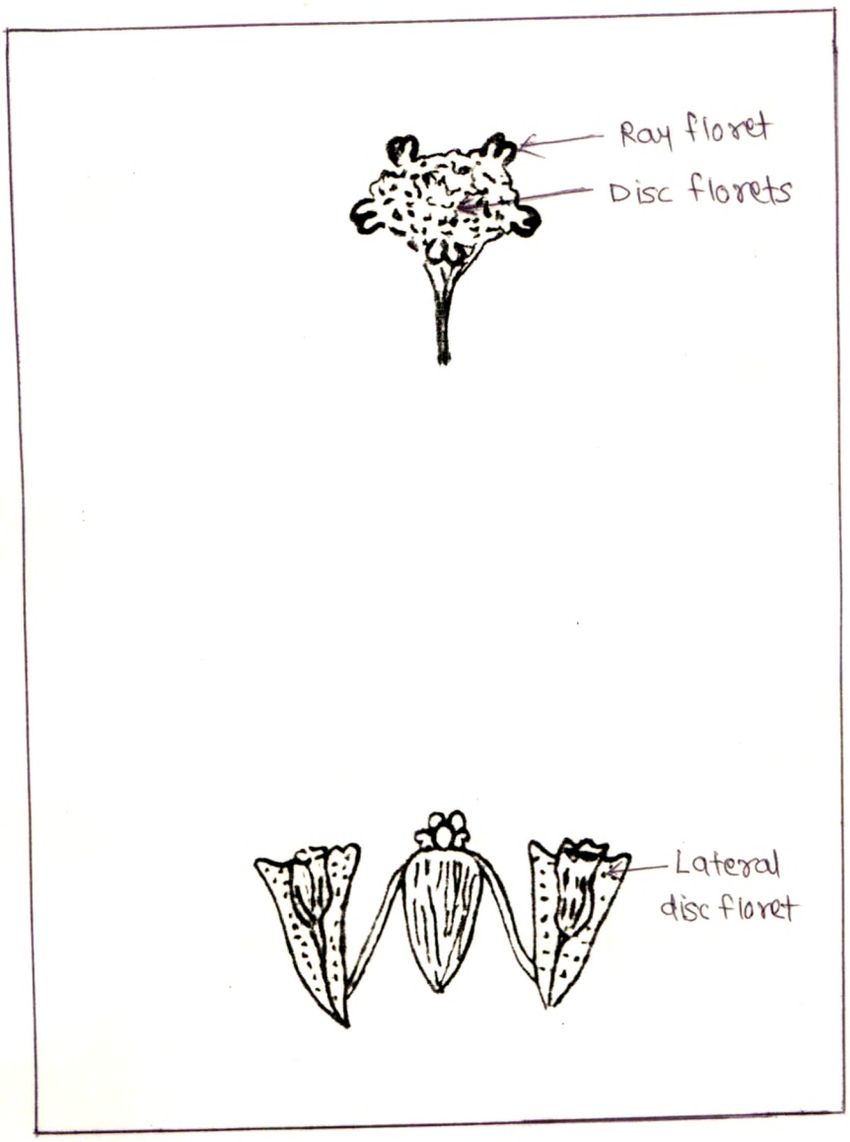
Plant	Frequency (%)	Abundance (%)	Density (%)
Healthy	93.33 ±1.5	32.85 ±1.8	30.66 ±1.1
Infected	73.33 ±2.1	4.09 ±0.5	3.00 ±0.7

± S.D.

Fig. 6 : A twig of healthy and MLO infected plant of P. hysterophorus.

Fig. 6

Fig. 7 : Ray floret (♀) showing attachment of two lateral disc florets (♂) in P. hysterophorus.


Ray floret
Disc florets
Lateral
disc floret

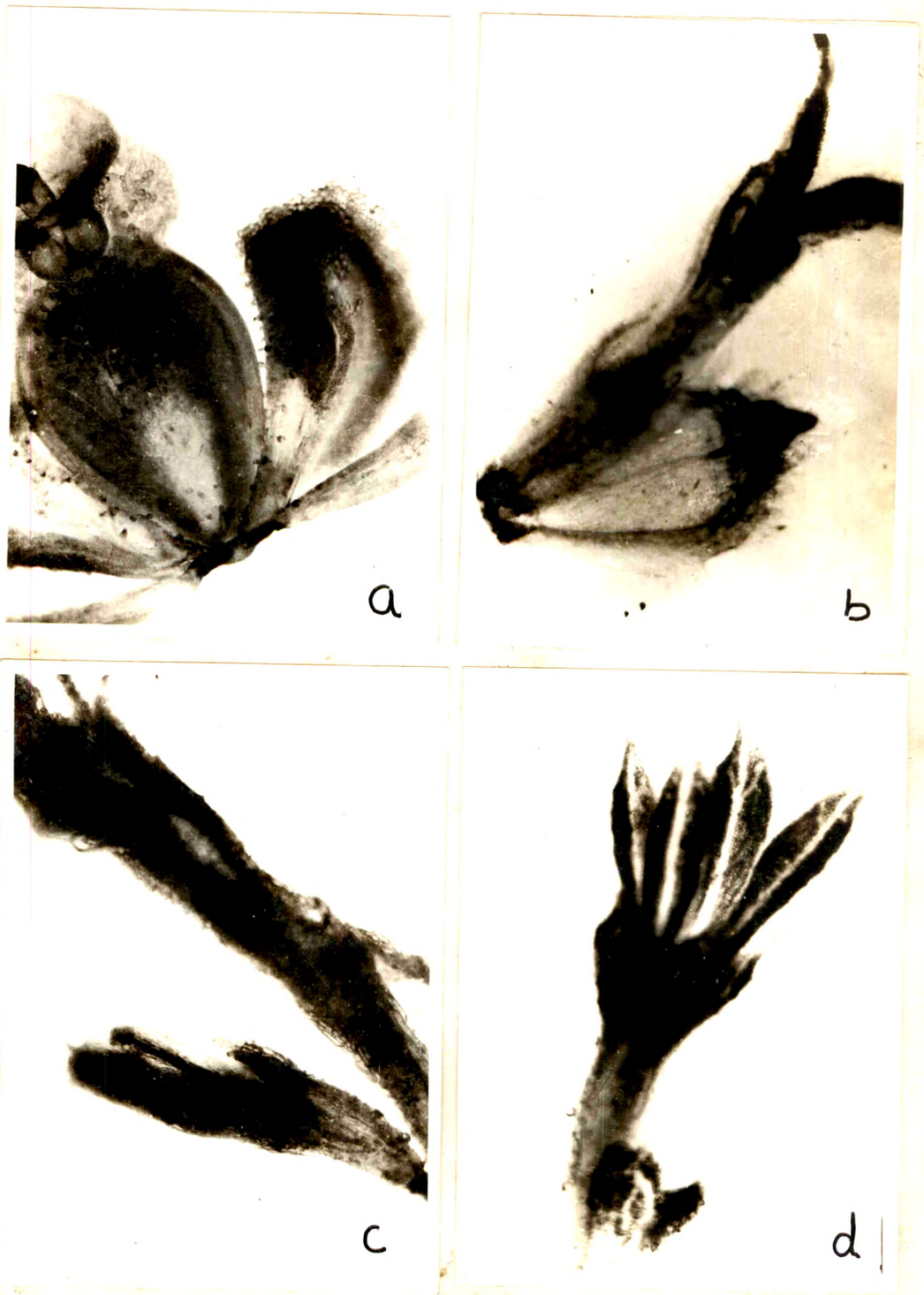


Fig. 8

Fig. 9 : Phyllody of P. hysterophorus showing conversion of inflorescence into a small seedling like structure.

Fig. 9

The phyllody disease of Parthenium hysterophorus was observed long back and reported by several workers (Sahambi 1970, Ghosh and Raychaudhuri 1974, Hegde and Patil 1976, Maharaj - Patil 1992). However, no further detailed study has been carried out to find out the causal organism and biochemical changes induced by the causal organism in the host plant.

B. MLOs : Casual organism of phyllody disease of P. hysterophorus

To study the causal organism of phyllody disease of Parthenium many efforts have been made by the several workers elsewhere. Chavan and Kulkarni (1974) have reported that a fungal Pathogen Physarum cinnereum is responsible to induce phyllody of Parthenium. Later on upon studying the infected plant under electron microscope Verma et al. (1974) and Phatak et al. (1975) have described the presence of mycoplasma like organisms in the sieve tubes of the diseased plant. They have attributed these MLOs as the causal organism of phyllody disease of P. hysterophorus. Microscopic observation of the diseased plants carried out by Hegde and Patil (1976) revealed that the plant showing early symptoms of phyllody were heavily infested by the aphids of Hemiptera group. They

have further reported that these aphids induce the leaf curling symptom upon transferring them to healthy plant. From this observation they have concluded that the phyllody disease may be transmitted by the vector aphid. As such the aphids of Hemiptera group are welknown for their quick fecundity and Aphis fabae is one of them (Sundra Rajulu et al. 1976). Formarly it was believed that the transmission of diseases caused by MLOs in nature is not possible through insect vectors. However, subsequently Singh and Shukla (1965) have indicated the possible involvement of aphids in MLOs transmission particularly in Grassy Shoot Disease(GSD) transmission. The critical and extensive study of several workers has confirmed the involvement of insects in GSD transmission. The mechanism of disease infestation studied by Moreu and Boulay (1967) revealed that the effective transmission of the disease needs reaching the stylet of the insect vector upto the phloem of the healthy plants which results in the establishment of connection between the phloem and the vector for the transmigration of MLOs. The alimentary canal helps in upward sucking of the sap while salivary canal injects the saliva into the phloem and thereby the MLOs are

transmitted and multiplied in the phloem tissue. The transmission of MLOs can also be achived through mechanical means (Edison et al. 1976), through artificial means (Rishi et al. 1973), by graft transmission (Dimock et al. 1971) and Dodder transmission (Jha et al. 1973).

C. MLOs Associated with Parthenium phyllody

Mycoplasma like organisms in phyllody of Parthenium are spherical to pleomorphic bodies of 140nm to 600nm were found in the phloem cells (Phatak et al. 1975). These bodies contained ribosomes and electrotransparent zones transversed by fine filaments and were enclosed by a trilaminar unit membrane. Cytoplasmic granular material was also seen at the periphery. No such bodies were observed in the sections cut from healthy tissues. Since Cousin et al. (1970) have also recorded association of strikingly similar organisms with Sesamum phyllody, hence Parthenium phyllody may be of the same as Sesamum phyllody.

Although the causal organism of phyllody of Parthenium was established by Phatak et al. (1975); the

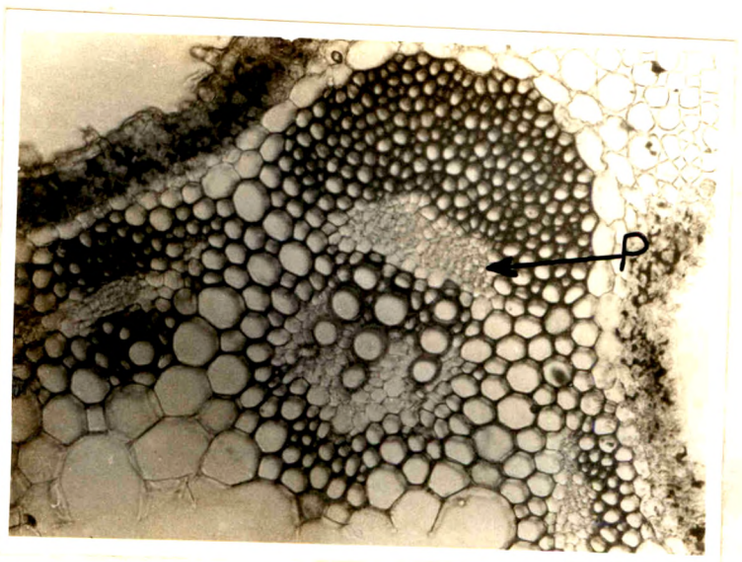
light microscopic histochemical technique was employed in the present investigation to confirm the same. The microphotograph of transverse section of healthy and infected plant tissue of P. hysterophorus stained with 0.2% Diene's stain indicated mycoplasma like organisms in the phloem of infected plants (Fig. 10). It is very clear from the figure that the Diene's stain is specific for MLOs which showed blue staining in the phloem region of the sections cut from infected plant tissue. Whereas the phloem region of the section cut from healthy plant tissue remain unstained. This quick, sure and diagnostic technique of MLO detection has been used by several workers (Deeley et al. 1979, Kondaiah and Nayudu 1981 , Srinivasan 1982) to detect and confirm MLO association with clover phyllody of Vinca rosea and many other MLO infected plants such as sandal, bringal, sesamum and rice respectively.

The symptoms of phyllody of Parthenium were also studied carefully and compared with the symptoms reported by Phatak et al. (1975) in MLO infected plant of P. hysterophorus. The symptoms showed by the infected plants had excessive branching, witche's broom type appearence, leafy inflorescence, stunted growth and

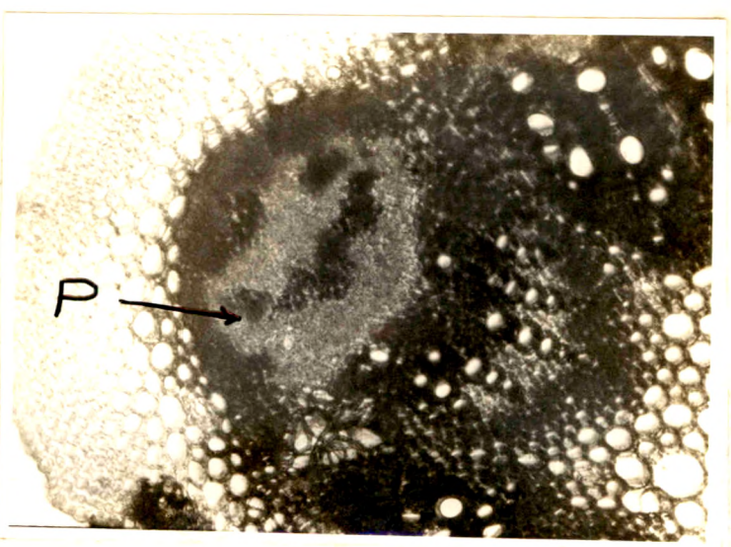
Fig. 10 : Histochemical localization of MLO using Diene's stain in *P. hysterophorus*.

a. T. S. of healthy stem showing absence of MLO bodies in phloem.

b. T. S. of infected stem showing presence of MLO bodies in phloem.



a



b

Fig. 10

typical phyllody nature. All these symptoms tallies very well with those reported by Phatak et al. (1975). From this it is concluded that the phyllody of Parthenium studied in present investigation is undoubtbly caused by MLOs.

D. Organic constituents

1. Chlorophylls :

Chlorophylls are the master molecules which harness solar energy and also are the energy trapping pigments in the process of photosynthesis. Hence the effect of MLOs induced phyllody of Parthenium on chlorophyll content was studied and the data is recorded in Table-2. It is vividly clear from the table that the infected plant contained less amount of chlorophylls than the healthy one. However, chlorophyll a/b ratio was found more in infected tissue. As high as 69.15% reduction was noticed in chlorophyll 'b' while 64.53% in chlorophyll 'a'. The total chlorophyll content was also reduced by 66.97% in infected plant tissue. The increase in chlorophyll a/b ratio is quite obvious and can be attributed to reduced level of chlorophyll 'b' in inflected plant tissue.

Table 2 : Chlorophyll content analysed from the leaves of healthy and MLO infected plant of Parthenium hysterophorus L.

Plant material	CHLOROPHYLL (mg 100^{-1}g fresh tissue)			
	Chl.a	Chl.b	Chl a/b ratio	Total chlorophyll
Healthy	93.4 ± 2.1	91.8 ±0.5	1.01	186.0 ± 3.5
Infected	33.13 ± 1.6	28.32 ± 0.8	1.16	61.43 ± 1.02

± S.D.

There are several reports which have indicated remarkable decrease in total chlorophyll content and especially chlorophyll 'b' concentration was adversly affected due to MLO infection (Waseem et al. 1979, Johri and Padhi 1981, Dhumal 1983, Maharaj-Patil and Patil 1989, 1992). According to Mitra and Sengupta (1980) low chlorophyll content in brinjal infected by MLOs was due to the lowered rate of synthesis and accelerated breakdown of chlorophyll. In the present investigation we have also observed reduction in chlorophyll content due to MLO infection. This is possibly because of disruption of photosynthetic apparatus and loss of chlorophyll's photosypthetic efficiency and perhaps they become increasingly dependant on reserve food material and dark respiration for energy and reducing power. This lowers the growth potential of infected plants. The reason why the MLOs infected Parthenium plant exhibit stunted growth. The available reports on the effects of MLO infection on chlorophyll content indicate similar pattern (Carroll and Kosuge 1969), Chen and Chen (1974) and Chen and Kong (1976) in sugarcane affectred by white leaf disease due to MLO, Parthasarathi et al. (1976) in sandal suffering from spike disease, Carling and Milliken (1977) in Vinca rosea affected by MLO, Purohit et al (1978) in Tephrosia

purpurea affected by witches' broom disease have reported quantitative reduction in total chlorophylls.

a) Photo-oxidative degradation of chlorophylls

The stability of chlorophyll concentration in healthy and MLO infected Plant studied after every 24 h over a period of 5 days by keeping the acetone extract of chlorophyll in diffused light at room temperature is given in Table-3. Similarly values of chlorophyll a/b ratio and per cent reduction in chlorophyll level of healthy and MLO infected plant after every 24 h over a period of 5 days are depected in Table-4 and 5 respectively. It is clear from the table that the rate of reduction of chlorophyll was gradually increased with increase in exposure time of chlorophyll extract to diffused light at room temperature. This reduction, in chlorophyll 'a' content was comparitively more in infected plant. However, the reduction rate of chlorophull 'b' content after 24 h of exposure to diffused light was comparitively less in inflected plant than that of healthy one. Thereafter this reduction rate was almost the same with increase in exposure time. No significant change was noticed in the per cent rate of photooxidative reduction of total chlorophyll in healthy

Table 3 : Photo-oxidative degradation of Chlorophyll* content upon exposing acetone extract of chlorophyll to diffused light (5 μE m^{-2} s^{-1}) at room temperature (30°C ±2).

Parameters	Days of incubation				
	1st	2nd	3rd	4th	5th
HEALTHY					
Chl a	93.4 ±1.1	63.17 ±1.0	25.14 ±1.0	0.16 ± 0.01	-
Chl b	91.8 ±0.4	62.2 ±0.2	29.08 ±0.9	0.19 ±0.01	
Chl a + b	186.0 ±3.1	125.2 ±1.8	54.23 ±1.3	0.35 ±0.05	-
INFECTED					
Chl a	33.13 ±0.7	18.4 ±0.51	8.81 ±0.2	0.065 ±0.0	-
Chl b	28.32 ±0.7	23.8 ±0.35	8.85 ±0.2	0.068 ±0.01	-
Chl a + b	61.43 ±1.2	42.31 ±1.2	17.5 ±1.0	0.13 ±0.0	-

* Values are in mg 100^{-1} g fresh tissue.

\- Not detected, ± S.D.

Table 4 : Effect of photo-oxidative degradation on Chl a/b ratio upon exposing acetone extract of chlorophyll to diffused light (5 μE m^{-2} s^{-1}) at room temperature (30°C ±2).

Parameter	Days of incubation									
	1st		2nd		3rd		4th		5th	
	H	I	H	I	H	I	H	I	H	I
Chlorophyll a/b ratio	1.01	1.16	1.01	0.77	0.86	0.99	0.83	0.95	-	-

H : Healthy

I : Infected

Table 5 : Per cent decrease in chlorophyll content upon exposing the acetone extract to diffused light (5 μ E m^{-2} s^{-1}) at room temperature (30°C±2).

Parameters	Hours of incubation			
	(24)	(48)	(72)	(96)
	(% decrease in chlorophyll content)			
HEALTHY				
Chl a	32.1	72.0	99.8	T.D.
Chl b	32.24	68.32	99.79	T.D.
Chl a + b	32.68	70.84	99.8	T.D.
Chl. a/b	00.0	14.85	17.82	T.D.
INFECTED				
Chl a	44.4	73.4	99.8	T.D.
Chl b	15.96	68.75	99.75	T.D.
Chl. a + b	31.12	71.5	99.78	T.D.
Chl a/b	33.62	14.65	18.1	T.D.

T.D. = Total destruction of chlorophyll

as well as in infected plant. Similarly chlorophyll a/b ratio was not changed in healthy plant after 24 h exposure to diffused light while in infected plant it was reduced by 33.62% (Table - 5). The complete photooxidative reduction of chlorophyll was observed after 96 h exposure to diffused light in both the cases.

This study led us to surmise that although chlorophyll stability found to be decreased in both the cases after 96 h exposure to diffused light at room temperature, the infection hastens the reduction comparitively at faster rate. According to Fletcher and McCullagh (1971) cytokinins stimulate chlorophyll reduction and the phenomenan is normally used to bioassay the cytokinins. The present study clearly indicated the reduction in the chlorophyll content due to MLO infection. From this observation it may be concluded that the cytokinin level in MLO infected plant gets hampered and perhaps this imbalance in the cytokinin level can bring about abnormalities in the growth of the plant. As such the MLO infected plant of Parthenium had brush like appearence and above all they exhibit stunted growth and considerable reduction in leaf size.

2. Starch test

In order to test the photosynthetic capability of MLO infected plant, the leafy part was tested for starch content using iodine solution. The results are shown in Fig. 11. It is very clear from the figure that the leafy part able to synthesize the starch as evidenced by the development of bluish colour. However, the persistance of the colour is not so prominent as compared with the starch test of healthy leaf of P. hysterophorus. From this observation we can say that the MLO infection reduce the starch forming ability of the plant by affecting the function of leat chloroplast. However, this conclusion can only be strengthened by studying photosystem I and II and the structure of chloroplast of MLO infected plant leaf under electron microscope. So also the rate of carbon assimilation in the isolated chloroplast of inflected plant.

3. Polyphenols

The role of polyphenols in disease resistance has been extensively worked out and is become a field of active research for many years (Sharma et al. 1979, Salem and Mitchail 1981). In the present investigation

Fig. 11 : Phyllody of Parthenium showing +ve test for starch content.

1. Untreated with iodine.

2. Treated with iodine.

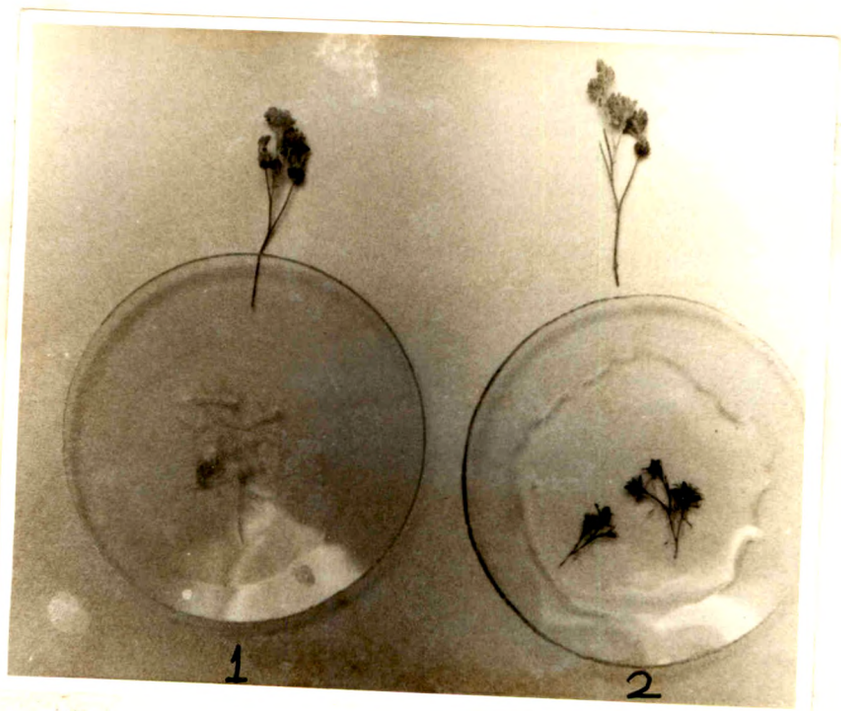


Fig. 11

the polyphenol content from the dried powder of entire plant of healthy and MLO infected P. hysterophorus was analysed and incorporated in Table-6. It is clear from the table that MLO infected plant contain bit higher concentration of polyphenols. The increase in polyphenol level due to infection was 31.22% as compared with healthy one.

Several workers have supported the view of increase in polyphenols due to MLO infection. To name few of them are Parthasarathi et al. (1970) in sandal affected by sandal spike disease caused due to MLO Purohit et al. (1979), Prasad and Sahambi (1980), Arya et al. (1981) in Sesamum affected by Sesamum phyllody. However, conflicting observations have also been reported pertaining to the effects of MLO infection on polyphenol content by Mitra and Majumdar (1977), Srinivasan (1983) and Maharaj Patil and Patil (1989) in brinjal, Areca palms and Vinca rosea respectively.

Several reasons have been ascribed to explain accumulation of polyphenols in diseased plants. Among them, accelerated synthesis of phendols via schikimic acid pathway by utilizing carbohydrates or amino acids

Table 6 : Polyphenol content analysed from healthy and MLO infected plant of *Parthenium hysterophorus* L.

Plant material	Polyphenols (g 100^{-1} g dry tissue)
Healthy	6.15 ± 0.75
Infected	8.07 ± 0.23

± S.D.

(Pridham 1965), increased content of copper and zinc (Sasikumaran et al. 1979) are worth mentioning. All these above mentioned factors may either individually or collectively contribute for greater synthesis of polyphenols in MLO infected Parthenium hysherophorus. However, it is too early to conclude that the increase in polyphenol content in MLO inflected P. hysterophorus contribute for disease resistance, unless studying the Schikimic acid pathway in both healthy and MLO infected P. hysterophorus using radio tracer technique.

E. Oxidative Enzymes

1. Polyphenol oxidase

The phenol oxidase system plays an important role in respiration by transferring electrons from respiratory substrates to other hydrogen or electron acceptors. Quinone is the oxidation product of phenol which may be reduced to their original phenol-form by respiratory carriers. The increase in polyphenol oxidase activity in diseased plant is generally accompanied by the increased concentration of phenolic substances. Hence the activity of polyphenol oxidase was scored in healthy and MLO infected plant of P. hysterophorus. The

data is given in the Table-7 and also the activity of polyphenol oxidase is represented graphically in Fig. 12. It is clear from the table that the MLO infected P. hysterophorus exhibited about more than four fold activity as compared to healthy one when expressed as Δ OD min^{-1} mg^{-1} chl. However, when expressed on the basis of fresh weight no significant difference was noticed. The cumulative activity of polyphenol oxidase represented graphically in figure indicated increase in the polyphenol oxidase activity for the first sixty seconds and thereafter slowly declined. The trend exhibited by the cumulative activity of the Enzyme is more or less same as compared with the noncumulative activity shown in Table-7. However, the increased activity of polyphenol oxidase on chlorophyll basis can be attributed to the reduction in total chlorophyll content in general and chlorophyll 'b' in particular due to MLO infection.

There are several reports which have indicated favourable as well as adverse effects of MLO infection on the activity of polyphenol oxidase. In sandal affected by spike (Parthasarathi 1977) and in brinjal affected by little leaf (Mitra and Majumdar 1977) have higher levels of Polyphenol oxidase. On the other hand,

Table 7 : Polyphenol oxidase activity in healthy and MLO infected plant of *Parthenium hysterophorus* L.

Plant material	Polyphenol oxidase	
	$\triangle$ OD min^{-1} g^{-1} fresh tissue	$\triangle$ OD min^{-1} mg^{-1} chl.
Healthy	0.45 ± 0.02	4.03 ± 0.02
Infected	0.40 ± 0.01	18.9 ± 0.57

± S.D.

Fig. 12 : Polyphenol oxidase activity in healthy and MLO infected plant leaves of P. hysterophorus.

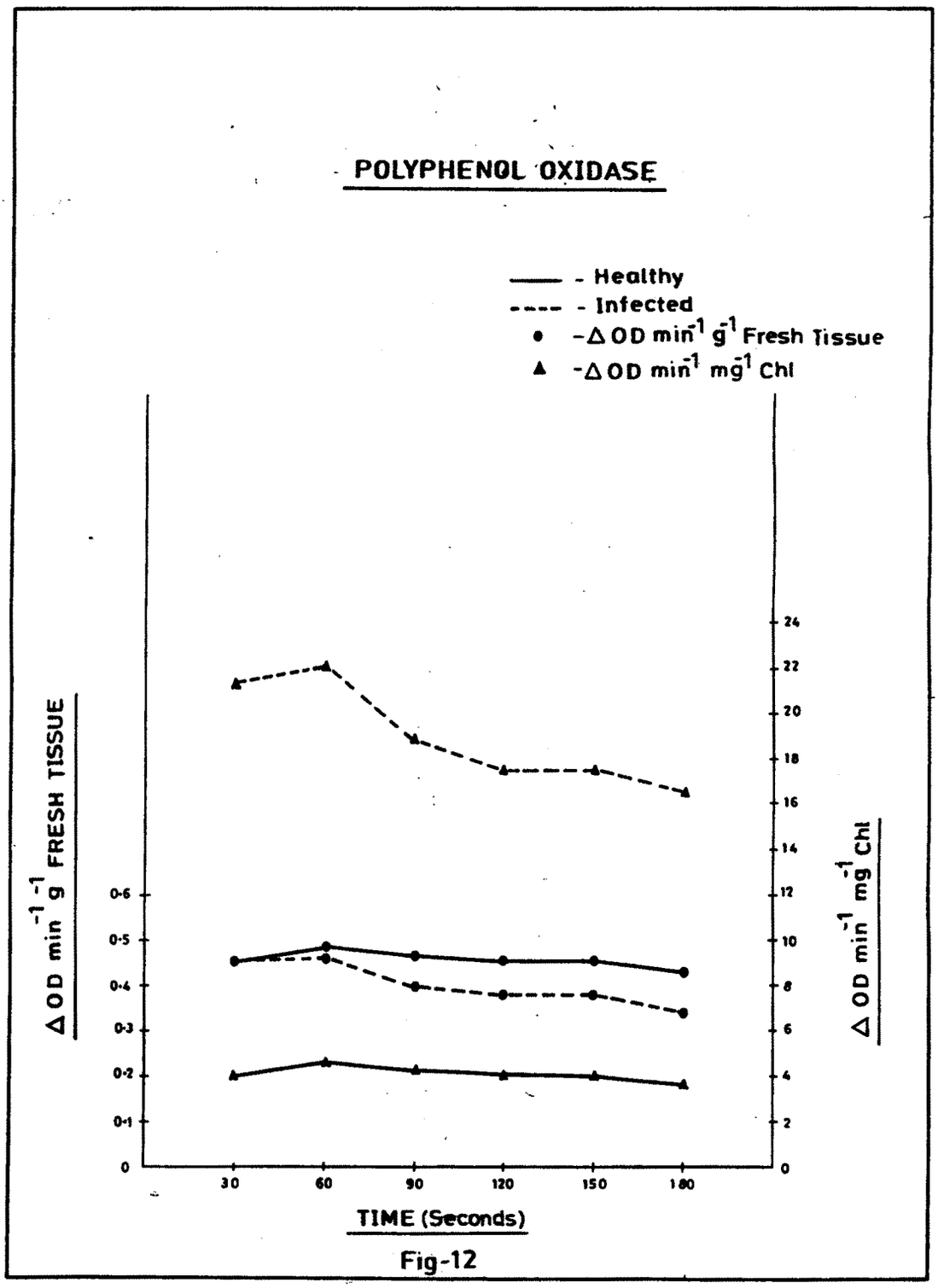

POLYPHENOL OXIDASE
- Healthy
- Infected
-ΔOD min-1 g-1 Fresh Tissue
-ΔOD min-1 mg-1 Chl
ΔOD min-1 g-1 FRESH TISSUE
ΔOD min-1 mg-1 Chl
0
0·1
0·2
0·3
0·4
0·5
0·6
2
4
6
8
10
12
14
16
18
20
22
24
30
60
90
120
150
180
TIME (Seconds)

Fig-12

Mitra et al. (1976) in brinjal affected by little leaf disease, Purohit et al. (1979) in Sesamum affected by Sesamum phyllody and Maharaj Patil and Patil(1989) in Vinca rosea affected by MLO have noted decreased activity of polyphenol oxidase. According to parthasarathi et al. (1975) though the increased polyphenol oxidase activity has a beneficial effect from the point of view of disease resistance, it may have adverse effect on IAA oxidase activity resulting in hyperauxinity. Thus in the present investigation the morphological changes such as stunted growth, profuse tillering, premature sprouting of buds, transformation of reproductive parts into vegetative parts observed in MLO infected P. hysterophorus possibly be due to hyperauxinity.

2. IAA oxidase

The endogenous IAA level is controlled by an enzyme IAA oxidase which is involved in plant growth (Waldrum and Davies 1981) and moreover IAA concentration in the host is inversly correlated with IAA oxidase activity. The results depicted in Table-8 clearly indicate that the MLO infection inhibits the activity of IAA oxidase.

Table 8 : IAA oxidase activity in healthy and MLO infected plant of *Parthenium hysterophorus* L.

Plant material	IAA oxidase ($\triangle$OD h^{-1} g^{-1} fresh tissue)
Healthy	1.14 ± 0.03
Infected	0.15 ± 0.01

± S.D.

The investigations of Daniels (1979), Davey et al. (1981) and Dhumal (1983) have indicated that MLO infection upsets the hormonal balance of the infected plant which causes various types of growth abnormalities. Arya (1982) while reviewing the effects of plant pathogens such as fungi, bacteria, viruses, MLOs, insects and mites has mentioned induction of abnormal growth in plants by these pathogens.

Several reasons have been attributed to the lowered activity of IAA oxidase in diseased plants. These includes increased polyphenol content and enhanced activity of polyphenol content and enhanced activity of polyphenol oxidase (Zenk and Muller 1963, Parthasarathi et al. 1970, 1975, Ramawat et al. 1980, Lee et al. 1982), while Siegel and Galston (1953), Morgan et al.(1966), Vidhyasekaran and Durairaj (1973) are of the opinion that decreased activity of catalase and low concentration of manganese may be responsible for the inhibition of IAA oxidase activity. On the other hand increased IAA oxidase activity has been ascribed to low phenolic content and stimulation of catalase activity (Ray 1958 , Kosuge 1969). Polyphenols like caffeic acid, chlorogenic acid (Mehrotra 1980) also inhibit the

activity of IAA oxidase. In the present investigation, the low activity of IAA oxidase may be due to stimulated activity of polyphenol oxidase, increase in total phenolics and the phenolic compounds such as caffeic acid possibly resulting in hyperauxinity causing morphological abnormalities in MLO infected P. hysterophorus plant.

F. Chromatography of polyphenols

After having an idea of total phenolics and activity of oxidative enzymes viz. polyphenol oxidase and IAA oxidase, healthy and MLO infected P. hysterophorus plants were also investigated for detection of phenolic compounds by employing unidimensional paper chromatography. The probable identity of different phenolic compounds was established by comparing the fluorescence under UV light, UV + NH3, fumes, 1 : 1 mixture of 0.3% $FeCl_3$ and 0.3% K_3 $Fe(CN)_6$ and corresponding Rf values of authentic standards. The probable phenolic compounds identified in healthy and MLO infected P. hysterophorus plants were enlisted in Table-9. The concentration of individual phenolic compound per spot is also given in Table-10. The

Table 9 : Detection of polyphenal content from healthy and MLO infected plant of *Parthenium hysterophorus* L.

Spot number		Colour under	Colour under	0.3% $FeCl_3$ +	Rf x 100	Probable
13	-	Yellowish green	Yellowish green	Faint blue	95.0	Kaempferol + Ellagic acid
-	14	Yellowish green	Yellowish green	Faint blue	97.0	Ferulic acid

Table 10 : Quantification of phenolic compounds separated on chromatogram from healthy and MLO infected plant extract of Parthenium hysterophorus.

Name of the compound	Polyphenol concentration ($\mu g\ spot^{-1}$)	
	Healthy	Infected
1. Proanthocyanodins	16.5	19.0
2. Flavan	14.5	14.0
3. Flavonoids	16.5	88.0
4. D-catechin	-	42.0
5. Quercetin	14.0	45.0
6. Myricetin	-	40.00
7. Quercetin derivatives	17.0	-
8. Quercetin derivatives	-	17.0
9. Tannic acid	27.5	-
10. Tannic acid + Gallic acid	-	56.00
11. Catechol + Quinic acid	9.5	45.0
12. Caffeic acid	-	46.0
13. Kaempferol + Ellagic acid	Tarace	-
14. Ferulic acid	-	61.00

phenolic compounds from the plant extract and the authentic standards of phenolic compounds separated on chromatographic paper are given in Fig. 13 and Figs. 14 a,b,c and d respectively. Similarly, tracing of all the authentic standards of phenolic compounds separated on chromatographic paper is represented in Fig.15. It is vividly clear from the table that MLO infected plant has got 11 different phenolic compounds, while healthy has 8 compounds, which clearly indicated that infected plant has more phenolic compounds. The phenolic compounds identified includes proanthocyanodins, flavan, flavonoids, D-catechin, quercetin, myricetin, quercetin derivatives, tannic acid, gallic acid, catechol, quinic acid, caffeic acid, kaempferol, ellagic acid, ferulic acid. Out of these phenolic compounds quercetin derivatives, tannic acid and kaempferol + ellagic acid were found missing in MLO inflected plant while D-catechin, myricetin, gallic + tannic acid, caffeic acid, ferulic acid and few derivatives of quercetin were not appeared in healthy plant. The concentration of phenolic compounds studied in healthy and MLO infected plant revealed that MLO infected plants contain more amount of polyphenols as compared to healthy one. The dominant

Fig. 13 : Chromatogram showing separation of phenotic compounds of healthy and MLO infected P. hysterophorus.

Band No.	Healthy	Infected
1.	Proanthocycanodins	Proanthocyanodins
2.	Flavan	Flavan
3.	Flavonids	Flavonids
4.	-	D-catechin
5.	Quercetin	Quercetin
6.	-	Myricetin
7.	Querectin derivatives	-
8.	-	Quercetin derivatives
9.	Tannic acid	-
10.	-	Tannmic acid + Gallic acid
11.	Catechol + Quinic acid	Catechol + Quinic acid
12.	-	Caffeic acid
13.	Kaempfeol + Ellagic acid	-
14.	-	Ferulic acid

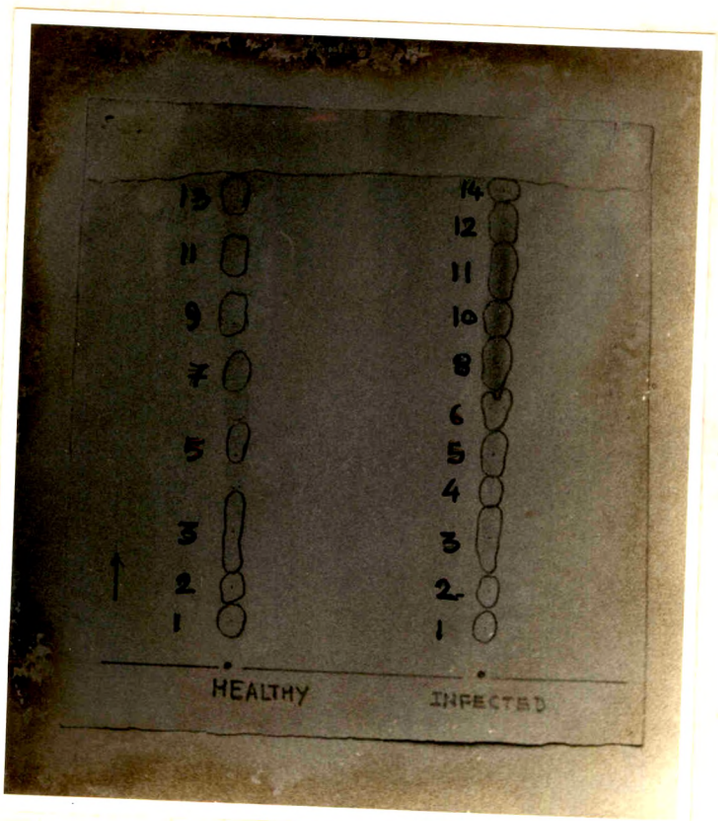


Fig. 13

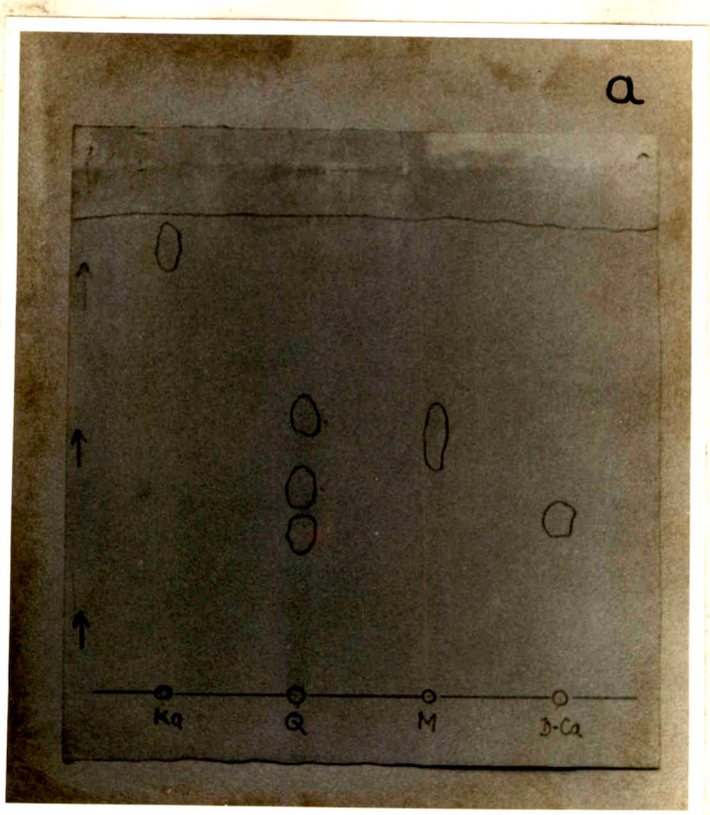
Fig. 14 : Chromatography of standard phenolic compounds

a. Ka -Kaempferol
Q - Quercetin
M - Myricetin
D-ca - D-catechin

b. Ta - Tannic acid
Ga - Gallic acid

c. Cou - Coumaric acid
Ella - Ellagic acid

d. F - Ferulic acid
Ca - Caffeic acid



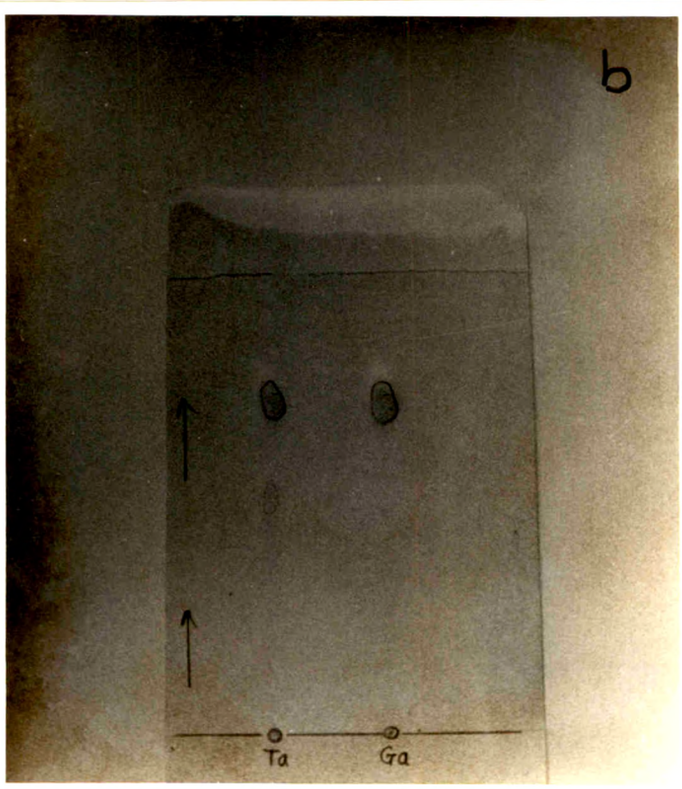
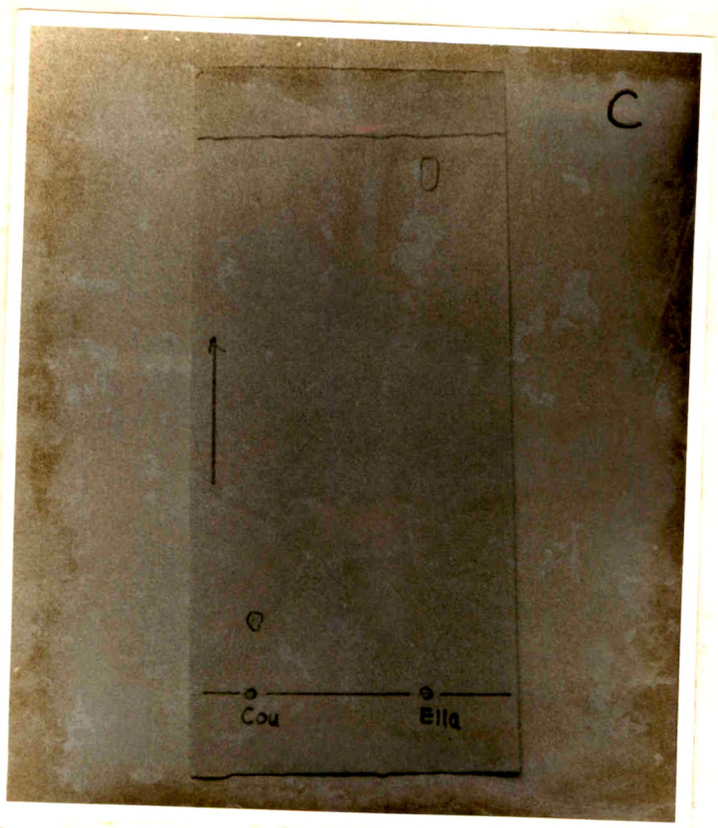


Fig. 14



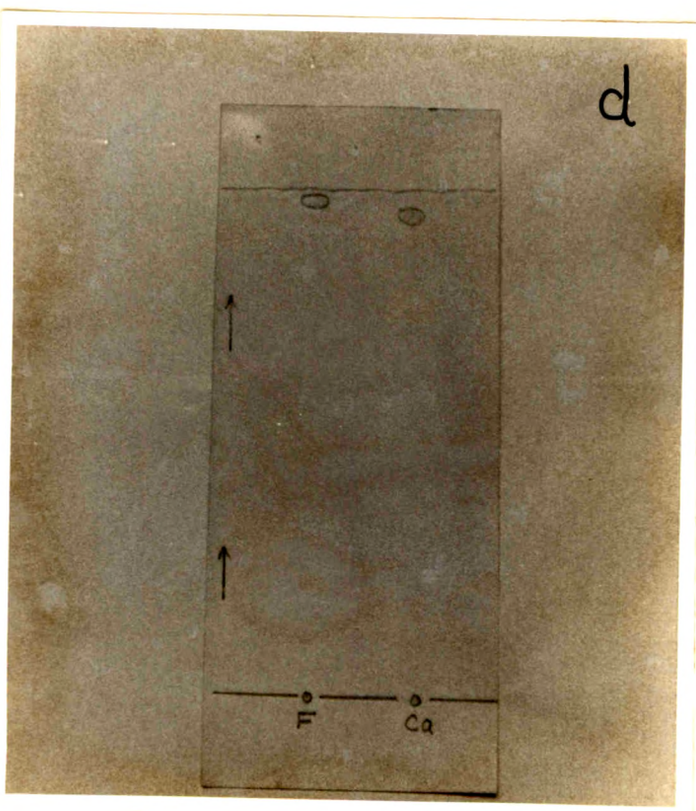


Fig. 14

Fig. 15 : Tracing of individual standard phenolic compounds separated on chromatographic paper.

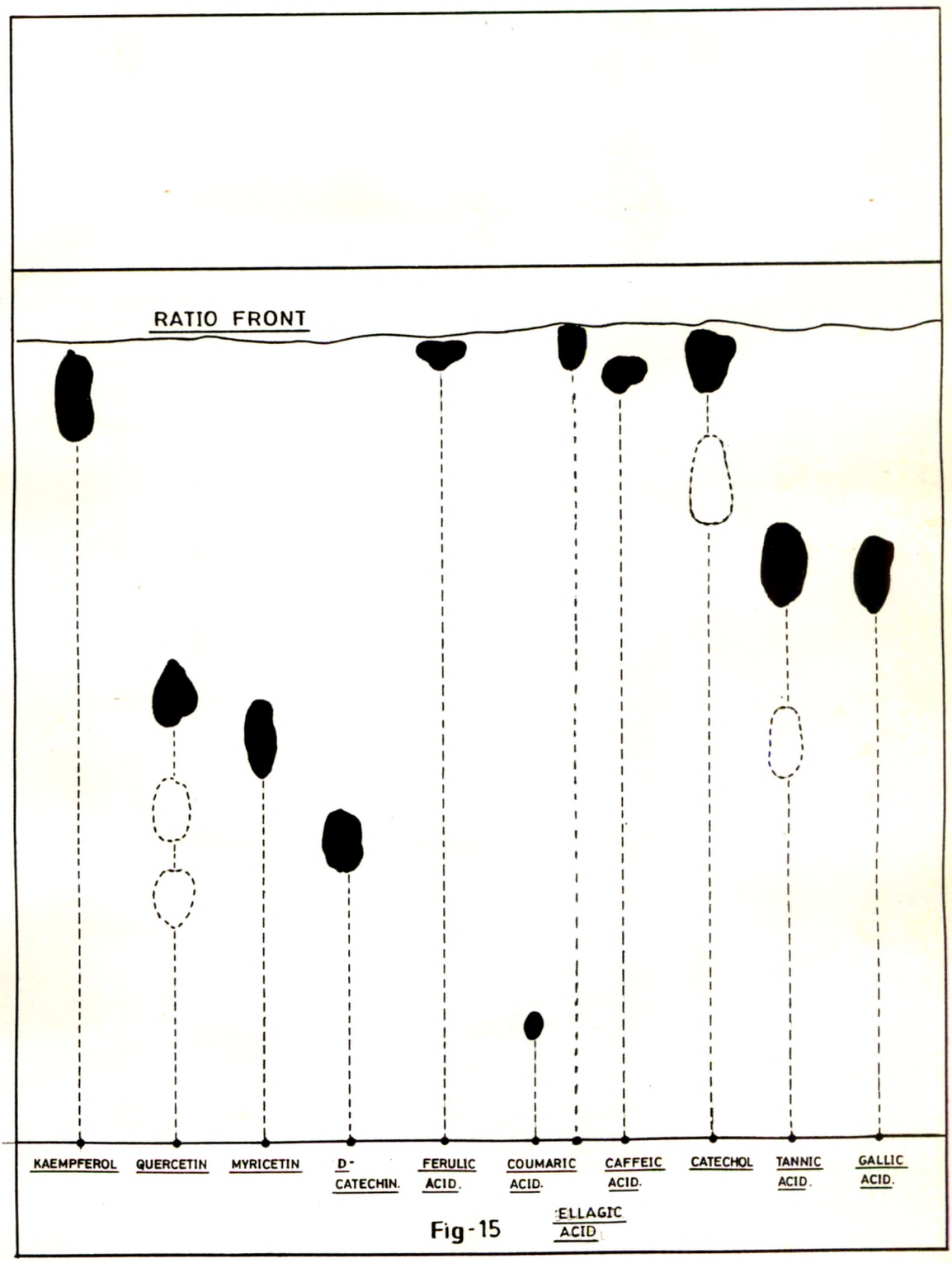

RATIO FRONT
KAEMPFEROL
QUERCETIN
MYRICETIN
D-CATECHIN.
FERULIC ACID.
COUMARIC ACID.
ELLAGIC ACID
CAFFEIC ACID.
CATECHOL
TANNIC ACID.
GALLIC ACID.

Fig-15

among them were flavonoids, tannic acid, caffeic acid, ferulic acid, quercetin, myricetin, catechol and quinic acid. The presence of caffeic acid in MLO infected plant is noteworthy because it inhibits IAA oxidase activity (Table-8). As such this type of inhibition has been reported by Mehrotra (1980) which supports our findings. Mears (1980) while studying flavonoids of Parthenium has reported kaempferol and quercetin. Venkataramaiah and Rao (1984) have reported presence of caffeic acid in mature and young leaves of healthy P. hysterophorus. It is interesting to note here that the caffeic acid which was reported by Venkataramaiah and Rao (1984) in healthy P. hysterophorus was found to be accumulated more in MLO infected P. hysterophorus also.

G. Amino acids

The green plants are the only organisms, harvest solar energy and convert it into chemical energy in the form of sugars. This mechanism in the broader sense is photosynthesis. In plants, photosynthesis and respiration are the major metabolic processes. These metabolic processes may get hampered due to biotic and abiotic stress. Disease stress or biotic stress not only

disturb the above processes due to their consumption of some metabolites or due to their presence but they may produce enzymes and toxins which interferes the metabolic activities.

Carbohydrates are the products of photosynthesis, largely absorbed by the pathogens as an energy source and utilize for the synthesis of some macromolecules and structural components required for growth of pathogens. The pathogens may require some amino acids of host for the synthesis of their own building blocks of protein. Thus the demand of these amino acids by the pathogen fulfilled only during photosynthetic process. Such derangement in photosynthesis can be visualized by studying the status of some simple metabolites like amino acids by employing paper chromatographic technique.

The amino acid composition studied from the dried sample of healthy and MLO infected P.hysterophorus plant is given in Table - 11. To support the table are the figures of amino acid composition of plant samples (Fig.16) and standard amino acids (Fig. 17, a, b & c and Fig. 18). As high as 14 different amino acids were visualized from both healthy and infected plant extract

Table 11 : Detection of amino acids from healthy and MLO infected plant of Parthenium hysterophorus.

Band No. on chromatogram	Probable identification	Concentration	
		Healthy	Infected
1.	Unidentified	+	+
2.	Cysteine monohydrochloride		+
3.	Cysteine		+
4.	Histidine monohydrochloride	Trace	++
5.	Serine + Glutamic acid	+++	++++
6.	Aspartate	+++	++++
7.	Alanine	+++	++
8.	Tyrosine	trace	++
9.	Amino butyric acid	+	+
10.	Tryptophan	+	++
11.	Methionine	+	
12.	Phenylalanine + Valine	+	+
13.	Leucine	trace	+
14.	Isoleucine	Missing	+

Fig. 16 : Chromatographic separation of free amino acids from healthy and MLO infected P. hysterophorus.

Band No.	Healthy (H)	Infected (I)
1.	Unidentified (+)	Unidentified (+)
2	Cysteine monohydrochloride	Cysteine monohydrochloride (+)
3.	Cysteine	Cysteine (+)
4.	Histidine monohydrochloride (Trace)	Histidine monohydrochloride(++)
5.	Serine + Glutanic acid (+++)	Serine + Gultanic acide (++++)
6.	Asparate (+++)	Asparate (++++)
7.	Alanine (+++)	Alanine (++)
8.	Tyrosine (Trace)	Tyrosine (++)
9.	Amino butyric acid (+)	Amino butyric acid (+)
10.	Tryptophan (+)	Tryptophan (++)
11.	Methinonine (+)	Methionine (+)
12.	Phenylalanine + valine (+)	Phenylalanine + valine (+)
13.	Leucine (Trace)	Leucine (+)
14.	-	Isoleucine (+)

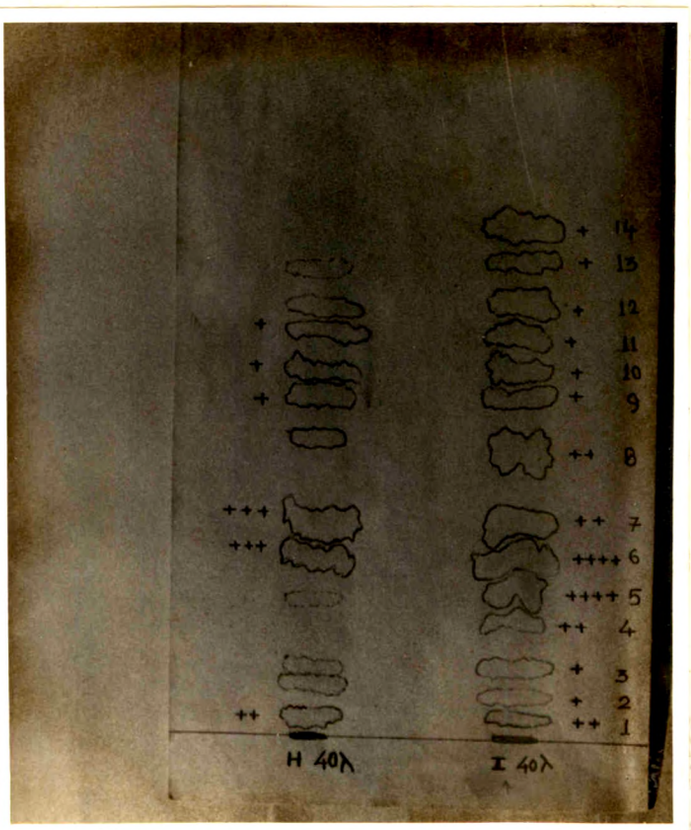


Fig. 16

Fig. 17 : Chromatography of standard amino acids.

a. 1 Serine
2 Hydroxyproline
3 Ornithine monohydrochloride
4 DL-DOPA
5 Tyrosine
6 Tryptophan
7 Threonine

b. 1 Histidine monohydrochloride
2 Amino butyric acid
3 Arginine monohydrochloride
4 Lysine monohydrochloride
5 Valine
6 Alanine
7 Glycine

c. 1 DL-nor Leucine
2 Cysteine
3 Cysteine monohydrochloride
4 Aspartic acid
5 Methionine
6 Isoleucine
7 Glutamic acid
8 Phenylalanine

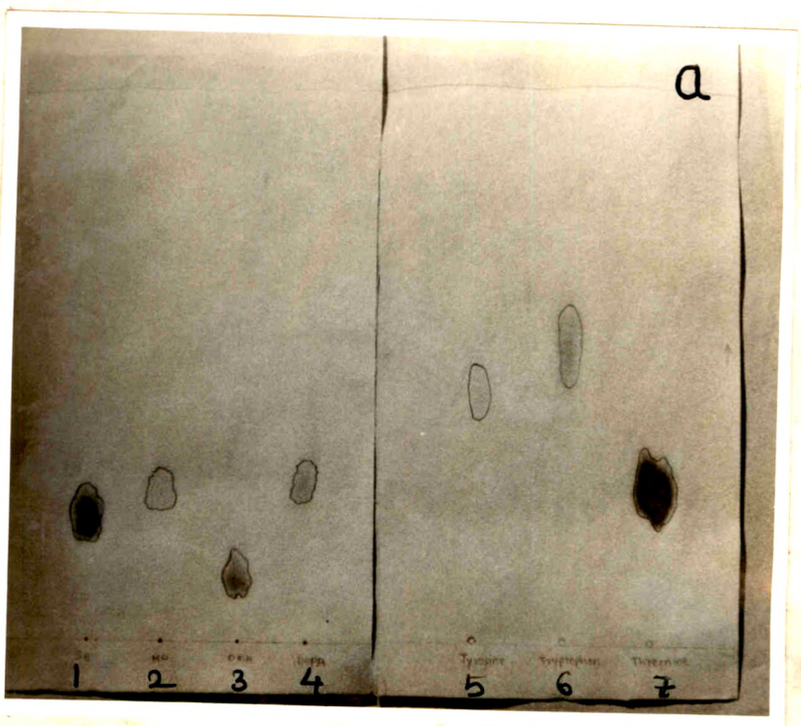


Fig. 17

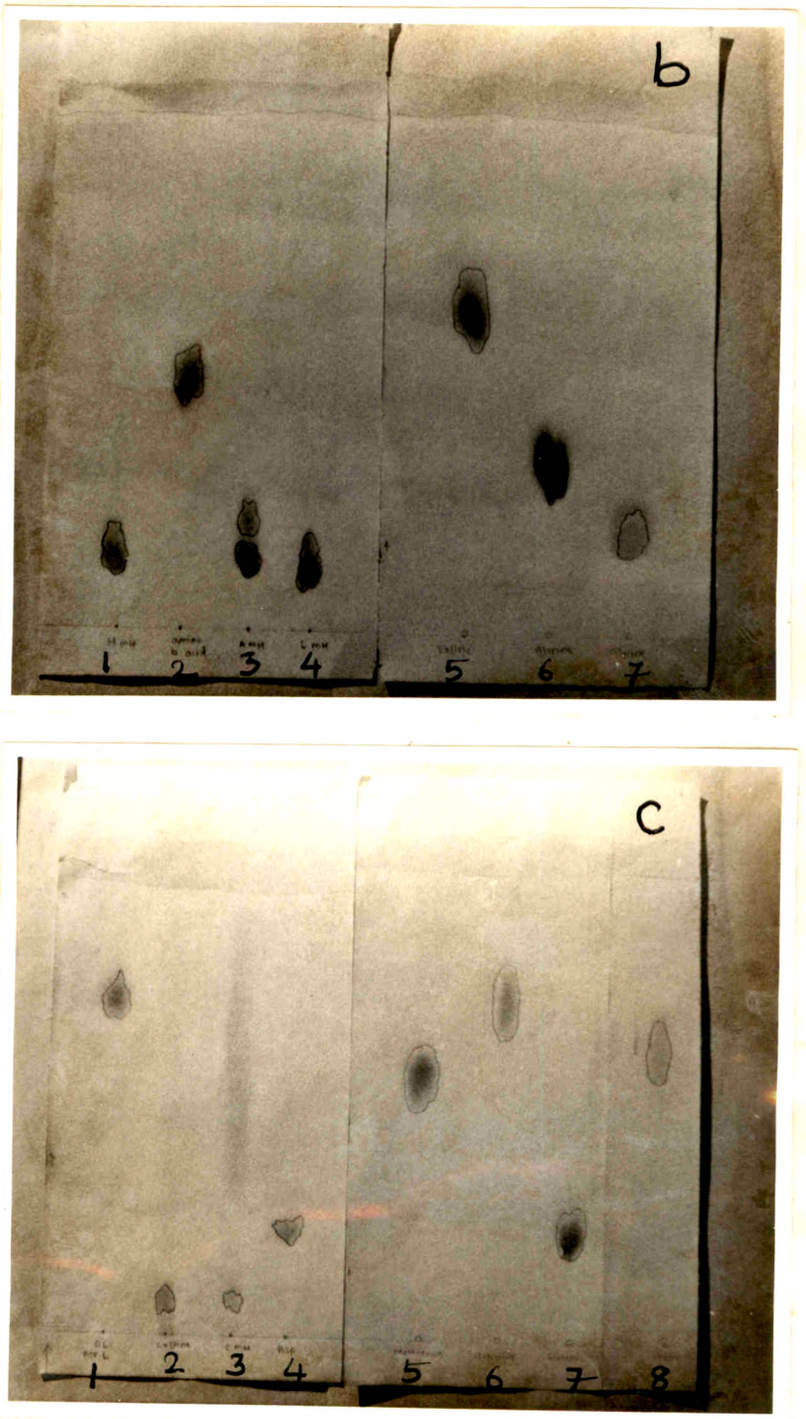


Fig. 17

Fig. 18 : Tracing of individual standard free amino acids separated on chromatographic paper.

1. DL-Serine
2. L-Hydroxyproline
3. L-Ornithine - mono hydrochloride
4. DL-POPA
5. L-Tyrosine
6. DL-Tryptophan
7. DL-Threonine
8. L-Histidine- mono hydrochloride
9. DL-2-Amino-n-butyric acid
10. L-Arginine - mono hydrochloride
11. L-Lysine- mono hydrochloride
12. DL-Valine
13. DL-Alanine
14. Glycine
15. DL-nor-Leucine
16. L-Cysteine
17. L-Cysteine- mono hydrochloride
18. DL Aspartic acid
19. DL-Methionine
20. DL-Isoleucine
21. L-Glutamic acid
22. DL- -Phenylalanine

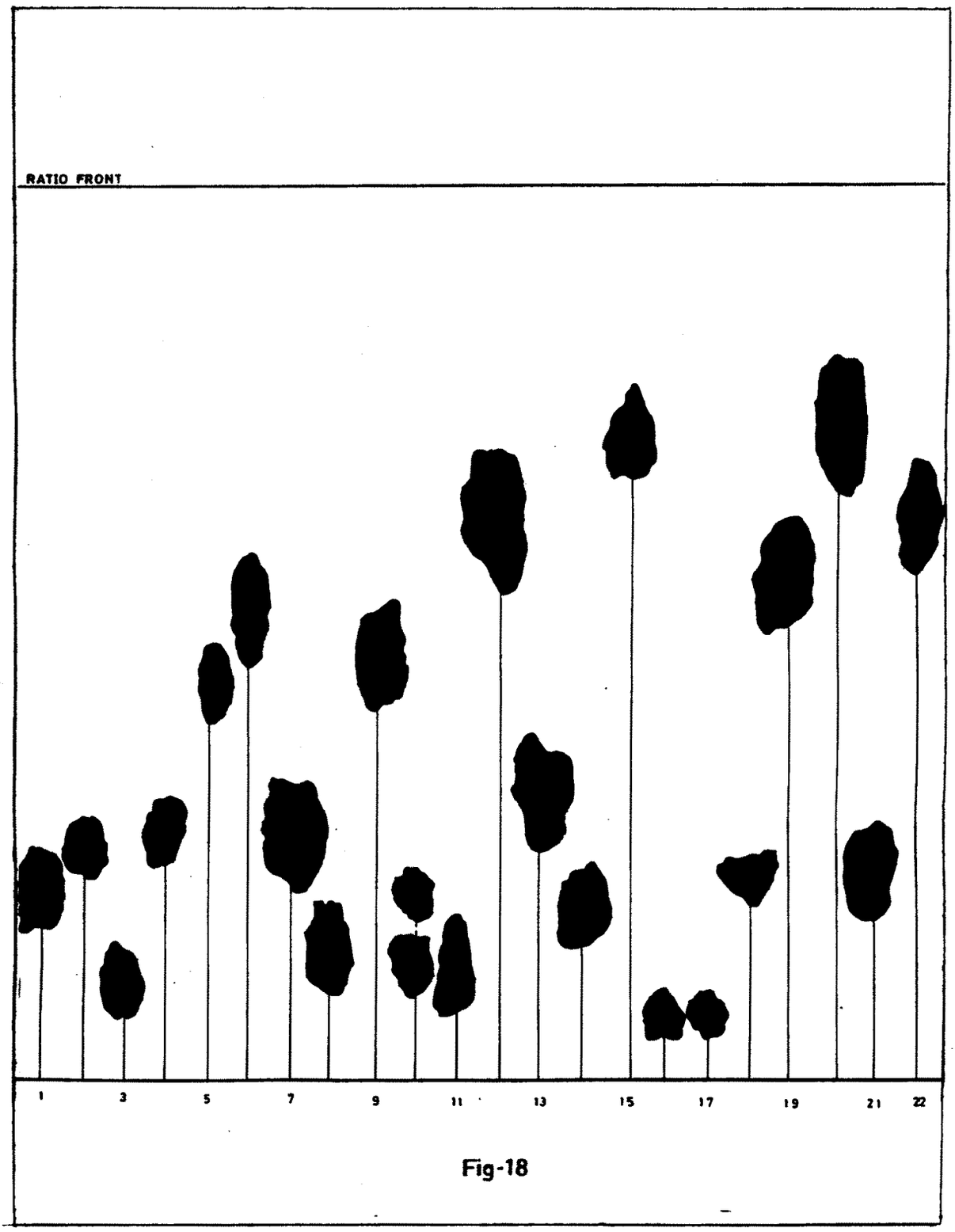


Fig-18

with varying band intensity. The amino acids such as glutamate, aspartate, alanine, tyrosine and trypotphan are found in appreciable amount in MLO infected plant as compared with healthy one. The other amino acids viz. cysteine monohydrochloride, cysteine, leucine have very low visilance as compared with their visilance in infected plant. Moreover isoleucine is totally missing and histidine monohydrochloride is in trace in healthy plant. Whereas the band intensity of amino butyric acid methionine and phenylalanine remained same both in healthy and infected plant extract. From this observation one thing is clear that the infected plant show more amount of amino acid concentration. This higher concentration of amino acid observed in infected plant extract led us to argue that the pathogen such as MLO is mainly responsible for the nonutilization of amino acids for further metabolic processes. This can be attributed to the reduced growth of plant due to MLO infection. Moreover it is well established that the MLOs are located in a conducting tissue 'phloem' and due to rapid multiplication of the organism conducting system gets blocked which affect the entire transpiration stream. The amino acids such as glutamic acid and aspartic acid are reported in phloem tissue

(McCoy, 1981). This clearly indicates that the greater accumulation of amino acid in infected plant possibly be due to hinderance in the translocation mechanism or nonutilization of these amino acids in the synthesis of secondary metabolites. Conflicting results have been reported on the effects of MLOs infection on concentration of amino acids in diseased plants. Jaiswal and Bhatia (1971) reported accumulation of free amino acids and amides in GSD infected sugarcane leaf tissue. Similarly Ramaiah et al. (1964) in sandal affected by spike disease reported increased content of aspartic acid, methionine and arginine while the work of Singh et al. (1976), Verma and Singh (1977) in citrus sp. affected by citrus greening disease. Srinivasan and Chelliah (1978) in brinjal infected with little leaf disease, indicated a marginal increase in the content of amino acids. They further noted that the content of aspartic acid, tryptophan and arginine of diseased plant was slightly greater than that of healthy one. On the contrary Goswami et al. (1971) in citrus sinensis suffering from citrus greening, Prasad and Sahambi (1980) in Sesamum affected by sesamum phyllody disease have reported reduction in the contents of amino acids and amides. Recently Maharaj - Patil (1992) has also

reported marked accumulation of amino acids such as amino buturic acid, arginine, glutamic acid, isoleciune, methionine, proline in MLO infected leaves of Justicia gendarussa as compared to healthy one.

In the present study we have also noted greater accumulation of amino acids such as aspartate, glutamic acid, tyrosine, histidine and tryptophan in MLO infected P. hysterophorus as compared with healthy one.

Various reasons have been attributed to account for the accumulation or reduction in amino acid content in diseased plants. The increase may be due to the accelerated proteolytic activity or due to the impaired translocation and utilization. While, the reduction in amino acid content may be due to their rapid utilization by the pathogen or increased protein synthesis. The accumulation of tryptophan in MLO infected plant is an interesting observation and can be attributed its presence as a precurser of auxin IAA. Possibly the changes taking place in the auxin concentration due to MLO infection may be responsible for induction of phyllody disease in P. hysterophorus. However, to draw a definite conclusion regarding the role of these amino acids in general and tryptophan in particular under pathogenic condition, further research using lebelled amino acid is needed.

H. Sesquiterpene lactone

Sesquiterpene lactones are characteristic constituents of the Asteraceae but also occur sporadicaly in other angiosperm families like Lawraceae, Magnoliaceae and Umbelliferae (Rodriguez et al. 1971). During the past three decades more than 1000 sesquiterpene lactones have been isolated, identified and in some cases, synthesized (Fischer et al. 1978). The information about their bioactive properties is gradually building up. Many SLs of the genus Parthenium, Ambrosia, Chrysanthemum and Frullania are known to cause allergic contact dermatitis and constitute a major class of allergens (Mitchell et al. 1972, Rodriguez et al. 1983, Patil and Hegde 1988). The major SL in P. hysterophorus which cause allergic action has been reported as 'Parthenin' ($C_{15}H_{18}O_4$) by many workers (Rodriguez et al. 1983, Picman et al. 1979, Picman and Towers 1982, Patil and Hegde 1988). In the present study we selected healthy and MLO infected P. hysterophorus for isolation and analysis of SL using Nuclear Magnetic Resonance Spectra (NMR) and High Peformance liquid chromatography (HPLC). The SL isolated from Parthenium both from healthy and MLO infected plants exhibited yellowish white mass. Further purification of this yellowish white mass showed white flakes(Fig. 19). These white flakes when observed under

Fig. 19 : Microphotograph showing purified flakes of parthenin isolated from P. hysterophorus.

Fig. 19

light microscope showed radiating type of crystals (Fig.20). The yield of SL in healthy Parthenium plant was more as compared with the yield of SL in infected plant (Table-12). The infection caused as high as 58.3% reduction in SL yield. This yield reduction of SL can be attributed to stunted growth of the plant and less number of trichomes (Fig. 21) on the plant.

1. Solubility of SL

Solubility of the sesquiterpene lactone in various solvents was studied and the data is depicted in Table-13. It is very clear from the table that the compound is sparingly soluble in acetone, methanol, chloroform and insoluble in solvent ether hexane, benzene, toluene, xylene and water.

2. λ max of SL

The λ max of the compound gives the absorption peak of the solute in a desirable solvent at particular wavelength. This was achived by studying the absorption spectra of the compound on double beam spectrophotometer at UV range by dissolving it in acetone. As such most of the SLs exhibit their absorption peaks in the UV region (Rodriguez et al. 1983). The absorption curve obtained

Table 12 : Quantification of sesquiterpene lactone isolated from healthy and MLO infected plant of Parthenium hysterophorus L.

Plant material	Sesquiterpene lactone g 100^{-1}g dry tissue
Healthy	2.4 ± 0.5
Infected	1.0 ± 0.2

± S.D.

Fig. 20 : Microphotograph showing radiating type crystals of parthenin.

Fig. 21 : Microphotograph showing trichome of P. hysterophorus present all over the plant body.

Fig. 20

Fig. 21

Table 13 : Solubility of the sesquiterpene lactone in various organic solvents.

Solvent	Solubility
Acetone	Soluble
Acetonitrile	Soluble
Ethanol	Soluble
Chlroform	Soluble
Methanol	Soluble
Solvent ether	Insoluble
Hexane	Insoluble
Benzene	Insoluble
Tolune	Insoluble
Xylene	Insoluble
Water	Insoluble

for SL isolated from both healthy and MLO inflected P. hysterophorus is shown in Fig. 22 : Both the curves represented similar pattern with slight variation in their values. However, the maximum absorption peak was obtained at 205 nm with spectral range 200nm - 220nm. Radriguez et al. (1983) have reported that 215 nm is the good average λ_{max} for SLs. In the present investigation when the compound was studied for its absorption peak using spectrally purified solvent acetonitrile, it also exhibited its highest peak at 215 nm.

3. Allelopathic effects of SL isolated from P. hysterophorus :

The allelopathic effects of SL isolated from healthy and MLO infected P. hysterophorus plant were studied on wheat germination and the data is reported in Table-14, represented histographically in Fig. 23, 24 and 25 and in support are the photographs (Fig.26a & b and Fig. 27a & b). It is clear from the table and figure that the SL isolated from healthy P.hysterophorus plant has inhibitory effect both on seed germination and root-shoot, length with increasing concentration. However,

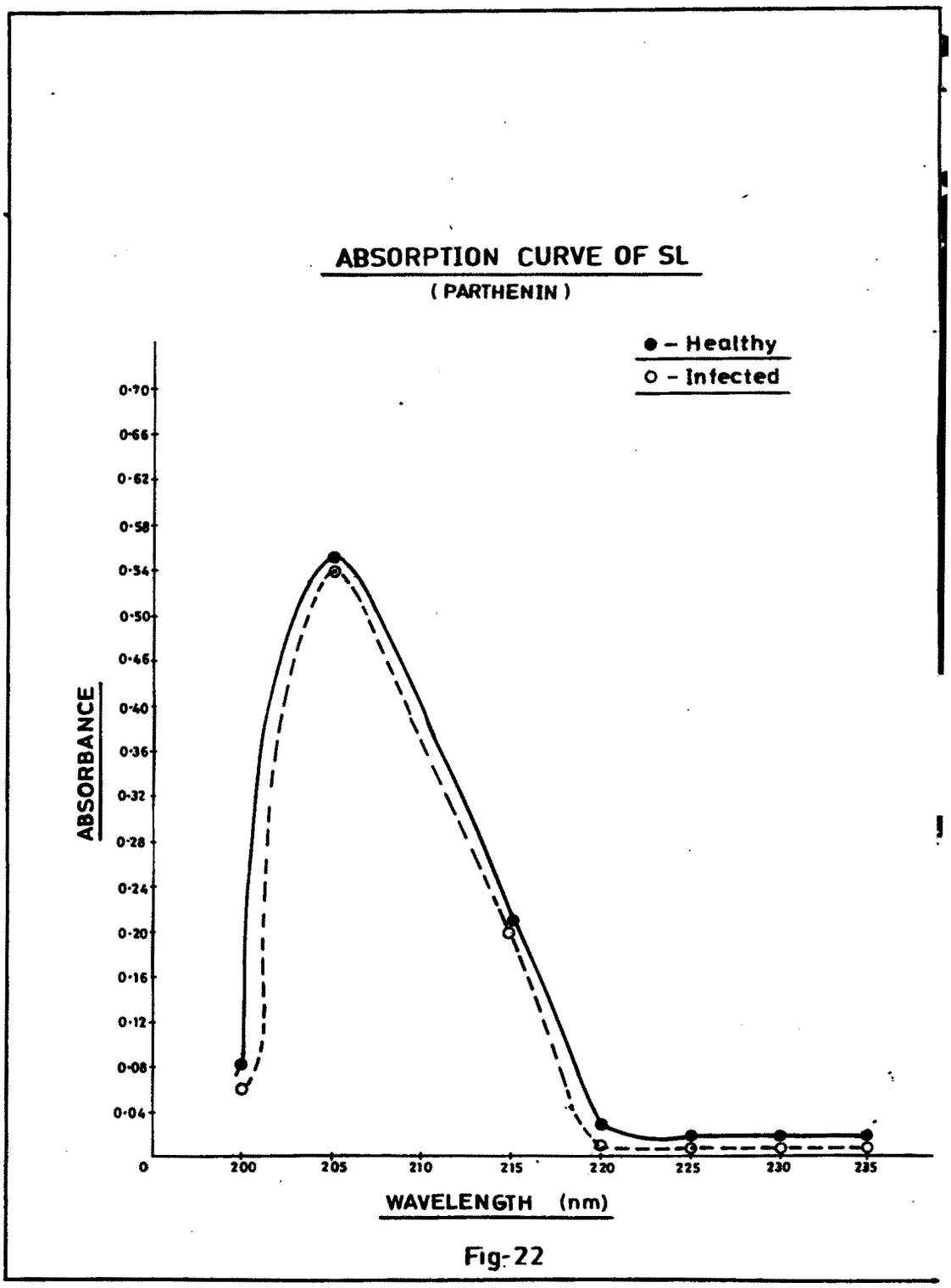


Fig-22

Table 14 : Effect of sesquiterpene lactone isolated from healthy and MLO infected plant of Parthenium hysterophorus on germination and growth of wheat after 72 h germination.

SL concentration (ppm)	Germination (%)	Root length (cm)	Shoot length (cm)
Control	100	7.38 ± 0.41	2.86 ± 0.40
Healthy			
250	92	2.6 ± 0.78	2.1 ± 0.56
500	63	1.6 ± 0.57	0.94 ± 0.55
1000	56	0.56 ± 0.51	0.72 ± 0.42
Infected			
250	96	3.6 ± 0.51	2.2 ± 0.40
500	96	2.04 ± 0.83	1.7 ± 0.38
1000	88	1.22 ± 0.47	1.16 ± 0.31

± S.D.

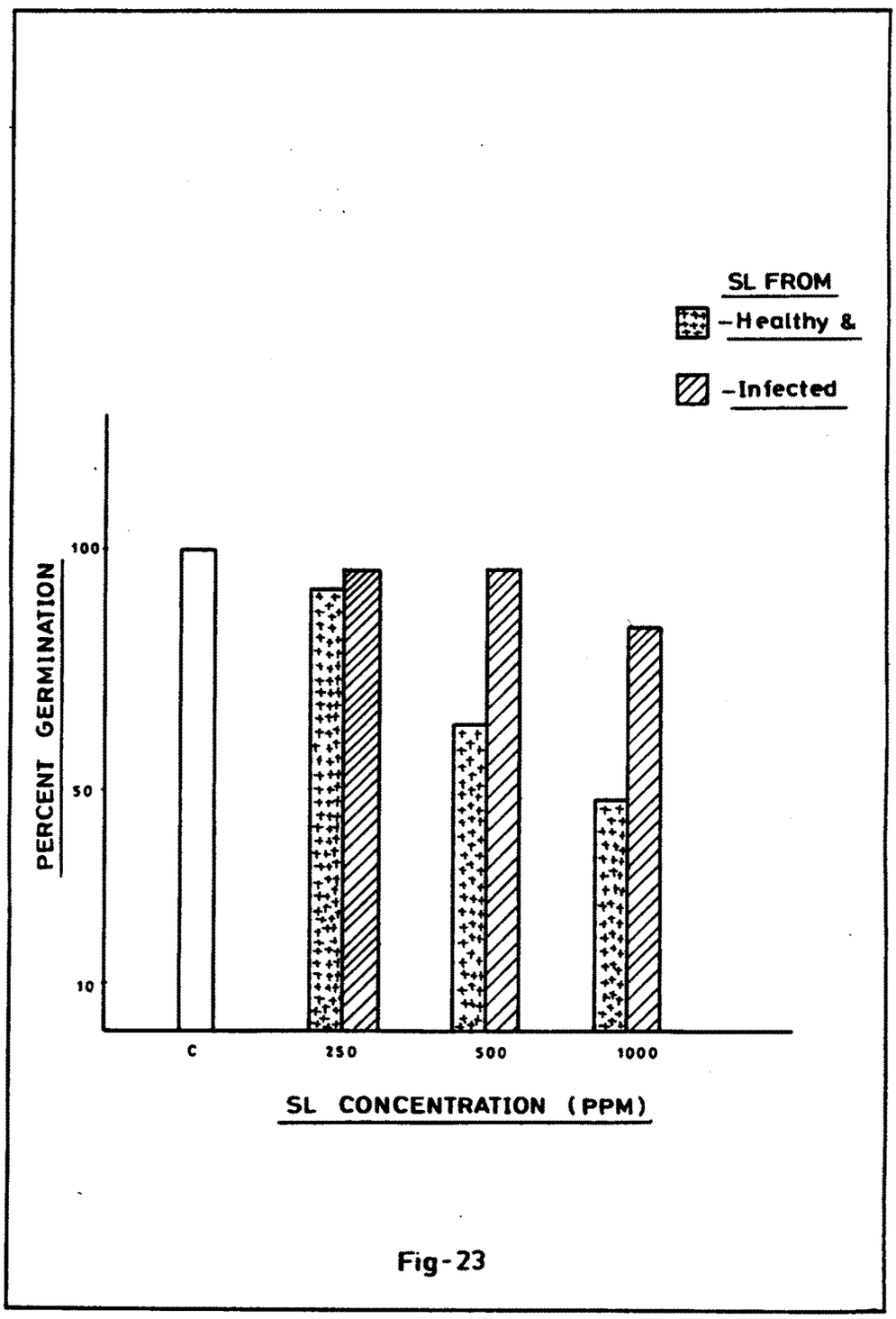

SL FROM
-Healthy &
-Infected
PERCENT GERMINATION
100
50
10
C
250
500
1000
SL CONCENTRATION (PPM)

Fig-23

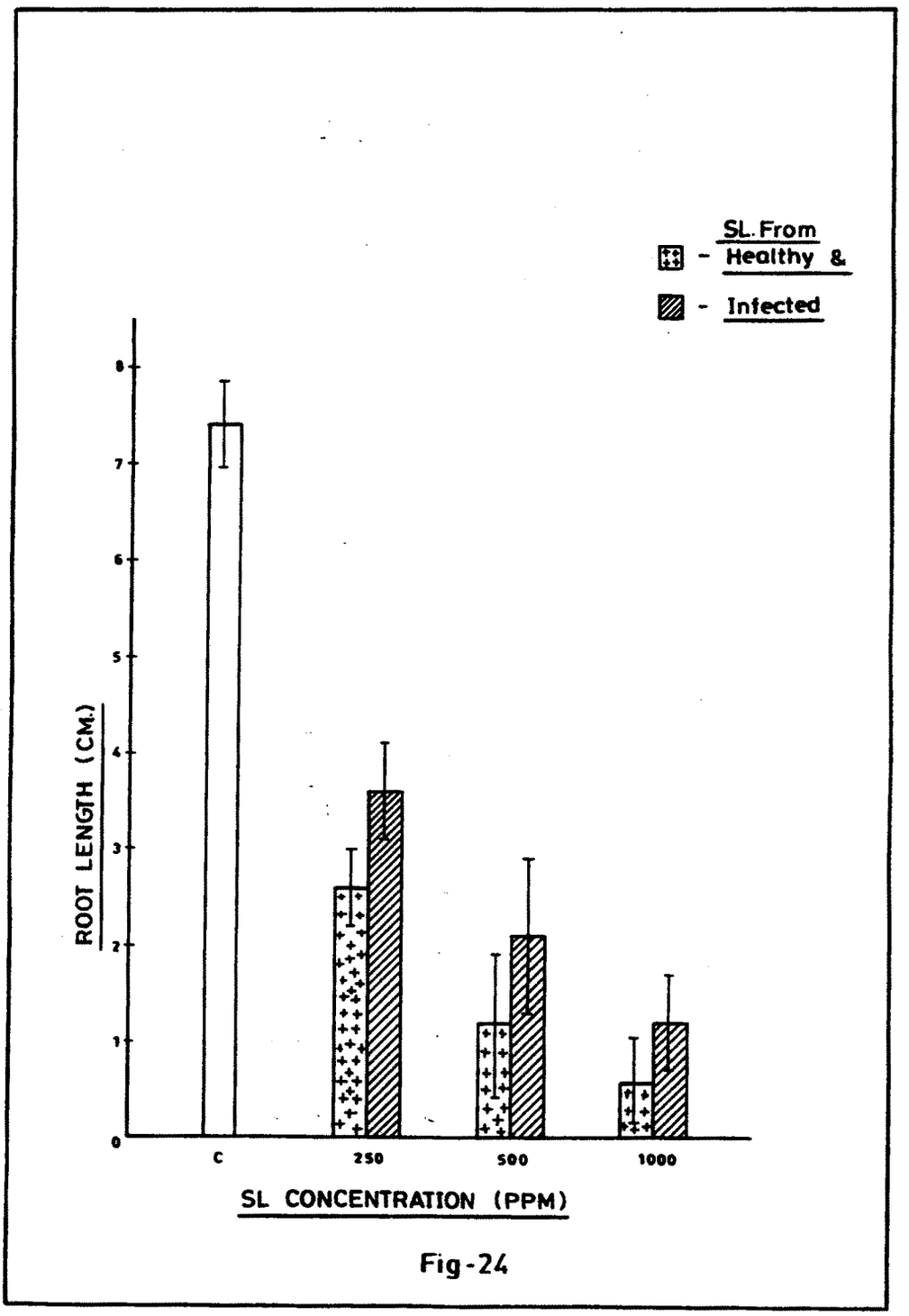


Fig-24

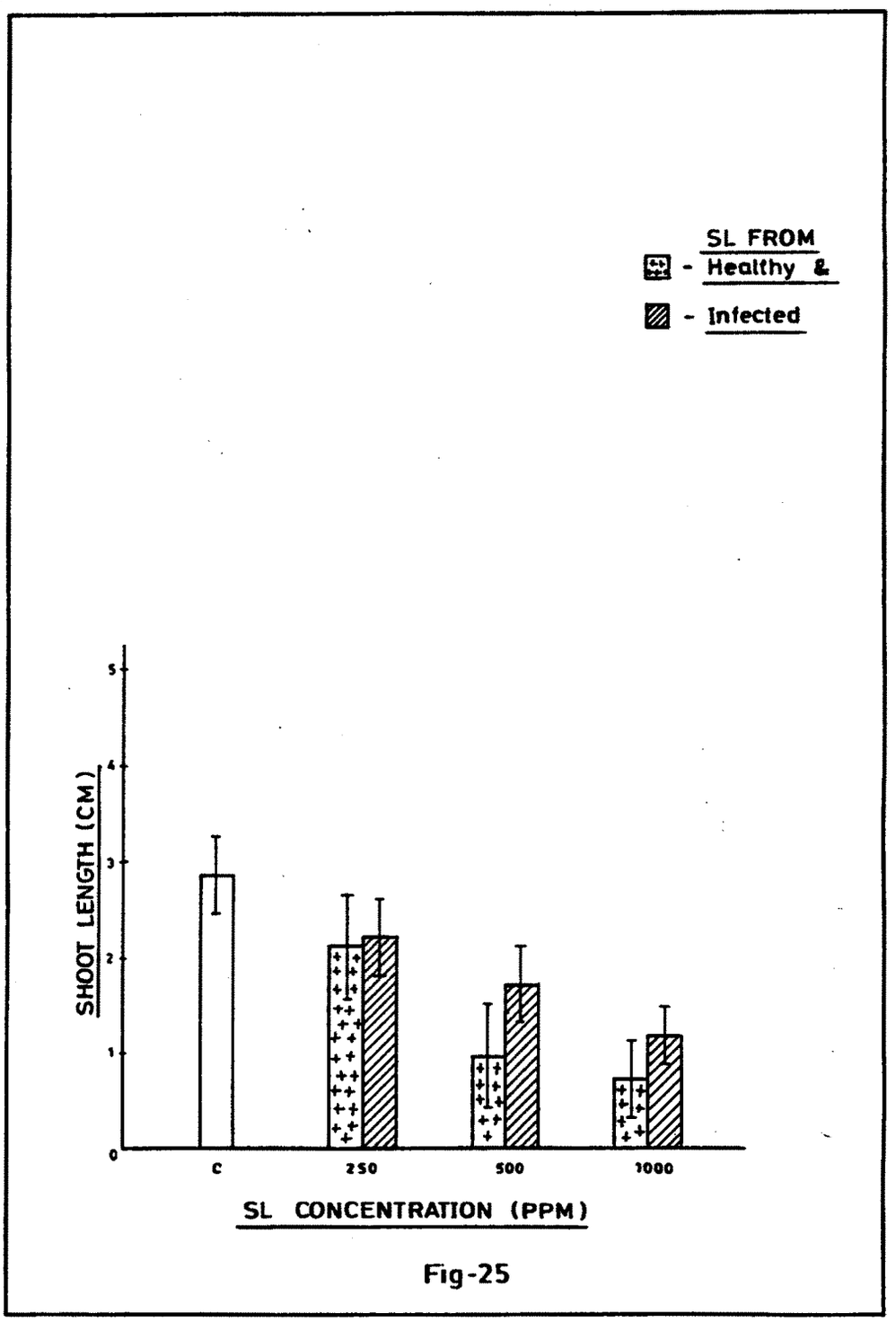
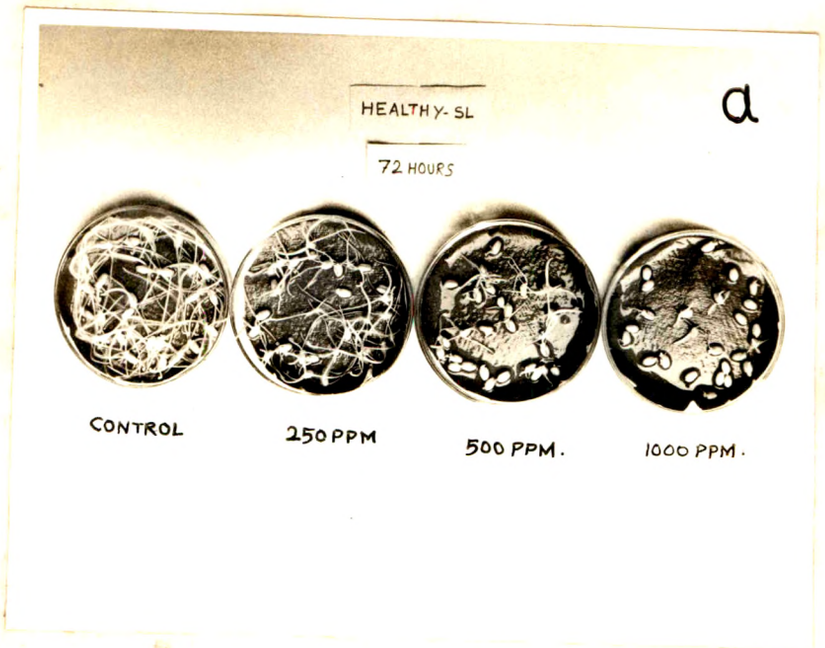

SL FROM
- Healthy &
- Infected
SHOOT LENGTH (CM)
0
1
2
3
4
5
C
250
500
1000
SL CONCENTRATION (PPM)

Fig-25

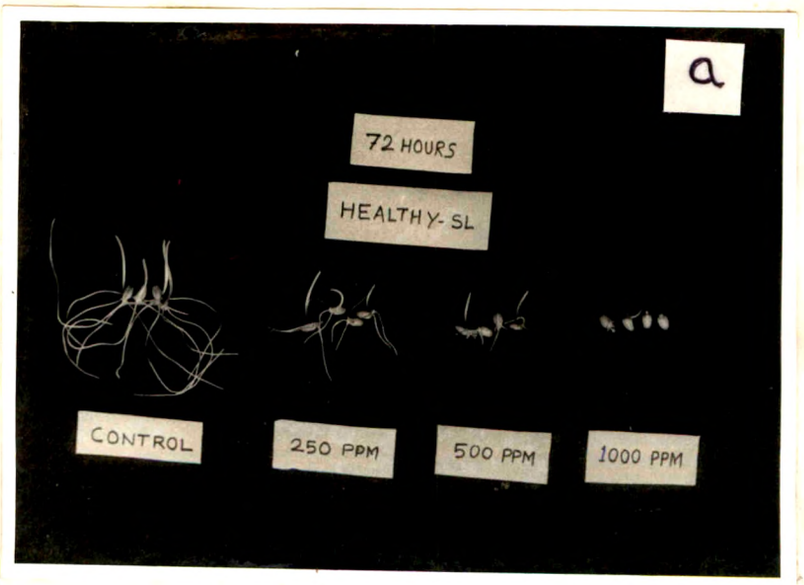
Fig. 26 : Photograph showing effect of different concentration of SL isolated from healthy (a) and MLO infected (b) P. hysterophorus on wheat germination after 72 h .


HEALTHY-SL
a
72 HOURS
CONTROL
250 PPM
500 PPM.
1000 PPM.


INFECTED-SL
b
72 HOURS
CONTROL
250 PPM
500 PPM
1000 PPM

Fig. 26

Fig. 27 : Photograph showing inhibitory effect of different concentration of SL isolated from healthy (a) and MLO infected (b) P.hysterophorus on root and shoot length of wheat after 72 h germination.


a
72 HOURS
HEALTHY-SL
CONTROL
250 PPM
500 PPM
1000 PPM

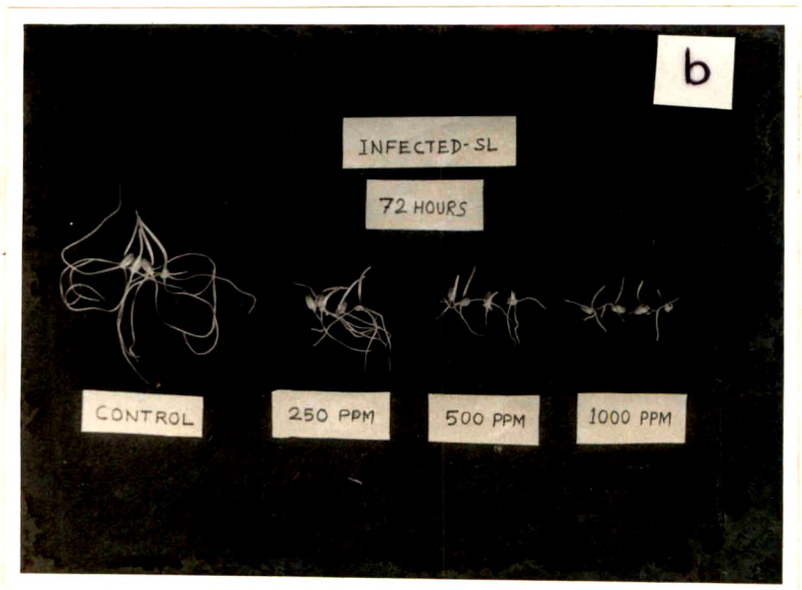

b
INFECTED-SL
72 HOURS
CONTROL
250 PPM
500 PPM
1000 PPM

Fig. 27

the SL isolated from infected plant though exhibit inhibitory effects on germination as well as root shoot length it was comparitively less toxic than that of SL isolated from healthy one. It is also evident from the table that root length was hampered to a considerable extent with increase in sesquiterpene lactone concentration. As high as 92.41% reduction in root length of wheat was noticed at 1000ppm SL isolated from healthy plant. This reduction at lower concentration was 78.32% and 64.76% at 500 ppm and 250 ppm respectively. On the contrary, SL isolated from MLO infected plant showed comparitively less reduction. It was 83.47%, 72.36% and 51.22% at 1000 ppm, 500 ppm and 250 ppm respectively. The similar trend was observed with shoot length. The reduction in shoot length at 1000 ppm SL of infected plant was 59.44% while it was 74.83% at 1000 ppm SL of healthy plant.. At other concentrations viz. 500 ppm and 250 ppm the per cent reduction observed in shoot length due to SL of infected plant was 40.56% and 23.08% and due to SL of healthy plant was 74.83% and 26.57% respectively. From the Fig. 26 and 27 the inhibitory effect of SL can clearly be seen. From these results it can be safely concluded that the SL isolated from infected plant has poor allelopathic effects as compared with SL of healthy one.

As early as 1832, De Candolle anticipated the

role of inhibitory substance in crops and natural communities, when he suggested that the deleterious effects of continuous one crop culitvation might be due to toxic root secretions. Since then a large number of reports have accumulated and while some investigators have denied the existance of growth inhibitory substances in root exudates, others have over emphasized the role of these inhibitors in the growth of plants in natural community.

In recent years several specific instances of toxicity of substances liberated by different species of plants have been reported (Das and Sadhu 1985) severe root interaction was noted in Guayule (P. argentatum) plants under certain conditions. The allelopathic effects of P. hysterophorus have also been reported elsewhere by many workers (Kanchan 1975, Kanchan and Jayachandra 1979, 1979a, 1980, Mall and Dagar 1979). However all these workers have tested the allelopathic effects by using either leaf extract, stem extract, root extract or root exudates. In the present investigation the principle allergen isolated from healthy and MLO infected plant has been used to study the allelopathic effects. From the results obtained it is very clear that the SL isolated from healthy plant has strong inhibitory effect on wheat germination as compared with SL isolated

from infected plant. Patil and Hegde (1988) have made similar observation, however by using only SL isolated from healthy plant. Further they have elucidated the antifungal and cytotoxic property of SL isolated from healthy Parthenium plant.

From the above discussion one thing is very clear that SL isolated from MLO infected plant exhibit low/less allelopathic effects. This observation led us to surmise that there must be structural change due to MLO infection in SL which may possibly reduce the allelopathic effects and also appears to be poor in allergic reaction. In this regard it is noteworthy to mention that the allergic reaction of SL is mainly because formation of adducts of SL (Parthenin) with cysteine and glutathion which are the sulfur containing amino acids (Picman et al. 1979). Subsequent biochemical investigations established that the α-methylene moiety is an important immunochemical requisite for the substance to be an active allergen and according to Mitchell (1975) the α-methylene group reacts with α-cysteine to form monoadduct with the endocyclic double bond on the cyclopentenone ring and become involved in the allergic reaction. The proposed mechanism of allergic contact dermatitis evoked by SL (Parthenin) is shown in Fig. 28. Now whether the same mechanism holds

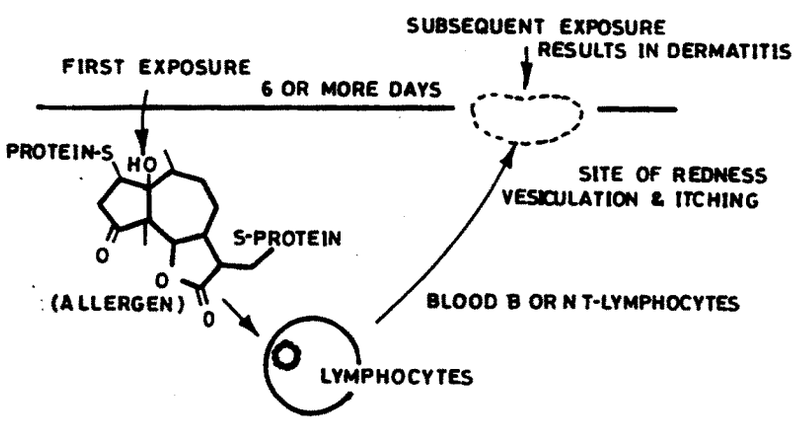


Fig-28:- PROPOSED PATHOGENETIC MECHANISM OF ALLERGIC CONTACT DERMATITIS EVOKED BY SESQUITERPENE LACTONES.

true for SL isolated from MLO infected Parthenium plant or not?, is still not known. However, we can only say that the SL is poor in its activity either due to inability of forming the adducts with amino acids or change in the structurral integrity of SL. This will only be made clear by studying the nuclear magnetic resonance spectra (NMR) and comparing with SL isolated from healthy plant.

As such most of the workers have studied the allelopathic effect of P. hysterophorus and according to them the growth inhibitors which are responsible for strong allelopathic effects are either plenolic compounds or amino acids. To name few are caffeic acid and P-caumaric acid (Kanchan 1975), SL viz. Parthenin (Patil and Hegde 1988), Ambrosin, Coronopillin and Hymenin (Behl and Behl 1984, Marchand et al. 1983). The amino acids of pollengrains of P. hysterophorus viz. arginine, proline, histidine have also been reported to be allergic and toxic by Gupta et al. (1980). In addition, the SL (Parthenin) has inhibitory effect on larval growth (Isman and Rodriguez 1983), cardiac inhibitory properties in the migratory grass hopper Melanoplus sanguinipes (Picman et al. 1981). Root and leaf extract of Parthenium proved as bacterial inhibitor viz. Rhizobium phaseoli and Azatobactar (Kanchan and

Jayachandra 1981) and also act as a inhibitor of cockroach head and gizzard cholinesterase (Rajakumar and Nandkumar 1984).

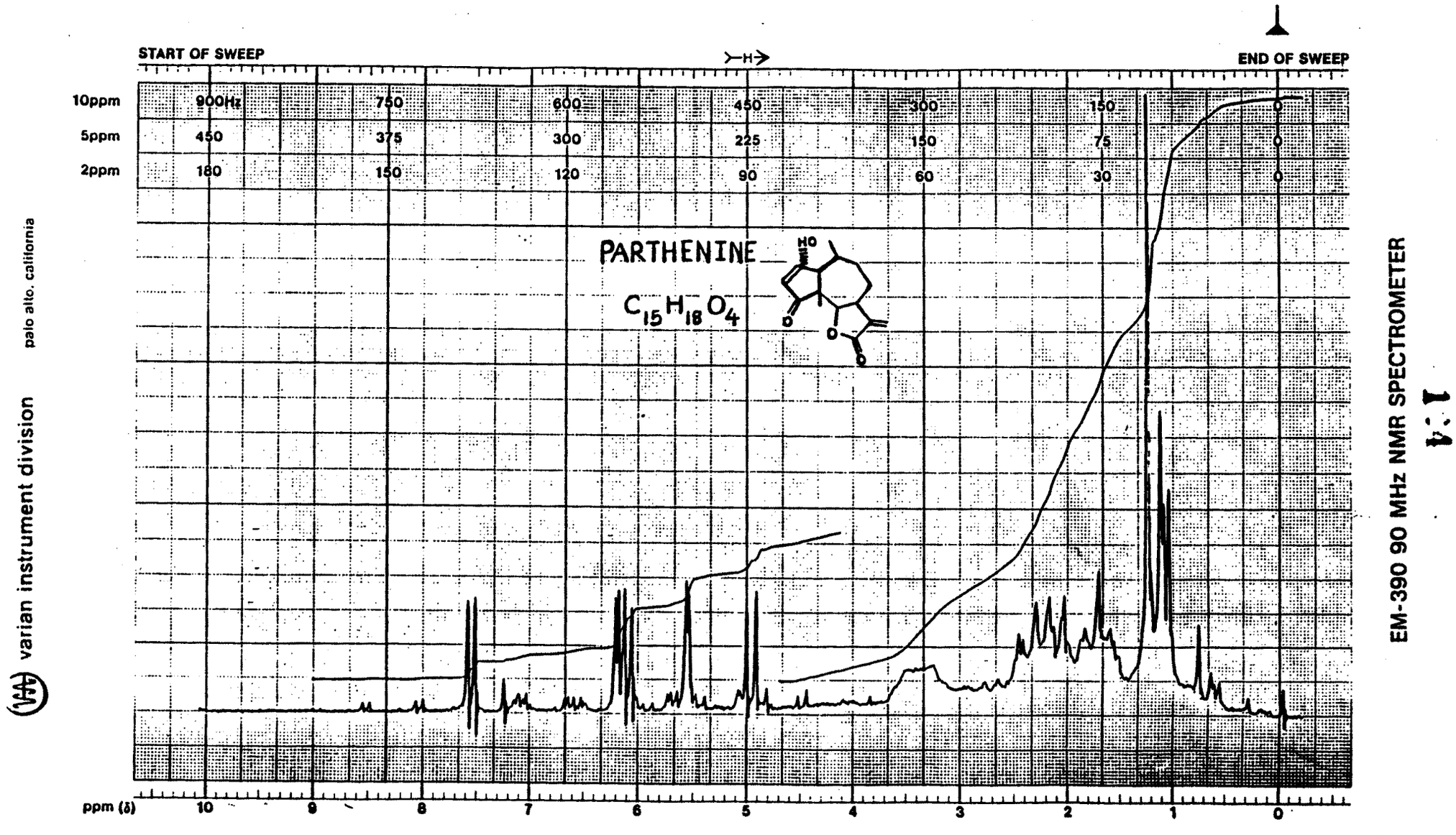
From the above discussion it is crystal clear that the SL (Parthenin), phenols and plant as a whole exhibit allelopathic effects. However, MLO infected plant and the sesquiterpene lactone isolated from it do exhibit the same but at lower ebb.

I. Characterisation of SL on NMR and HPLC.

1. Nuclear Magnetic Resonance (NMR) of SL

In order to establish the identity of SL isolated from healthy and MLO infected Parthenium hysterophorus, NMR spectra was studied on EM 390 90 NHz spectrophotometer by the courtesy of Dr. Manuel Aregullin and Prof. Eloy Rodriguez, Irvine, California U.S.A. The NMR spectra of SL isolated from both healthy and MLO infected plant are represented in Fig. 29 and 30. The NMR study of SL revealed that the major SL in both healthy and infected plant is 'Parthenin' having chemical formula $C_{15}H_{18}O_4$ and structural formula as shown in Fig. 29 and 30. Although NMR analysis proved that both healthy and MLO infected plant contain the

Fig. 29 : NMR spectra showing structure and chemical formula of parthenin isolated from healthy P. hysterophorus.



varian instrument division palo alto, california

EM-390 90 MHz NMR SPECTROMETER

LOCK POS. _____ ppm
LOCK POWER _____ mG
DECOUPLE POS. _____ ppm
DECOUPLING POWER _____ mG

SPECTRUM AMPL. 600
FILTER 0.05 sec
RF POWER 0.15 mG

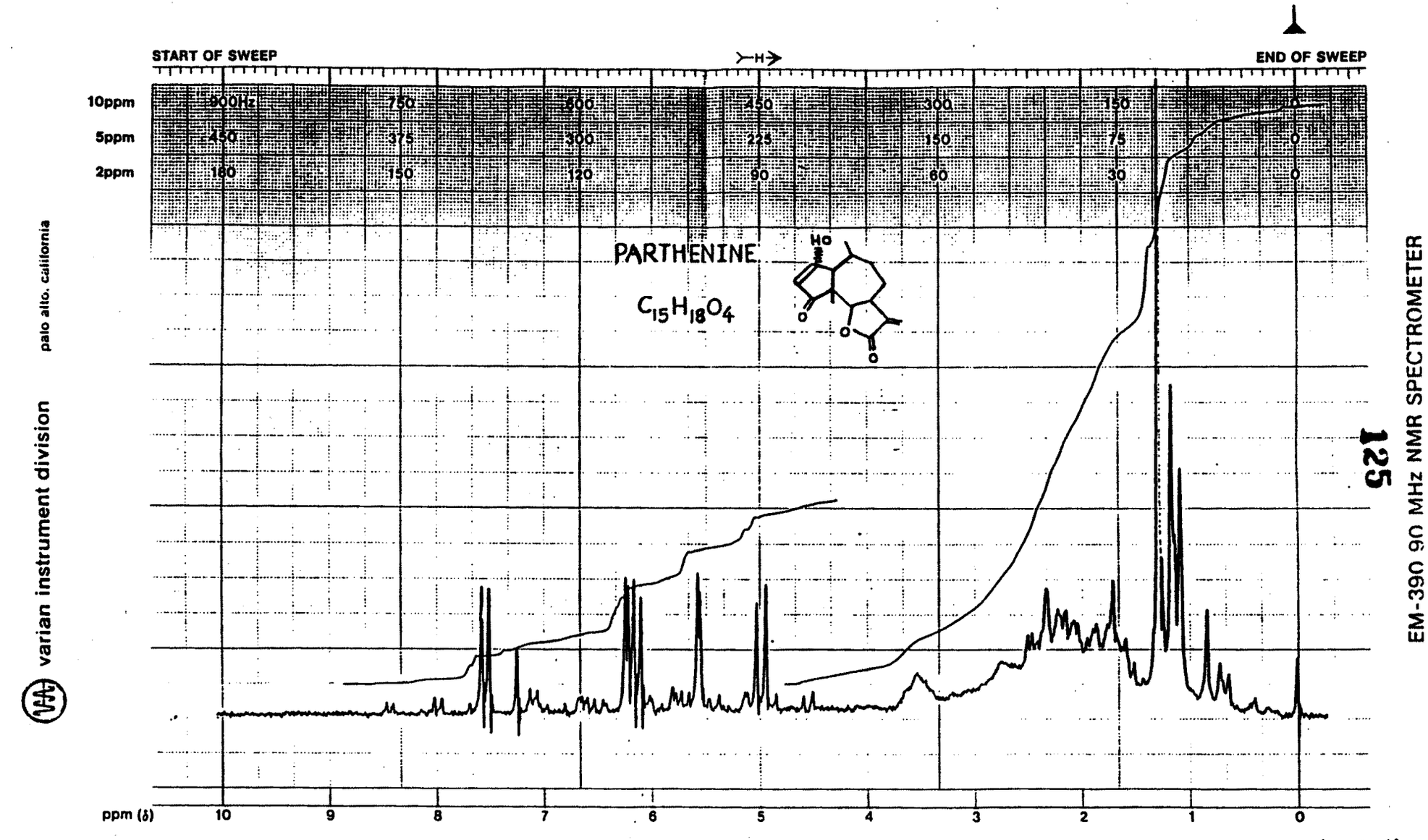
SWEEP TIME 5 min
SWEEP WIDTH 10 ppm
END OF SWEEP 0 ppm

NUCLEUS H
ZERO REF. TMS
SAMPLE TEMP. RT °C

SAMPLE: Sample A
SOLVENT: $CDCl_3$

OPERATOR M. Arezzini
DATE 1/5/92
SPECTRUM NO

Fig. 30 : NMR spectra showing structure and chemical formula of parthenin isolated from MLO infected P. hysterophorus.



varian instrument division palo alto, california

PRINTED IN U.S.A.

PART NO 950200 02

EM-390 90 MHz NMR SPECTROMETER

125

LOCK POS. _____ ppm
LOCK POWER _____ mG
DECOUPLE POS. _____ ppm
DECOUPLING POWER _____ mG

SPECTRUM AMPL. 2080
FILTER 0.05 sec
RF POWER 0.15 mG

SWEEP TIME 5 min
SWEEP WIDTH 10 ppm
END OF SWEEP 0 ppm

NUCLEUS H
ZERO REF. TMS
SAMPLE TEMP. 25 C

SAMPLE: Sample B
SOLVENT: $CDCl_3$

OPERATOR M. Arguello
DATE 1/5/92
SPECTRUM NO. _____

same SL 'Parthenin', has exhibited marked difference in its allelopathic effects. However, NMR is not a very good analytical technique for purity and hence the high performance liquid chromatographic (HPLC) analysis of both the samples were carried out by using C-18 RP column 25 : 75 water acetonitrile isocretic system with UV detection at 215 nm.

2. HPLC of SL

The peaks obtained on HPLC are represented graphically in Fig. 31 and 32. It is very clear from the figure that both the samples contain approximately 91% of Parthenin with variable peaks of contaminants which are different in both the samples. The different peaks obtained for SL isolated from healthy and infected plants, on high performance liquid chromatrography (HPLC) are given in Table - 15 and 16. It is vividly clear from the table that SL isolated from healthy exhibited 11 peaks while SL isolated from infected plant exhibited only 6 peaks. The major peak of SL was at retention time 3.58 and 3.5 with peak area 91.667% and 91.53% was confirmed as parthenin of healthy and infected plant respectively. The other peaks observed at retention time 9.49, 11.64, 14.74 and 20.54 are totally missing in the SL isolated from MLO infected plant. Possibly these unknown peaks of different compounds may be responsible for strong allelopathic

Fig. 31 : HPLC analysis of SL isolated from healthy P. hysterophorus and visualized chromatogram of parthenin.

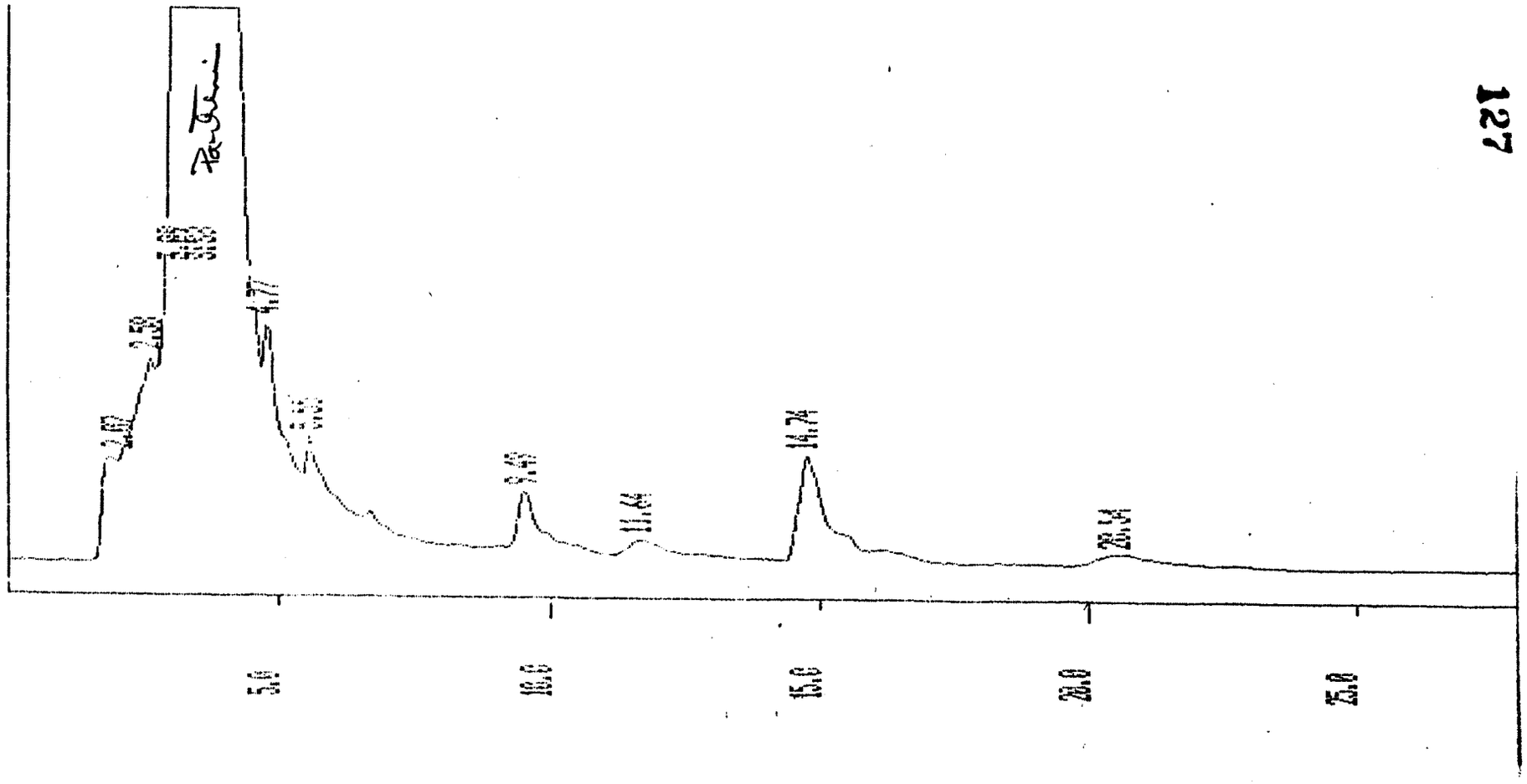
SAMPLE: A
METHOD: SesaLact
ANALYST:

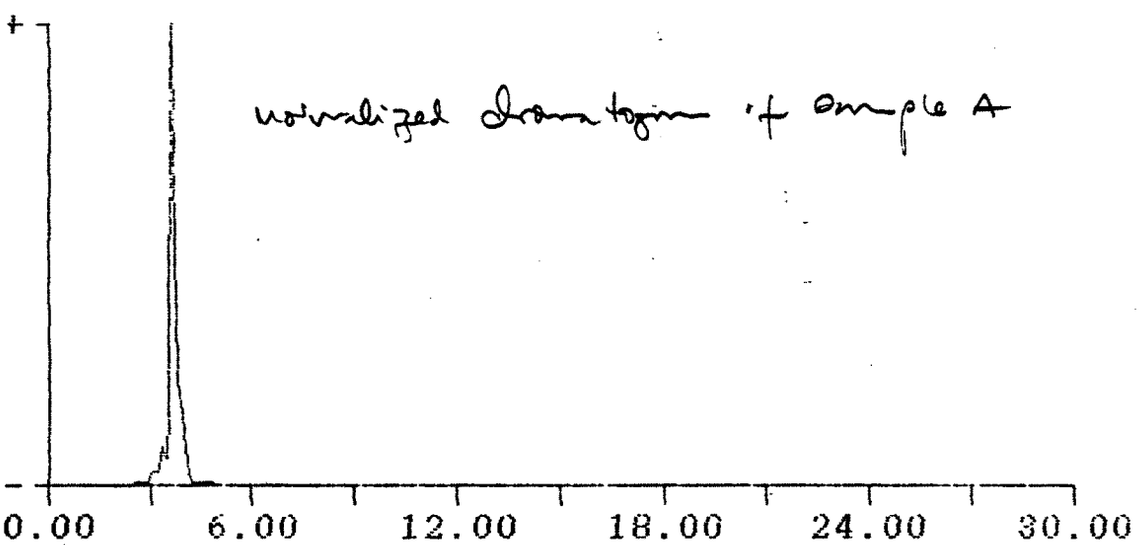
DATA FILE: SESQLACW.BFF
METHOD FILE: SESQLA60.MTH
SYSTEM FILE: 1

DATE: 01/06/93
TIME: 09:30:00

PEAK WIDTH: 12 PEAK THRESHOLD: 1500 OFFSET: 5 AUFS: 0.034

WAVELENGTH: 215nm





Chromatogram Analysis: \FOCUS\SESQLACW.BFF

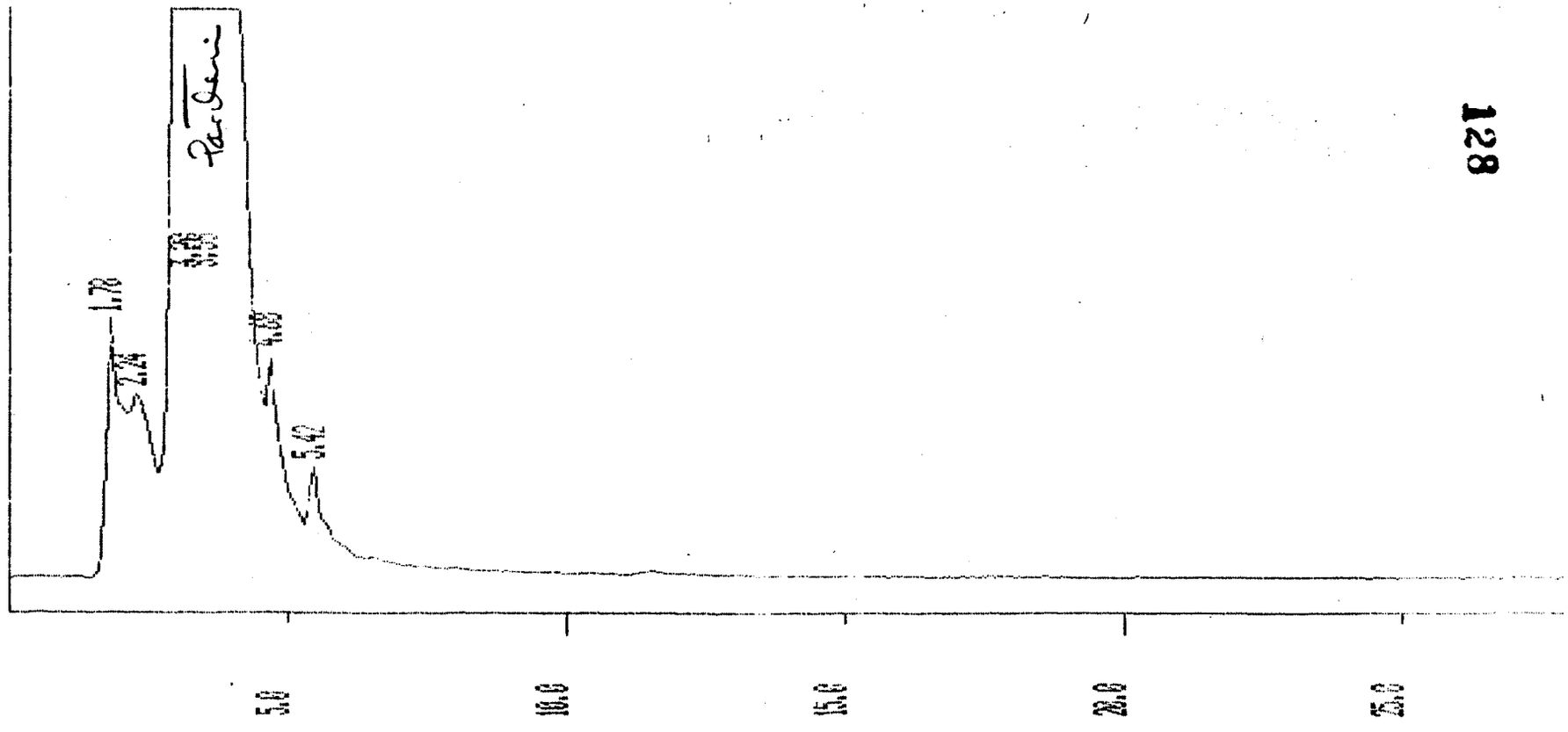
Fig. 32 : HPLC analysis of SL isolated from MLO infected P. hysterophorus and visualized chromatogram of parthenin.

SAMPLE: B
~~METHOD: Sesqlact~~
ANALYST:

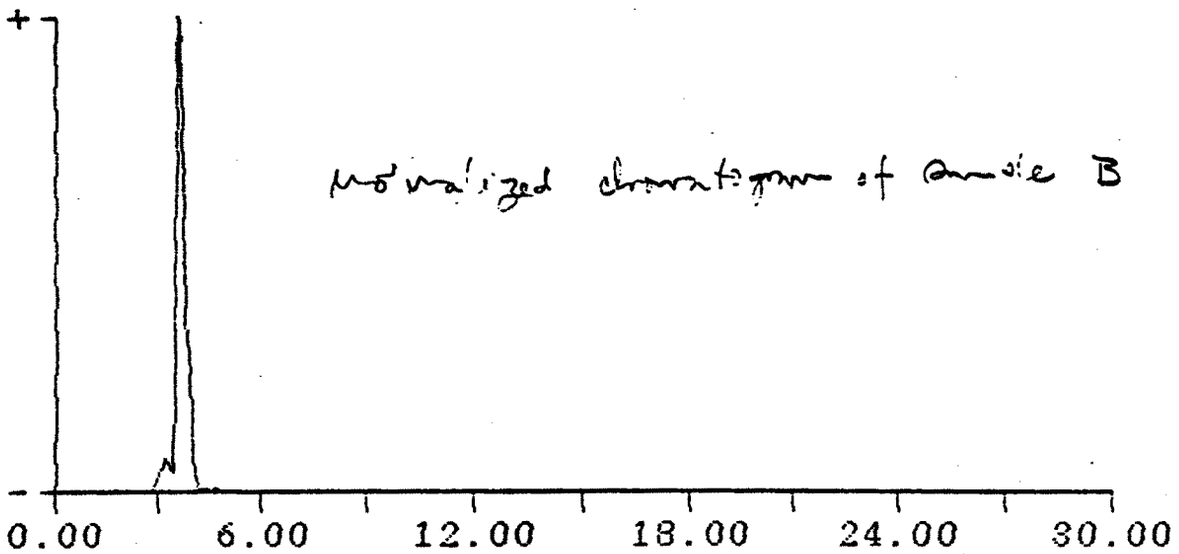
DATA FILE: SESQLACX.BFF DATE: 01/06/93
METHOD FILE: SESQLA82.MTH TIME: 10:54:00
SYSTEM FILE: 1

PEAK WIDTH: 12 PEAK THRESHOLD: 1500 OFFSET: 5 AUFS: 0.034

WAVELENGTH: 215nm



128



Chromatogram Analysis: \FOCUS\SESQLACX.BFF

Table 15 : HPLC analysis of sesquiterpene iactone isolated from healthy plant of P. hysterophorous showing peak numbers, retention time and the peak area.

Peak No.	Retention time	Peak area (%)
1	2.07	0.1111
2	2.58	0.081
3	3.09	2.108
4	3.33	5.12
5	3.58	91.667
6	4.77	0.234
7	5.55	0.062
8	9.49	0.095
9	11.64	0.065
10	14.74	0.382
11	20.54	0.075

Table 16 : HPLC analysis of sesquiterpene lactone isolated from MLO infected plant of P. hysterophorus showing peak numbers, retention time and peak area.

Peak No.	Retention time	Peak area (%)
1	1.78	0.4
2	2.24	0.403
3	3.2	7.151
4	3.5	91.53
5	4.68	0.359
6	5.42	0.157

effects. The identification of these unknown compounds which have exhibited different peaks on HPLC scan and the NMR of these compounds will throw light on their structure and chemical nature, which needs further investigation and the work on this line is in progress. At present one thing is very clear that allelopathic effects of P. hysterophorus are not due to Parthenin alone but there are other compounds (as evidenced by HPLC analysis) which contribute towards allelopathic effects. As such often a minor constituent happens to be responsible for the biological properties observed and hence, there is a strong possibility that one or few compounds appeared in HPLC analysis of SL isolated from healthy plants, along with parthenin, has synergestic allelopathic effect as compared with the allelopathic effects exhibited by SL of MLO infected plant.