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"STUDIES ON THE ALLELOPATHIC POTENTIAL OF SELECTED PLANT SPECIES AND CHARACTERIZATION OF ALLELOCHEMICALS"

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As two academics, we always considered it our responsibility to harness the research capabilities of our respective Departments, and further strengthen the facilities available in the University of Colombo, both in terms of technical training for technical staff and students and purchase of new scientific equipment. Both of us worked untiringly towards meeting these goals, and looking back, we firmly believe that we have achieved some of these goals through our Grant, to the maximum extent that was possible. Needless to say that such achievements are only possible with the ready co-operation of the two Departments, particularly the respective Heads of Departments, the Dean of Faculty of Science and the Vice-Chancellor of our University, in general. We are extremely grateful to Professor G. L. Peiris, our Vice-Chancellor, during whose tenure in office, we won this award and carried it to its successful end. Grateful acknowledgement is made of the Dean of Faculty of Science, and the Heads of both Departments of

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SUMMARY

The present research had the following broad objectives: (a) To identify from among selected common plant species those that have a high potential for allelopathic interactions; (b) To explore the possibility of utilizing the residues of such species in biological weed suppression in field crop production; and (c) To characterize the inhibitory (or stimulatory) allelochemicals from them with a view to obtaining biological pesticides and/or plant growth regulators.

After preliminary investigations, two strongly allelopathic species, namely Gliricidia maculata (Fabaceeae) and Tithonia diversifolia (Asteraceae) were selected for detailed study. After establishing that leachate and blended aqueous extracts of both species had considerable bioactivity (both stimulatory and inhibitory), the leaf material applied as surface mulches and/or soil-incorporated residues, was tested in field experiments as well as in glasshouse-based pot experiments for weed suppression ability and other effects. Field studies as well as pot experiments indicated that both types of residues very significantly stimulated the growth of crops (Tomato, Brinjal, Chilli), whilst controlling weeds in the treated plots very significantly, generally at rates of 2 or 4 kg/m^2. In these studies, the promotion of growth of the three field crops caused by both types of residues was quite spectacular.

Pot experiments clearly established that surface applied residues, as well as incorporated residues stimulated the growth of crop species, and caused significant inhibition of seed germination and seedling growth of weed species. Surface-placed residues were more effective than incorporated residues. When the effect of time of decomposition on the phytotoxicity of the residues was studied, evidence was obtained that Gliricidia residues were bioactive (stimulating the growth of crop species) mostly after one or two weeks of decomposition. With Tithonia residues, as decomposition progressed, there was evidence of the soil being ammended to be phytotoxic to the test species.

Bioassay-guided, activity-directed extraction and fractionation of bioactivity of the two species was carried out, using a tomato seed germination and seedling growth bioassay. Characterization of chemicals was carried out using standard spectroscopic methods. Coumarin, a well-known seed germination inhibitor was identified as the major active allelochemical in Gliricidia fresh leaf residues. From dried leaf residues of Gliricidia, a an active triterpene

compound was found to be released into the environment. This compound was identified as the 22 - epimer of Soyasapogenol, which appears to be a previously unknown compound.

A series of sesquiterpene -lactone germacronolides have been found as the active allelochemicals in dried leaves of Tithonia, as the leaf residues decompose in fields. Of the active allelochemicals isolated, previously known Tagitinin A, and Tagitinin C, and a previously unknown compound (proposed name Tagitinin G) appear to be the most important.

The significance of these findings indicate that Gliricidia and Tithonia leaf residues could be effectively used in field crop production for their combined effect of promoting the growth of crops and suppression of weeds.

CHAPTER 1

GENERAL INTRODUCTION

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1.1 Allelopathy: A newly understood Phenomenon

The concept that one plant can influence the growth of another is well known in agricultural plant science, beginning historically with the first discussions on the subject of 'allelopathy' by DeCandolle (1832), to the investigations of Pickering (1917, 1919), the classic writing on allelopathy by Molisch (1937), and more recent reviews of Bonner (1950), Grummer (1955), Borner (1960), Evenari (1961), Whittaker & Feeny (1971), Rice (1974; 1984) and Putnam (1985a, b; 1986).

Over the years it has become increasingly apparent that some interactions among plants involve substances released from one plant which have an influence upon another plant, the phenomenon that has come to be known as 'allelopathy'. This subject has received increasing worldwide attention within the past few decades, particularly in relation to its significance in both natural and man-managed ecosystems. Allelopathic phenomena refer to all biochemical interactions (stimulatory and inhibitory) among plants including micro-organisms (Molisch, 1937). The fact that the term "allelopathy" is literally translated as "mutual harm or suffering" led to some initial confusion and other interpretations. Many scientists including Rice (1974), initially interpreted the definition to include "only higher plants and other harmful interactions." However, this confusion has been now cleared and there is general agreement that Molisch's broad definition is probably the most appropriate, because considerable research has indicated the involvment of micro-organisms and lower plants in phytotoxin production. Also natural compounds that inhibit growth at certain concentrations often enhance growth at lower concentrations (Rice, 1984;

Putnam, 1985a, 1986). In the recent past, Bhandari and Sen (1983), suggested that the term allelopathy should refer to the effects of one plant species, including micro-organisms (the 'donor'), on the germination, growth, development and metabolism of plant of another or same species (the 'recepient'), through reciprocal biochemical interactions, a view that is supported by Rice (1984) and Putnam (1985a), as well.

The very crucial point concerning allelopathy is that its effects depend on a chemical compound being released by one plant species and added to the environment of another. Chemicals that impose allelopathic influences are called 'allelochemicals' or allelochemics. If present in low enough concentrations, they may stimulate, rather than inhibit the growth of a neighbouring plant. Allelochemicals are present (usually in conjugated form) in virtually all plant tissues, including leaves, stems, fruits, buds, seeds and roots (Rice, 1974, 1979, 1984; Putnam, 1985a, b, 1988a). Under certain conditions, these compounds may be released into the environment (atmosphere or rhizosphere) in sufficient quantities and with enough persistence to affect a neighbouring or successional plant.

The phenomenon of allelopathy is therefore separated from "competition", a substractive process, which involves a removal or reduction of some factor (water, minerals, light, energy) from the environment that is required by some other plant sharing the same habitat. The latter phenomena has been termed "allelospoly" (Bykov, 1970). Both mechanisms are not easily separated in field ecological studies and Muller, (1966) suggested the term "Interference" to be used whenever the cases of mutual inhibition are not clealy separated. Szczepanski (1977) has described three posible mechanisms for plant- plant interference which include: (a) competition for necessary growth factors ("allelospoly"), addition of toxic substances or factors to the environment ("Allelopathy") and possession of herbivore toxicant or repellent substances that prevent grazing ("Allelomediation").

The concept of allelopathy has been supported and developed by many workers who have written on the subject at various times (Evanari, 1961; McCalla & Haskins, 1964; Tukey, 1969; Muller & Chou, 1972; Datta & Sinha-Roy, 1974; Rice, 1974, 1979, 1984; Roy 1974; Chatterji, 1975; Chou & Lin, 1976; Altieri & Doll, 1978; Putnam & Duke 1974, 1978; Sen, 1981; Chou, 1982; Bhandari & Sen, 1983; Putnam, 1985a,b, 1986). In all these reviews there is extensive evidence that allelopathy may contribute to patterning of vegetation in natural ecosystems and plant succession. Distinct zones of inhibition are present under and adjacent to a variety of woody species. Often toxins from their residues and litter are implicated. It is also suggested that aggressive perennial weed species quickly gain dominance in given localities by exploiting allelopathic mechanisms. A very large number of individual weed species are known to possess allelopathic potential (Rice, 1974, 1984; Bhandari & Sen, 1983; Putnam, 1985a,b, 1986; Putnam & Weston, 1986). Chapter 2 of this Report provides a broader literature review and an overview of the developments and advances of knowledge that

has been accumulated over the last 30- 40 years. Alleged allelochemicals represent a myriad of chemical compounds from simple hydrocarbons and aliphatic acids to complex polycyclic structures. They represent acids, aldehydes, cyanogenic glycosides, thiocyanates, lactones, coumarins, quinones, flavonoids, tannins, alkaloids, terpenoids and a variety of other chemicals, as reviewed in Chapter 2.

1.2 Proof of Allelopathy

According to Fuerst and Putnam (1983) and Putnam and Tang (1986), although numerous studies have provided excellent evidence of allelopathy, seldom have investigators followed a specific protocol (similar to Koch's postulates for proof of disease) to achieve convincing proof. They suggest that proof could generally involve the following sequence of studies:

(1) Demonstrate interference using suitable controls, describe the symptomology, and quantitate the growth reduction.

(2) Isolate, characterize, and assay the chemicals against species that were previously affected. Identification of chemicals that are not artifacts is a key step in allelopathy.

(3) Obtain toxicity with similar symptomology when chemical(s) are added back to the system.

(4) Monitor release of chemicals from the donor plant and detect them in the environment (soil, air, etc.) around the recepient and, ideally, in the recepient.

1.2 Allelopathy, as a Tool in Agriculture

The potential impacts of allelopathy on agriculture have been also reviewed extensively during the past 2 -3 decades (Tukey, 1969; Altieri & Doll, 1978; McCalla & Haskins, 1964; Patrick et al 1964; Putnam & Duke, 1978, Putnam, 1983a, 1985a) Emphasis has been placed on the detrimental influences of plants or their residues that may provide reduced crop growth or productivity. In particular, problems with certain crop rotations, toxicty of surface residues in reduced-tillage systems and 'stubble-mulch farming, problems of autotoxicity and orchard-replanting or forest regeneration and strong interference by certain weed species are frequently mentioned. As Putnam (1985a) states, it is not clear whether reduced plant (crop) growth is a direct result of released toxins, or whether the toxins precondition the plant to invasions by plant pathogens, or predispose them to other detrimental effects. Rice (1974, 1984) indicated that allelopathy may contribute to weed seed longevity problems through at least two mechanisms: chemical inhibitors in seeds prevent seed decay by microbes or

inhibitors function to keep seeds dormant, yet viable for many years.

Putnam (1983a, 1983b, 1985a, 1986) and his associates (Putnam & Duke, 1974; Putnam & De Frank, 1983; Putnam et al, 1983) have sharply focussed the attention of agriculturists all over the world, on exploiting allelopathy as a weed control strategy. They have recognized the fact that strongly allelopathic weeds such as quackgrass [Agropyron repens (L.) Beauv.], yellow nutsedge (Cyperes esculentus L.) or plants such as black-mustard [Brassica nigra (L.) Koch] can dominate agricultural sites. One of the main ideas being tested is whether crop species also could be made to become 'dominant' over weeds and suppress the latter, using allelopathic mechanisms. One of the approaches followed has been to find allelopathic characteristics within the germplasm of crops, and to attempt the introduction of these genetically into other cultivars (Putnam & Duke, 1974). Another major approach has been the possible use of allelopathic species as 'rotational crops' or 'intercrops', or allelopathic plant residues as 'mulches'.

Plant materials, viz; fresh leaves, dried leaf materials, play a vital role in agriculture as a source of fretilizer. Plant materials including 'green manure' have been applied successfully to increase the availability of nitrogen for plants in most parts of the world. A 'green manure' is a plant leaf material often incorporated into soil while green or soon after its maturity, for soil improvment (SSSA, 1979). Green manuring is the practice of incorporation into soil, undecomposed plant material which can be grown in situ or brought from outside, with the object of increasing soil fertility, especially soil organic matter and inorganic soil components.

In addition to green manure, a variety of plant residues are left or placed on the surface of the soil deliberately as 'surface mulches'; 'mulches are worldwidely regarded as very effective in controlling soil erosion by wind and water. However, the presence of plant residues has been reported to both increase and decrease crop yeilds (Putnam & Duke, 1974; Putnam & De Frank, 1983; Guenzi et al, 1967). The availability of greater amounts of nitrogenous materials and other nutrients have been regarded as the cause for well-documented yield increases, while yield reductions have been attributed as being due to phytotoxic substances present in the residues. Reports also indicate that the presence of certain plant residues either as incorporated residues or as surface mulches, can reduce the biomass of certain weeds (Overland, 1966; Putnam & Duke, 1974; Putnam & De Frank, 1983; Putnam et al, 1983) and thus allow for higher crop yields. Thus, it is now accepted that under certain conditions, plant residues can suppress weed species and be an effective tool in weed control. The phenomenon of allelopathy is recognized as being involved (Rice, 1974; 1984).

Allelochemicals which are contained in such incorporated residues

or surface mulches, may be directly released to the soil system by leaching in rain water or daily watering, and also by gradual decomposition of residues by microorganisms. The liberated toxic chemicals may cause significant damage to weed seeds which lie shallowly buried on the fields. Most probably the damage to established seedlings can be expected to be much less. The quantity or concentration of a toxic chemical required to elicit an adverse response may be high and such amounts may not always occur in fields, although in many laboratory experiments these effects are easily demonstrable. The transient and ephemeral nature of most allelo-chemicals and their concomittant short persistences in natural habitats also raises doubts about long-term effects in every situation. Alteiri and Doll (1978) stated that cultural practices may interfere with the dynamics of inhibitors, affecting their concentration either in plants or in the soil and the presence of several weed species in close proximity, could result in interactions between inhibitory and stimulatory substances.

Guenzi and McCalla (1966a, b) showed that most crop residues contain water-soluble substances that can depress growth of corn (Zea mays L.), wheat (Triticum aestivum L.) and sorghum (Sorghum bicolor L.). They identified five phenolic acids from a number of plant residues and showed that all inhibited the growth of wheat. Ferulic acid was the most common phenolic acid in wheat residues. Chou and Patrick (1976) isolated and identified a number of phytotoxic compounds from soil amended by rye (Secale cereal L.). Many other studies have also indicated that compounds from plant residues can adversely affect plant growth (Overland, 1966; Liebl & Worsham, 1983; Shilling et al, 1985). Shettle and Balke (1983) have further demonstrated that allelopathic compounds can selectively suppress certain weed species. Cheema et al (1990) recently reported that wheat residues, particularly roots and straw, left on the surface or incorporated in cotton fields, were effective in controlling both grass and broad-leaf weeds.

The possibility that microbes play a pivotal role in the production and persistence of phytotoxic compounds from plant residues has also been demonstrated (Guenzi et al, 1967; Chou & Patrick, 1976; Liebl & Worsham, 1983). Guenzi et al (1967) found that aqueous extracts of weathered corn and sorghum residues were most phytotoxic to heat growth after 4 and 16 weeks of decomposition, respectively. The greatest phytotoxicity exhibited by aqueous extracts of wheat and oat (Avena sativa) straw occurred at or near harvest time, with essentially all toxicity gone after 4-weeks of decomposition. Kimber (1973a, b) found that aqueous extracts of several grasses and legumes that had been rotting for periods of up to 21 days were more inhibitory to the growth of wheat. He also showed that straws cut while still 'green' produced a higher level of toxicity than those cut when fully mature. Toussoun et al (1968) found that production of water-soluble phytotoxins of barley (Hordeum vulgare L.) residues in soil require a soil moisture content above 30% of soil and residue dry weight. Extracts became toxic 7-10 days after incorporation into soil with phytotoxic activity reaching a maximum in 3-weeks. Thus, microorganisms can enhance and/or

reduce the phytotoxicity of plant residues.

The implication that 'living crops' can allelopathically suppress weeds has been made by Putnam and Duke (1974) and Leather (1983a, b). Their studies demonstrated that the potential for suppression of weeds will be enhanced by crop selection. Putnam and Duke (1974) suggested that there is a potential for breeding crops to better suppress weeds by utilizing and improving allelopathic characteristics. Even if it is not always possible to get excellent weed control by applying into fields allelopathic plant residues, it is generally accepted that isolation and characterization of such chemicals could be valuable. Since allelochemicals have been described as the plant's own herbicides, it is thought that such allelochemicals may provide clues for novel herbicide chemistries. Detailed knowledge of allelopathic phenomena and allelo-chemicals involved in natural plant communities can provide excellent models for new strategies in developing highly selective herbicides (Putnam, 1988a, b). Allelochemicals should serve as the model for future herbicides if environmental compatibility is a required feature. Novel agricultural chemicals may be synthesized based on the structure and characteristics of these compounds. Synergistic or antagonistic compounds may be designed to amend the soil, thus enhancing or suppressing the allelopathic interaction in the interest of crop management. Recent and continuing trends towards reduced or no-till agriculture have increased the need for newer more cost-effective and efficacious herbicides (Shilling et al, 1985).

Herbicides with new or novel mechanisms of action are desired to prevent the development of weed resistance to long-used herbicides. Use of selective herbicides have encouraged shifts in weed floras to weed species more closly related to the associated crops, a situation requiring that future herbicides have been greater selectivity. As the need for new and novel herbicides are increasing. Clasical synthesis and screening are yeilding fewer returns at the same time, licensing and registration of new herbicides are increasingly difficult and expensive. Thus, studies of natural products are becoming attractive to produce new agro-chemicals.

1.4 Methodologies used in Allelopathic studies

For any suspected allelopathic species, leachates of its leaves, root exudates, residues and soil previously in contact with its root are normally tested against potential receptor species from the same locality. The most common technique has been the preparation of extracts/leachates by grinding or thorough soaking of either dried or live plant parts in water. After soaking plant material for varied lengths of time or grinding, the exuded material is usually filterd or centrifuged before bioassaying mainly by petridish method or in soil under glasshouse and field conditions or nutrients solution using crop or weed seeds as test material.

measurement of radicle elongation so widely used to express growth and development of seedlings, is often complicated by curling and other alterations in morphology. This problem is usually alleviated by using fresh weights or dry weight to measure growth.

Though many researchers have been using petridish bioassays to demonstrate allelopathic potential of suspected plant parts, several drawbacks inherent in this technique under laboratory conditions have also been noted. Mainly, the effects shown in petri dish assays may not appear in field experiments, Sometimes the laboratory trials conducted with plant parts result in negative effects, whereas in natural ecosystem the same plant exbitits positive effects. This is due to the contribution of microbial activity. Even inactive chemicals leached out from the plant parts may get converted to active chemicals, due to the microbial transformations.

1.4.2 Isolation and Characterization of Allelochemicals

Once the presence of strongly bioactive chemicals has been demonstrated from an aqueous extract or leachate, isolation and chemical separation of the compound(s) may be accomplished by partitioning on the basis of polarity into a series of solvents from non-polar to a polar solvent like methanol. The compounds may also be separated by molecular size, chargre, or adsorptive charasteristics, etc. Various chromotography methods are utilized, including column, thin layer (TLC), Gas- Liquid (GLC), and more recenty High Presure Liquid (HPLC) systems. HPLC has proven particularly useful for separations of water solubles compounds from relativily crude plant extracts.

Previously, the major effort toward compound identification involved chemical tests to detect the specific functional groups, whereas characterization is now usually accomplished using series of spectroscopic analysis. Initially Ultraviolet (UV) spectroscopy is useful in this regard, to detect specific functional groups. More recently, Infra Red (IR) spectroscopy and multi dimentional Nuclear Magnetic Resonance (NMR) and sophisticated Mass Spectroscopic methods including Electron Impact (EI), Chemical Ionization (CI), Fast Atomic Bombardment (FAB), Field Desobtion (FD), etc. have helped immensely in determining the structures of active principles.

Fig. 1.1 below is a general scheme of approaches used in allelopathy research and followed in the research embodied in this Report.

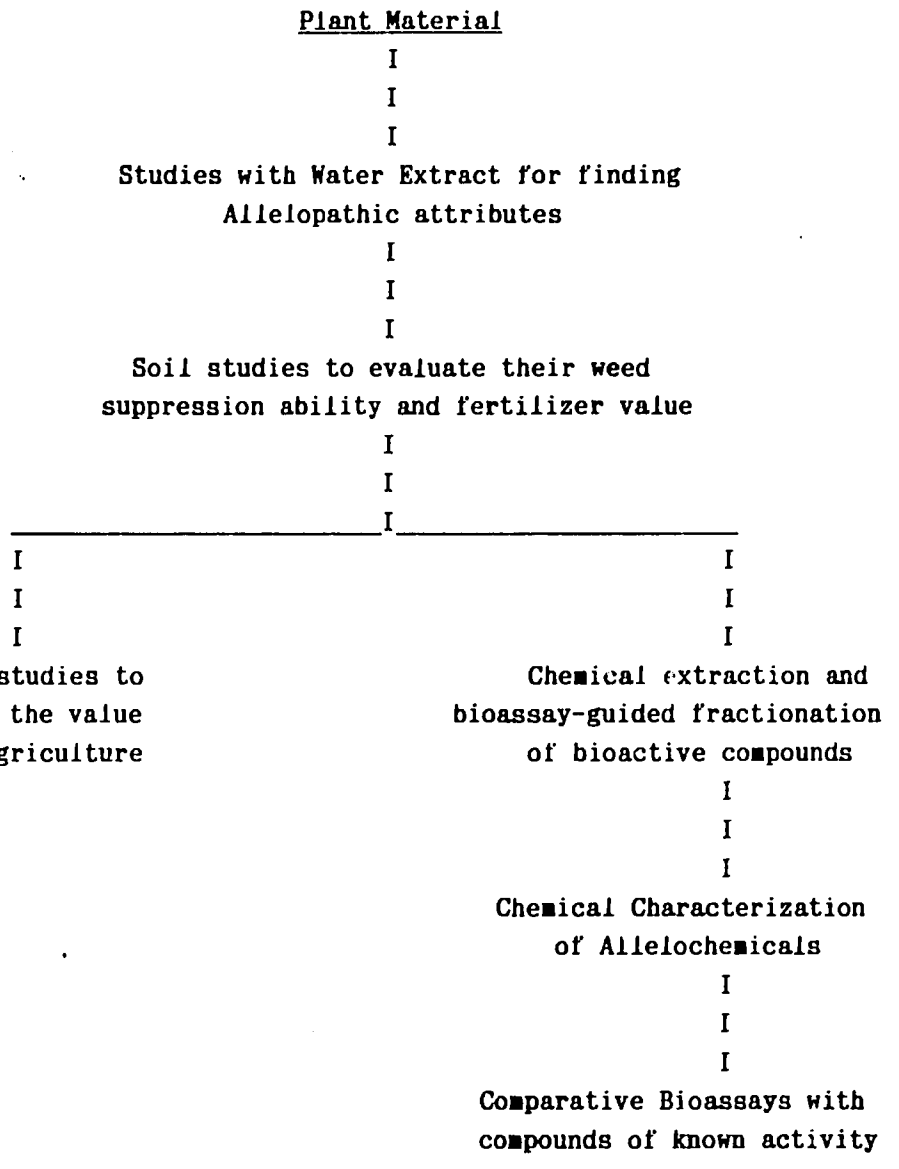


Figure 1.1: An approach for studies on Allelopathic potential of plant species

1.5 The Objectives of the current Research

This Report embodies research carried out in Sri Lanka with the following specific Objectives:

(a) To investigate and identify from among selected plant species, those that have a high potential for allelopathic interactions.

(b) To explore the possibility of manipulating or utilizing allelo-

pathic species (or their residues) in biological weed suppression in field crop production.

(c) To extract and characterize the inhibitory (or stimulatory) allelochemicals from species having high activity, with a view to obtaining clues for synthesis of biological pesticides and/or plant growth regulators.

After preliminary research, reported in Chapter 3, efforts were concentrated on two major plant species, namely Gliricidia maculata (H.B.K.) Steud. (Plate 1.1) and Tithonia diversifolia (Hemsl.) A. Gray (Plate 1.2). The leaves of both species, in fresh or dried state, are very commonly used in upland farming, to act as a source of nitrogenous compounds and as 'mulches'. The leaf material of the two species were investigated for their potential allelopathic bioactivities, when used as 'mulch', or incorporated residues. The studies concentrated mostly on the exploration of the posibility of manipulating or utilizing these allelopathic residues for weed control purposes. Given below are reviews of existing knowledge about the two species, as relevant to the investigations of the present research.

1.5.1 Gliricidia maculata

Gliricidia maculata (H.B.K.) Steud. is a fast-growing, deep-rooted, medium-sized, legume tree species, known by the vernacular names "wetahiriya", "maculatha", "girithisiya", "ginisiriya" and "albizzia". The plant, a native of Central America and West Indies, had been introduced to Asia in 1600's by the Spaniards who were involved in the spice trade. After first introductions to Sri Lanka in late 1880's from Trinidad for use in tea plantations as a 'shade tree', Gliricidia has become naturalized in the island. Although the species was introduced into the colonial plantations by the British, it became a popular species among local farmers and has been exploited in Sri lanka for multiple uses in agroecosystems (Seneviratne Banda et al, 1992; Bandara et al, 1992). One main reason for its wide use in a variety of land-use systems is the easy establishment through 'stakes' or 'cuttings' (Liyanage & Marasighe, 1991; Perera, 1992). The National Academy of Sciences of U.S.A. identified Gliricidia as an important fuelwood tree for the humid tropics, and listed many of the attributes that make it a valuable species.

The foliage of Gliricidia is known to provide excellent 'green manure' to a variety of upland crops and the lowland crop rice. For this purpose the leaves are incorporated heavily into soil by ploughing or left on the soil surface as a 'mulch' for decomposition to take place. Research in Sri Lanka has shown that application of two tonnes of Gliricidia leaves per hectare to paddy-field can result in a saving of about 25 kg of urea. On a dry weight basis Gliricidia leaves are known to contain ca. 3.7-4% nitrogen, 0.38% phosphorous, 2-2.1% potassium, 1.4% calcium and 0.4% magnesium. Gliricidia has been recommended for use as a green manure in

Figure 1.1: Gliricidia maculata

Figure 1.2: Tithonia diversifolia

coconuts as well (Liyanage & Marasinghe, 1991). Application of 30 kg of foliage per palm per year appears sufficient to supply the nitrogen requirements of a tree.

The use of Gliricidia leaf material as 'green manure' has been already studied in detail, and the positive effects noted (Gunasena et al, 1991; Bandara et al, 1992; Gunathilake & Liyanage, 1992). Jayawickrama and Thenabadu (1989) made a significant contribution to the existing knowledge by comparing the breakdown of Gliricidia foliage added to soil at 5% w/w as 'green manure', with other popular species Leucaena leucocephala, Tithonia diversifolia and Croton lacciferus (Jayawickrama & Thenabadu, 1989) (Table 1.1). A highly significant increase of water-soluble and exchangeable-N in soil was found due to the incorporation of green manures, compared with untreated controls (Table 1.2). Legume green manures (i.e. Gliricidia and Leucaena) which contained high amounts of N, released more N into soil, when compared with the other two non-legume species. Incorporation of the green manures caused a significant increase in microbial activity as measured by CO_2 liberation. Jayawickrama and Thenabadu (1989) concluded that green manures should be applied 'in bulk', and if so used, they would have high potential as excellent sources of fertilizer elements, especially N.

Table 1.1: Characteristics of four green manures (Jayawickrama & Thenabadu, 1989)

Green manure	N% *	C% *	C/N Ratio
Croton lacciferus	2.63	51.20	19.20
Tithonia diversifolia	3.92	48.30	12.32
Gliricidia maculata	4.24	51.20	12.07
Leucaena leucocephala	5.42	52.70	9.72

*** g/ 100g leaf dry matter**

Table 1.2: The amounts of maximum released nitrogen in treatments during the incubation period (Jayawickrama & Thenabadu, 1989)

Green manure	Maximum Released N *	Time (Dates)
Croton lacciferus	72.70	28
Tithonia diversifolia	77.00	24
Gliricidia maculata	80.70	24
Leucaena leucocephala	86.24	24
Control	6.44	28

* g/ 100g soil

Gliricidia is also widely grown in tea estates as a 'shade plant'. soil stabilizer and also to act as wind breaks. More recently, with the interest on 'alley-cropping' and conservation farming, Gliricidia has been promoted as an extremely useful tree for use as a 'hedge-row' plant to be grown in rows 4 - 5 m apart and food crops in between (Wijewardene and Waidyanatha, 1984). Its ability to grow fast, 'coppice' in response to frequent lopping of branches, and establish itself rapidly from stem-cuttings, has made Gliricidia one of the most suitable hedge-rwo species in alley-cropping systems, a vital aspect of which is the use of 'loppings' of the legume as 'mulch' in the alleys (Wijewardene & Waidyanatha, 1984). This 'mulch' appears to prevent many of the annual weeds from getting established during the cropping season, in addition to returning a major part of the nutrients to the soil when they decompose in the avenues, while the crop plants grow. This system of alley-cropping, using Gliricidia as hedge-rows, has resulted in enhanced yields of rice and maize in Sri Lanka, at reasonable levels of sustainable productivity Weerakoon (1982).

Handawela and Kendaragama (1992) recently summarized the results of many years of research trials conducted at Maha-Illuppalama in Gliricidia alley-cropping systems. The prime objective of these studies had been to study the effect of Gliricidia on soil fertility, productivity of rain-fed uplands of the dry zone of Sri Lanka and weed control ability of the leaf loppings applied as mulches. They reported the improvement in bulk density, organic matter content, exchangeable K, ca, Mg, Nitrate-N, Ammonium-N and

earthworm activity in the soil of Gliricidia tree plots, compared to bare plots without trees.

A suppression of grass weeds and dominance of broad-leaved species was also reported under Gliricidia alley-cropping (Table 1.3). Grain yields of maize and sesame obtained with and without post-planting weeding in these trials had shown that weeding was more important in the treeless, bare plots than in the tree plots (Handawela & Kendaragama, 1992), and that Gliricidia loppings had a profound effect on weed composition and weed density. The weed control ability of Gliricidia foliage has been noted for a long time, since the beginning of "alley-cropping" systems in Sri Lanka and elsewhere (Ray Wijewardene- personal communication).

Table 1.3: The Effect of Gliricidia on Density and Composition of weed flora in unweeded plots (Handawela & Kendaragama, 1992)

	Weed density and composition					
	1985/86 Maha			1986/87 Maha		
N level (kg/ha)	Total weed Dry matter	Broad leaved	Grasses	Total weed Dry matter	Broad leaved	Grasses
	(kg/ha)	(%)	(%)	(kg/ha)	(%)	(%)
(a) With trees:						
0	1419	86	14	2403	50	50
30	1251	79	21	1279	86	14
60	805	82	18	1742	74	26
100	971	89	1	1301	76	24
150	855	87	13	1717	82	18
Average	1060	85	15	1690	73	26
(b) Without trees:						
0	1825	13	87	1738	10	90
30	2038	14	86	1874	20	80
60	2288	06	94	1786	05	95
100	1973	17	83	1688	23	77
150	1906	13	87	2038	10	90
Average	2006	13	87	1825	14	84

* N-fertilizer added in addition to Gliricidia loppings added as normal treatments in the Trials.

Handawela and Kendaragama (1992) also provided an estimate of removal of N from N zero plots (Table 1.4). The total N removal from the field amounted to 304 and 129 kg N/ha in treatments with and without Gliricidia trees, respectively. On average, the crops in the tree treatments were estimated to have removed 175 kg N/ha from the field compared to those in treatments without trees. The authors suggested that this could be the amount of N supplied by the tree loppings. At the modest level of yield of tree loppings estimated at 5000 kg/ha/year and N analysis of the loppings at 3%, N supply to the field over the seven year study period amounted to 1050 kg N/ha. Thus, Gliricidia loppings supplied about 150 kg/N/ha/year of which about 16% was removed by the maize and sesame.

Gliricidia leaves have also been popularly used as 'green fodder' for livestock (cattle, sheep and goats) in Sri Lanka, particularly in dry seasons where quality grass-feed is scanty. As a fodder it is regarded as superior to other fodders fed to livestock because the leaves contain a high % of crude protein (26%), with high digestability (60%) (Perera, 1992). Its protein is also known to be very rich in essential amino acids.

Table 1.4: Removal of N from N zero plots during 1981-1987 in Gliricidia Alley-cropping Trials (Handawela et al, 1992)

	With trees	Without trees
Maize grain yeild, kg/ha	13264	4942
Sesame grain yeild, kg/ha	2376	1272
N removal via Maize, kg/ha	212	79
N removal via Sesame, kg/ha	92	50
Total N removal, kg/ha	304	128

However, due to continuous feeding of animals on increasing Gliricidia leaf meals, particularly with poultry, goats and cattle (Perera, 1992). Although none of these effects have been thoroughly investigated, toxic compounds in leaves are suspected (Perera, 1992). The fact that Gliricidia and several other tropical legumes contain 'Coumarin' is well-known (Duke, 1986). Coumarin is known to undergo bacterial conversion to 'dicoumarol' (Fig. 1.2), a substance chemically similar to Vitamin K in

function (Hochman, 1966). Dicoumarol interferes with Vitamin K in blood clotting. Dicoumarol is also used in rodenticides ('rat-poison') as anti-clotting agents. It has been observed that coumarin is converted to the toxic dicoumaryl during spoilage of Gliricidia leaves; the same conversion may be taking place during drying and storage, but silage making appears to make the feed safe (Perera, 1992).

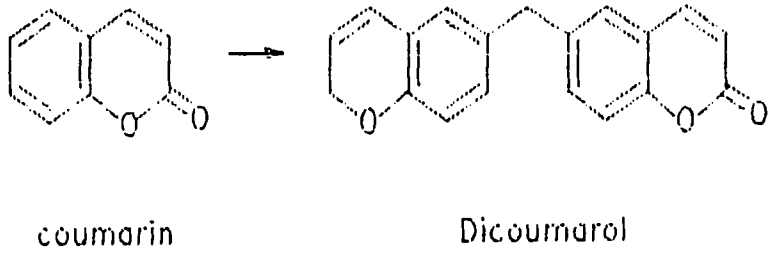


Figure 1.2: Dicoumarol formation from Coumarin

Although Gliricidia has been identified as a major legume tree species which can be exploited in a multitude of ways in tropical countries, and has been the subject of a vast amount of research, very little research has been done on the chemical compounds of its foliage. The roots, bark and seeds have also been reported as poisonous; the leaves may also be toxic to humans, although they are eaten in some parts of the tropics, possibly because cooking inactivates the toxin.

1.5.2 Tithonia diversifolia

Tithonia diversifolia (Hemsl.) A. Gray is a plant that belongs to family Asteraceae (Compositae) and is a common and widely spread weed in the mid- country of Sri Lanka, and occasionally in the low-country as well. It has also been recorded as a common weed in parts of India and Mexico. The plant is native to South America from where it has been introduced to India, Burma, Malaya, China and South Africa. A perennial bush usually not

exceeding three or four meters in height, the plant reproduces with seeds borne on conspicuously large yellow flowers. The plant has been known locally for its bitter tasting leaves and has been called 'Thitta gas'. The other vernacular names by which the plant is known are; 'Wal Sooriyakanta' (S) and 'Wild Sunflower' (E) both names possibly reflecting the similarity to true 'Sunflower (Helianthus annus). Tithonia diversifolia also has a medicinal reputation as a 'remedy' for malaria and in the treatments of sores in coastal Mexico and farther south.

Tithonia has also been used as a common 'green manure' plant in Sri Lanka's agriculture. Jayawickrama and Thenabadu (1989) in their work on the mineralization of common 'green manures', reported that Tithonia leaves also contained nitrogen comparable to that of legumes (Table 1.3). They argued that Tithonia compared favourably with legumes as source of exchangerble nitrogen and confirmed the advantage of using Tithonia 'green manure' species in upland farming.

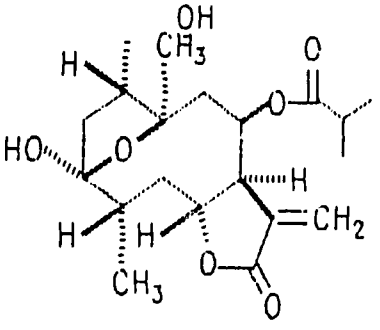
Leaves of Tithonia diversifolia have been chemically analysed by Baruah et al. (1979) who have isolated many sesquiterpene lactones as being the major bitter principles in these leaves. Sesquiterpene lactones Tagitinin-A, Tagitinin-C, Tagitinin-F and Tagitinin-D have been isolated from T. diversifolia and their stereochemistry described by these workers. Baruah et al (1979) and Raghwendra et al (1976, 1977) reported the presence of six sesquiterpene lactones, namely Tagitinin-A (1), Tagitinin-B (2), Tagitinin-C (3), Tagitinin-D (4), Tagitinin-E (5) and Tagitinin-F (6) from a related species, T. tagitiflora, and suggested that these compounds are the bitter-tasting principles of the leaves.

As part of a search for secondary metabolites of Compositae with potential biological activity, Herz and Sharma (1975) reported on the isolation and structure determination of two germacranolides, tifruticin (7) and deoxyfruticin from Tithonia fruiticosa Canby & Rose, and another germacranolide tirotundin and its ethyl ester from Tithonia rotundifolia (Mill.) Blake.

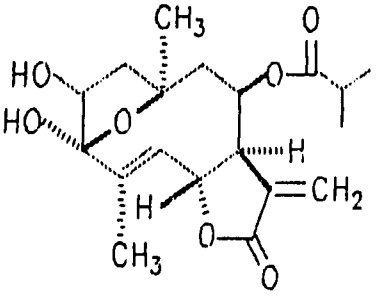
1.6 Preliminary Research

At the initial stages of this project, several plant species suspected of potential allelopathic activity or being involved in allelopathic phenomena, were screened in preliminary experiments based on activity of leachate or blended aqueous extracts (as described in Chapter 3) on a variety of indicator species.

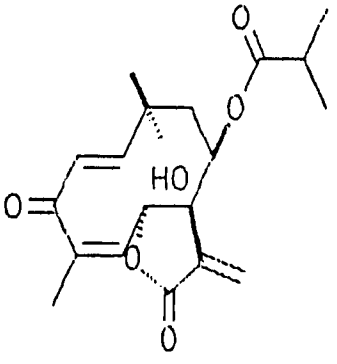
Among leguminous species, Gliricidia maculata H.B.K, Leucaena leucocephala (Lam.) de Wit, and Pueraria phaseoloides R. Br., three of the commonest plants used in upland agriculture in Sri Lanka, were extensively tested for bioactivity. As stated previously, Gliricidia and Leucaena are



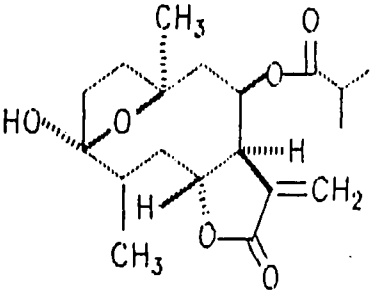
Tagitinin A (1)



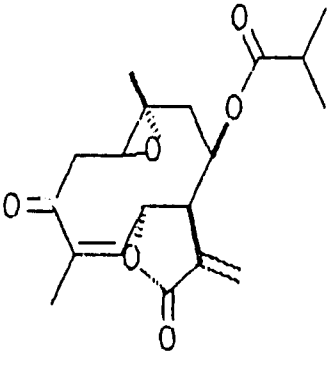
Tagitinin B (2)



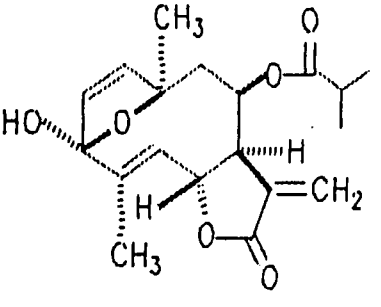
Tagitinin C (3)



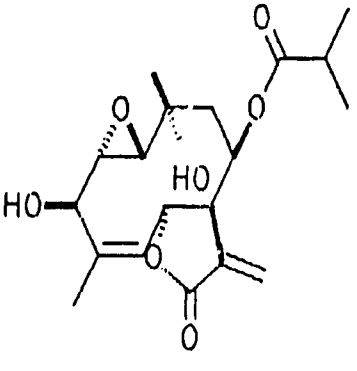
Tagitinin D (4)



Tagitinin E (5)



Tagitinin F (6)



Tifruticin (7)

the most widely grown hedge-row species in 'alley-cropping', in addition to being extensively used as 'green manure' plants. Pueraria is one of the most extensively used creeping 'cover crops' in rubber and coconut plantations of the island. In addition to contributing heavily towards stabilization and conservation of soil, replenishment of soil nutrients, Pueraria 'living mulch' is known to suppress weed growth as well. After many preliminary screening trials described in previous Progress Reports of the Grant, Gliricidia maculata, potentially the strongest allelopathic species of the three legumes tested, was selected for further biological studies, chemical extraction, bioassay-directed fractionation and characterization of allelochemicals.

Three common weed species of the family Asteraceae, namely, Tithonia diversifolia, Eupatorium odoratum and Mikania cordata were also subjected to preliminary screening for potential allelopathic activity, as described in previous Progress reports. Of these three species, Tithonia was chosen for further biological studies and characterization of allelochemicals, because it showed the highest potential allelopathic effects.

This Final Report therefore, deals with the research carried out to meet the objectives stated earlier, focussing attention on the allelopathic interactions of Gliricidia maculata and Tithonia diversifolia and characterization of the allelochemicals responsible.

CHAPTER 2

LITERATURE REVIEW

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2.1 Historical Background
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2.3 Allelopathy and Interference
2.4 Sources of Allelochemicals and their Release
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2.6.5 In Horticulture
2.7 The Nature of Allelochemicals
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2.11 Allelochemicals as Herbicides

2.1 Historical Background

Man has been aware of a "chemical warfare between plants" for a long time. As early as 300 years B.C., Theophrastus had stated that chick pea (Cicer arietinum) did not reinvigorate land as other related plants (legume) did, but "exhausted" it instead, and also that chick pea destroyed weeds. Plinius, as noted by (Rice 1984), in the first century A.D. had reported that chick pea, barley (Hordeum vulgare), fenugreek (Trigonella foenum-graceum), and, bitter vetch (Vicia ervilla) all scorched up cornland.

According to Lee and Monsi (1963), a japanese document of 300 years ago reported that rain or dew which washed the leaves of red pine (Pinus densiflora) caused harmful effects to crops which were grown under the pine. De Candolle (1832), a pioneering 19th century Botanist reported that some plants excreted substances from roots and that these were harmful to other plants. He noted specific inhibition of oat (Avena) by thistles (Cirsium) and wheat (Triticum) by ryegrass (Lolium) plants. De Candolle also referred to a "soil sickness" problem in agricultural land caused by exudates of crop plants and that rotation of crops helped to alleviate the problem.

In spite of these many early observations and suggestions, convincing evidence for a naturally occurring chemical warfare between plants was not published prior to the present century (Rice, 1974). Between 1907 and 1911, U.S. scientists presented evidence that exhaustion of soil by single- cropping was due to addition of growth inhibitors to soil by some crop plants (Schreiner & Reed, 1907; Schreiner & Sullivan, 1909). An unidentified substance was extracted from the soil fatigued by the growth of cowpeas (Vigna catiang); this soil was inhibitory to the growth of cowpeas sown subsequently. McCalla and Duley (1949) showed that the widespred use of decaying wheat residues as 'stubble mulch' to reduce wind and water erosion in the U.S.A. during 1930s, resulted in severe yield losses of corn. With these picneering research articles the evidence for a most interesting relatively new field of chemical interactions between plants emerged.

2.2 Terminology

A German scientist Molisch (1937) coined the term "Allelopathy" to refer to 'reciprocal biochemical interactions between all type of plants including micro-organisms, that were both inhibitory and stimulatory'. The chemical battle that seemed to take place between certain plants became known by this new term. Allelopathy is characterized by a reduction in plant emergence and growth, reducing the performance of at least some individuals in an association.

The word "Allelopathy" (root word "ALLELON and PATHOS) is derived from Greek; "allelon" meaning 'of each other' and "pathos" meaning 'to suffer'; hence the word literally means 'the injurious effect of one upon another'. Rice (1984), later pointed out that Molisch's choice of the word 'Allelopathy' was technically incorect because the root word 'pathy' implied detrimental interactions only.

In the early 1970s, many researches favoured the definition of allelopathy 'as any direct or indirect harmful effect by one plant including micro-organisms on another, through the production of chemicals that are released in to the environment' (Rice, 1974; 1984). Putnam and Duke (1978) used the term to refer to the detrimental effects of higher plants of one species (the donor) on germination, growth and development of plants of another species (the recipient). However, after examination of numerous evidence, there has been general agreement that the effect of any given compound may be both stimulatory and inhibitory depending on its concentration in the surrounding medium. It is therefore, now agreed that the interactions between plants that may arise out of some plants releasing chemicals into the environment, may be inhibitory or stimulatory to other plants (Rice, 1984). Hence, the concept of 'allelopathy' now encompasses the original context with which Molisch presented his view.

As essential feature of this phenomenon is that the effects are exerted through chemicals (allelochemicals) added in to the environment by one plant species, 'the donor'. The effect of these compounds may be inhibitory or stimulatory depending upon the concentration of the chemical, the type of a 'recipient' species or other factors involved. Micro-organisms associated with higher plants may also play a significant role in the production or release of these compounds in nature (Guenzi & McCalla, 1966a, 1966b; Rice, 1984; Putnam & Duke, 1978).

Muir and Majak (1983) suggested that within the broad definition of allelopathy there exists two distinct forms of allelopathy. There is a general toxicity which is usually indiscriminate, affecting both the species producing the compound, as well as unrelated species. This appears to be a widespread phenomenon, and is usually associated with the decomposition of plant residues. A second type occurs where one species actively produces a chemical that inhibits the growth of other adjacent species. This may result in a competitive advantage to the species producing the allelochemical.

2.3 Allelopathy and Interference

Plants may interact with each other in a positive, negative or neutral manner. The beneficial associations, mutualism and symbiosis are common and well-known. For example, roots of higher plants and fungi form mycorrhizal associations with certain bacteria to form a mutually beneficial relationship. It is rare to find instances where plants are unaffected by neighbours.

It is however, more common to find neighbouring plants interacting in a negative manner, where the emergence and growth of one or both is inhibited. Generally these negative interactions are regarded as the outcome of 'competition' for resources of the environment (space, light, nutrient etc) which are in limited supply. Many plant ecologists in the early days expressed the view that allelopathy was not a separate phenomenon but was a part of 'competition' between species. However, Rice (1974) and Putnam (1986) argued that the term competition has been misused by many agricultural scientists to describe a broader 'interference' between neighbouring plants. Muller (1966) had earlier proposed that the adverse effects of one plant (or microorganism) on a neighbouring plant be termed 'interference', whenever the causes of inhibition are not clear in the interaction of the two species. The adoption of this term has allowed the confusion to be lessened considerably.

It has been now accepted that allelopathic phenomena and competitive interactions are different in one essential feature. Competition always implies limitation of resources and therefore, involves a

removal or reduction of resources (light, space, nutrient or water) or some factor by one species from the environment, which may adversely affect another neighbour. Allelopathy on the other hand depends on a chemical compound being added by one plant into the environment of another. In keeping with this new thinking, 'competition' which involves a removal of growth factors has come to be known as "Allelospoly", and the possession of herbivore toxicant or repellent substances that prevent grazing as "Allelomediation". Hence, "interference" between two plant species could be regarded as a combination effect of both "Allelospoly and Allelopathy".

Sometimes allelopathy appears to occur among individuals of the same species. Intraspecies toxicity appears to be a manifestation of allelopathy in nature and is termed "Autotoxicity". The ecological purpose of autotoxocity has been dificult to interpret. Scientists hypothesize that at least in perennial species, it may function as a mechanism to encourage furthe spreading of a species by vegetative propagules etc., rather than allowing them to concentrate in one area (Putnam, 1986).

Sometimes allelopathy may produce obvio s and even startling effects (Putnam, 1988a, 1988b). For example, tomatoes growing near black walnut (Juglans nigra) may suddently wilt and die, a phenomemon attributed mainly to the presence of naphthaquinone 'juglone' which is released in large quantities by walnut roots and litter. However, Putnam (1988a) suggested that more commonly, allelopathic influences may be subtle, perhaps never detected, and hence difficult to prove and separate from the competitive aspects of 'interference'.

2.4 Sources of Allelochemicals and their Release

Chemicals with allelopathic potential are present in virtually all plants and in all types of tissues, including leaves, flowers, fruits, buds, seeds, stems, rhizomes, tubers, and roots. They may get released to the environment by four general routes (Tukey, 1969; Rice 1984), as depicted in Figure 2.1, and briefly reviewed below:

(a) Weathering or Decomposition of Plant Residues-

Leaves and other plant parts falling on the ground may be decomposed by weathering and soil microbes, thereby releasing various phytochemicals (Overland, 1966). Loss of membrane integrity that previously imposed permeability barriers may then allow the release of compounds which may impose their toxicity in an additive or synergistic manner (Putnam, 1985a). In addition to direct release of compounds from tissues, microbes in the rhizosphere may induce production of phytotoxic compounds by enzymatic

degradation of conjugates or polyymers present in plant tissues (Putnam, 1985a). Toxicity arising from plant residues provides a major challenge for agronomists and weed scientists. Toxins from 'stubble-mulch' farming have proven to be toxic to certain rotational crops (Patrick, 1971; Guenzi & McCalla, 1962). Putnam (1985a) states that, not only can decaying plant residues influence crop emergence, growth and productivity, but they can influence similar aspects of weed growth. The duration of allelochemical production and toxic effects from decomposition of residues, is known to vary among different types of residues. Guenzi and McCalla (1966a) found sorghum, oats, and corn residues were nearly as toxic at harvest, as after partial decomposition and that the toxicity of decomposing residues persisted for 4-weeks in oat and for 22-weeks in corn and sorghum residues. Chou and Lin (1976) found that the autotoxic effect of decomposing rice straw persisted upto 4 months, although maximum toxicity was in the first month after rice straw had been ploughed in.

(b) Leaching-

Vast quantities of metabolites are emitted and leached from aerial parts of plants or from plant residues, by the action of rain, fog or dew. These may be added to soil causing inhibition of growth of nearby species (Tukey, 1969). Among compounds shown to be leached out from plants are organic acids, sugars, amino acids, pectic substances, gibberellic acid, terpenoids, alkaloids and phenolic compounds. Juglone, an allelochemical from walnut (Juglans nigra) leaves, has been known to be leached out with water drippings and added to soil around the plant, causing toxicity to tomato plants (Rice, 1984). Colton and Einhellig (1980) found leaf leachates of velvetleaf (Abutilon theophratsi Medic.) to be inhibitory to soybean. A suspected ..-napthol derivative and scopolin were identified in the leaf leachate of sunflower (Helianthus annus L.) (Wilson & Rice, 1968). Leaf leachates of johnsongrass were also found to be inhibitory to the growth of several species (Abdul-Wahab & Rice, 1967). Leachates of Chrysanthemum leaves were shown to be toxic and cause a failure of understory plants to grow in forest stands (Lee & Monsi, 1963; del Moral & Muller, 1970).

(c) Root Exudation-

Plants are known to exude/libarate a myriad of chemical compounds through their roots or underground organs such as tubers and rhizomes (Rovira, 1969). The compounds are probably actively exuded, leaked or they may be released by dead cells which are sloughed-off the roots. Much of the evidence for root-mediated allelopathy has come from studies in which solutions passing by root systems of one plant are recycled into media containing an indicator species. Common lambsquater (Chenopodium album L.) plants were found to exude toxic levels of oxalic acid into solution, when

they reached the flowering stage (Caussanel & Kunesch, 1979). Exudates from giant foxtail (Setaria faberii Hermm.) have been shown to inhibit the growth of corn (Bell & Koeppe, 1972). The inhibition of wild mustard [Brassica kaber (DC.) L.C. Wheeler], caused by Avena species was though to be due to exudation of scopoletin and other allelochemicals (Fay & Duke, 1977). Sixteen inhibitory compounds exuded from the roots of bigalta limpograss (Hemarthia altissima) have been identified as a variety of benzoic, cinnamic, and phenolic acids (Tang & Young, 1982). Parthenin, a strong allelochemical has been found in root exudates of Parthenium hysterophorus (Kanchan & Jayachandran, 1979a).

(d) Volatilization -

Volatile toxins from plants are known to escape into the environment and affect the growth of other plants growing nearby (Muller & Muller, 1964). This phenomenon appears to be particularly common in plants of arid or semi-arid environments. Among the genera known to release volatiles are Artemisia (Muller et al, 1964), Eucalyptus (Moral & Muller, 1970) and Salvia (Muller, 1965). Volatile inhibitors are usually terpenoid essential oils of the monterpene or sesquiterpene group (Fischer, 1986), and are released into the environment from special glands on stems and leaves. Such volatiles may be absorbed through cuticles of neighbouring plants directly from air, or as condensation in dew. It is also possible that they may be absorbed on dry soil, released into soil solution in wet soil and taken up by plants from soil solution (Muller & Moral, 1966)

2.5 Fate of Allelochemicals in the Environment

Once allelochemicals are released into the immediate environment, by a donor plant or their residues, they may persist for some time and accumulate in sufficient quantity to affect other plants; or else the chemicals may be constantly released in to soil to have lasting effects (Rice, 1974; 1984). The potential fate of an allelochemical in the environment has been discussed by Fisher (1987) and can be summarized as below (Fig. 2.1)

Whatever the quantity, the phytoactive compound released from a plant enters the soil where several things can happen. The order of 2nd, 3rd and 4th steps in Fig. 2.1 is not necessarily the order in which the reactions actually occur. In fact if the compound is volatile, these steps may be skipped entirely. In most cases, however, it appears that allelochemicals enter the soil. Soil colloids are capable of adsorbing most allelochemicals. Such adsorption would result in temporary loss of its toxic or stimulatory activity. Chemical changes could occur during adsorption that would permanently deactivate the compound. The adsorptive reactions are

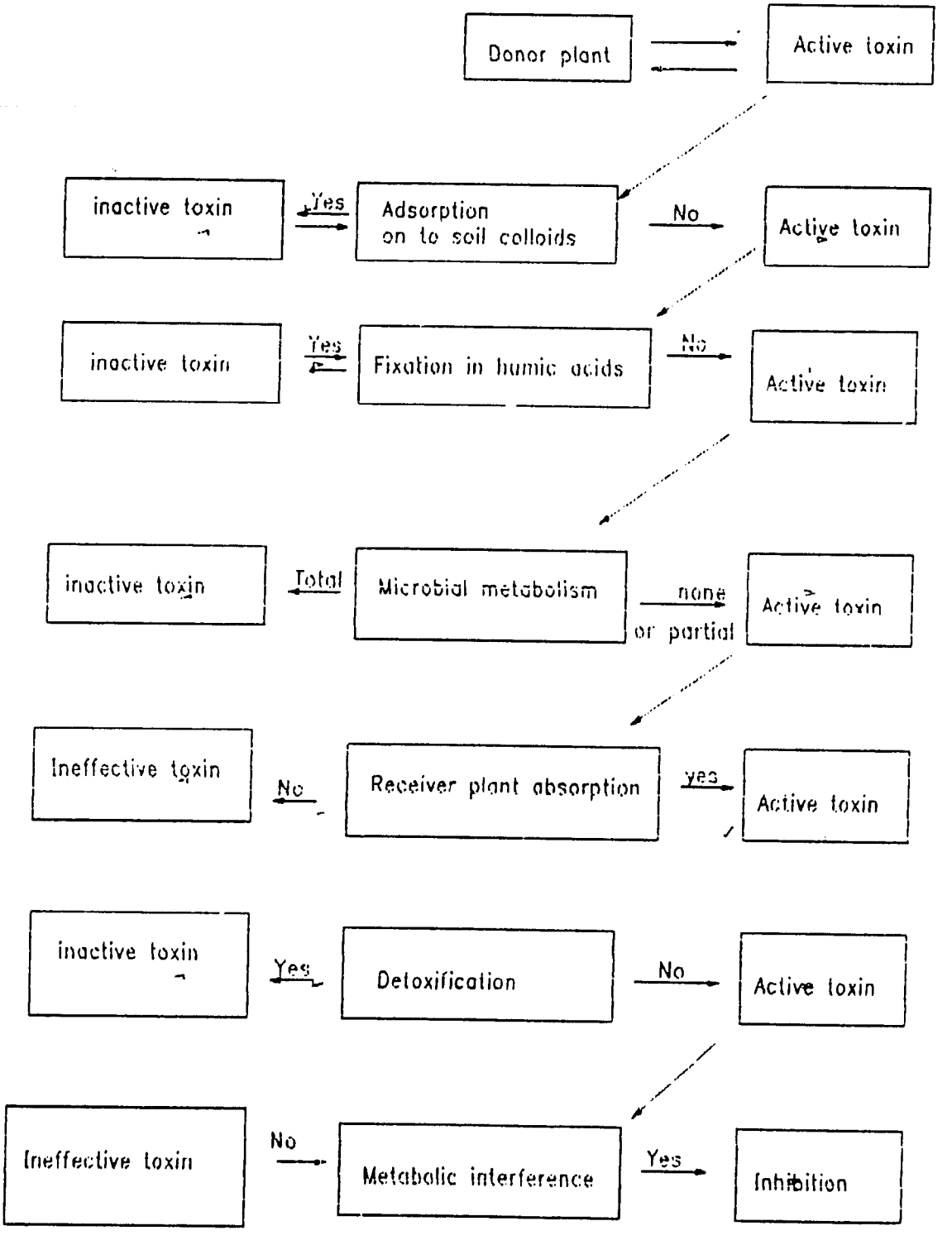


Figure 2.1: The posible fate of allelochemical in the environment (Fischer 1987)

usually reversible, so that some or all of the chemical would still be available for uptake by a receiver plant.

The phytoactive compound is also likely to be adsorbed or complexed by soil humic acids. If the reaction is a simple adsorption reaction, all or part of the toxin might later become available for absorption by a receiver. If the compound is complexed or precipitated by its reaction with soil humic substances, then it would be deactivated.

The allelochemical may undergo microbial degradation either while it is free in soil solution or while it is adsorbed. This could destroy all or part of the compound; there is evidence that most of the natural organic chemical groups that are contained in allelochemicals can be metabolized by some microorganisms. The possibility always exists however, that the microbial degradation product from the metabolism of an active chemical will itself be an allelochemical. The reactions that a chemical undergoes in soil are largely controlled by edaphic factors such as moisture regime, nutrient status or organic matter content. Soil moisture regime determines whether aerobic or anaerobic decomposition takes place, which in turn helps to fix the quantity of the chemical metabolized and the nature of the decomposition products. Soil nutrient status and soil temperature helps to determine the rate of microbial activity. The nature and amount of organic matter determines whether simple adsorption or complexing by humic substances takes place. Microbial degradation is also controlled by the spectrum of microorganisms present in soil. The edaphic effects mean that different things may happen to the same allelochemical introduced into different soils, or even into the same soil at different times (Fisher, 1987).

It seems unlikely that all allelochemicals that may be extracted from a donor plant are actually those that reach a host plant. Some compounds such as juglone may remain unchanged in soil under some circumstances, but many compounds such as ferulic acid or salicyclic acid, are converted to other chemicals in the soil. A chemical compound that is free in soil solution is available for uptake by receiver plants. Most, if not all allelochemicals are taken up by plants, but plants may discriminate against certain chemicals on the basis of molecular size (m.w.) or some other factor; if a receiver plant does not absorb the compound, it becomes ineffective in nature.

Once an allelochemical is absorbed, it must be translocated to the site where it is capable of interfering with metabolism. If translocation does not happen the phytoactive compound will be ineffective. Some plants may be capable of detoxifying a phytotoxic allelochemical that is absorbed. If a toxin is absorbed and translocated, but not detoxified within a receiver plant, it will interfere with the host plant's ontogenic processes or its metabolism.

2.6 Allelopathic Phenomena in Nature

Many workers, during the last two decades, have drawn attention to the fact that allelopathy phenomena may be involved in natural ecosystems as well, under natural conditions. Only some of the best known instances are cited below:

2.6.1 In the patterning of Vegetation:-

In many ecosystems, plants tend to pattern themselves as pure stands or as individuals spaced in rather specific densities or configurations. Many desert species, both woody shrubs and trees, show obvious zones of inhibition around which few, if any, alien species are able to invade. These patterns often cannot be adequately explained by competition alone, and are probably caused by a combination of factors including allelopathy (Putnam, 1985a).

Muller (1966) who studied the unique pattern of herb exclusion caused by Salvia bushes in semi- and arid-regions of a mediterranean community in California, attributed the effect to volatile compounds released by Salvia. Phytotoxic monoterpenes such as α-pinene, β-pinene, cineole and camphor, were released from the leaves of Salvia, and these effectivily suppressed the growth of adjacent spp., creating bare zones and an inhibition zones between two vegetations.

The vegetation under the trees in Japanese Red Pine (Pinus densiflora) forests has been found to be sparse, despite the fact that the interior of these forest do not suffer from greatly reduced light intensities. Various parts of the red pine and the soil underneath it contained chemicals toxic to many potential understory plants (Lee & Monsi, 1963). Leucaena leucocephala plantations exhibit a unique exclusion of many weeds beneth the Leucaena canopy, particularly in situations where a subtantial amount of leaf litter had accumlated. Chou and Kuo (1986) demonstrated that this was due to presence of large quantities of phytotoxins in the Leucaena leaf litter which included mimosine, and a large range of phenolic acids. In addition, in the exclusion of understories by the bamboo species Phyllostachys edulis, water soluble phenolics, O-hydroxy phenylacetic, p-hydroxycinnamic, p-hydroxybenzoic, ferulic, vanillic and syringic acids have been implicated (Chou, 1982). Thus, it was concluded that allelopathy plays an important role in retarding understory growth.

Prostrate knotweed (Polygonum aviculare) has been known to rapidly encroach bermudagrass (Cynodon dactylon) lawns, causing the grass to die in patches of the weed. AlSaadawi & Rice (1982a, 1982b) collected soil minus litter from areas under a P. aviculare stand and a bermudagrass stand. Soil collected under knotweed markedly inhibited the seed germination and

seedling growth of bermudagrass, compared to soil from under bermudagrass. Decaying roots and shoots, as well as root exudates of knotweed were also found to reduce seed germination and seedling growth of bermudagrass. Eleven allelochemicals inhibitory to the growth of bermudagrass were isolated from soil under knotweed, whereas none of these occurred in soil under the grass.

2.6.2 In Plant Succession:-

There is ample evidence to implicate allelopathy in natural plant succession. In the tall grass prairies of Oklahoma and Kansas in the U.S.A. four main successional stages have been noted, when infertile fields are abandoned from cultivation: a pioneer weed stage that persists for only 2-3 years, an annual grass stage that lasts for 9-13 years, a perennial grass stage that remains for 30 years or longer after abandonment, and finally the climax prairie. The evidence is strong that the pioneer weed stage disappears rapidly because the species are eliminated through strong allelopatic interactions (Rice, 1984).

For instance, Aristidia oligantha and other grass species which displace the pioneer weeds of the frist two stages, appear to produce allelochemicals that inhibit growth of Rhizobium and free- living nitrogen fixing organisms, and nodulation in legumes, thus reducing the biological nitrogen fixation. This slowing-down of nitrogen- fixation probably gives Aristidia and other grasses a selective advantage in competition with species that have higher nitrogen requirements and causes them to remain for a lengthly period (Rice, 1984). There is also evidence that nitrification is slowed down in the later stages of succession causing available nitrogen to be present chiefly as ammonium nitrogen (Rice & Pancholy, 1972; 1973), and increasing the total nitrogen content of the soil. Evidence suggests that as succession progresses there is less inhibition of nitrogen fixation, but more inhibition of nitrification, so that eventually the nitrogen concentration is increased to a point where the climax species can invade. Tannins, phenolic acids, flavonoids and coumarins which are all important inhibitors of nitrification have been implicated.

Urbanization around large cities in Japan has greatly changed plant communities because of the creation of bare areas or serious disturbances of natural ecosystems. Weed succession on urban waste sites is similar to that in old-fields in some parts of U.S.A., according to Rice (1984). In Japan, at these sites, Ambrosia artemisiifolia dominates in the first year followed by Solidago altissima and Erigeron spp. for a few years, and next by Miscanthus sinensis, a dominant grass. Numata and co-workers (as reported by Rice, 1984) found that S. altissima and Erigeron annus both produce polyacetylenic methyl esters which inhibit seed germination of A. artemisiifolia, M. sinensis and a species of Tagetes. The phytotoxin found in Solidago was

also extracted from soil at concentrations sufficient to regulate germination and growth of associated spp.

Kobasayshi et al (1980) reported that the roots of Solidago altissima contained 250-400 ppm of the C_{10}-polyacetylene, cis-dehdro-matricaria ester (cis-DME). They found that the soil under a stand of Solidago contained 6 ppm of cis-DME plus trans-DME, both of which were inhibitory the growth of other species. Three C_{10}-polyacetylenes were identified from methanol extracts of Erigeron annus, E. canadensis, E. floribundus and E. philadelphicus. These were the cis- and trans-matricaria ester and the cis-lachnophyllum ester, all of which were inhibitory to seed germination of Ambrosia artemisiifolia. Kobayashi et al concluded that the dominance of S. altissima and Erigeron spp. in the second stage of secondary succession was probably due to their production of acetylenic compounds which strongly inhibit other species. They also suggested that the relatively short period of occupation by S. altissima and Erigeron spp. may be due to the accumulation of such polyacetylenes in soil to a point where they are toxic to these species as well.

2.6.3 In Forestry:-

Tubbs (1973) reported that Sugar Maple (Acer saccharum) seedlings inhibited the growth of yellow birch (Betula alleghaniensis) seedlings despite the absence of competition in nursery experiments. Root elongation of birch was retarded by exudates of actively growing root tips of maple, when both were grown together in aerated nutrient solutions, suggesting that allelochemicals exudated by maple were responsible.

Walters and Gilmore (1976) reported that the height of sweetgum (Ligidambar styraciflua) was less in plots containing the grass fescue (Festuca arundinacea), than in adjacent plots without fescue. In these studies it was proven that leachates from the rhizosphere of live fescue, dead fescue roots and dead leaves caused decreased absoption of phosphorous and nitrogen by sweetgum, and that this caused the reduction in the growth of sweetgum, by fescue.

Certain reforestation problems have also been linked to allelopathy. Rice (1984) reported that there are many logged-over sites in Northern Pennisylvania, U.S.A. that have remained essentially treeless for many years. Several annual weeds, particularly Solidago and Aster species, have been shown to produce toxins that inhibit establishment of black cherry (Prunus serotina) seedlings that normally reinfest thes sites.

2.6.4 In Agriculture:-

Since the early works of Schreiner and associates and McCalla and Duley, previously referred to, evidence of allelopathy in agriculture have been accumlating rapidly. The emphasis has been generally on the detrimental influences of plants or their residues on crop growth or productivity. In particular, problems with certain crop rotations, toxicity of surface residues (mulches), autotoxicity and replanting problems, and strong interference by certain weed species are frequantly mentioned in this studies (Putnam, 1985a; Putnam & Weston, 1986).

It is well known that a certain quantity of unharvestable parts of a plant is left in soil on farmlands. The debris of the crops in soil has always been thought to be beneficial to succeding crops. However, the negative impact of certain crop residues on subsequent plantings is also well documented (Borner, 1971; McCalla, 1971; Patrick, 1971). As stated previously, phytotoxins present in residues may be leached out directly, released upon their breakdown, or produced by microbes that use the plant material as food sources. Many of these have caused major problems in agriculture. Putnam and associates (Barnes & Putnam, 1983; Putnam et al. 1989) have produced proof of allelochemicals in rye, which inhibit the growth of many species. Patrick and associates had earlier demonstrated that residue of rye, timothy, tobacco and barley that remained in soil released great amounts of phytotoxins 6-8 weeks after decomposition (Toussoun et al, 1968). Phytotoxins arising during decomposition of plant residues in soil inhibited the respiration, germination and growth of several kinds of plants (Patrick & Koch, 1958).

In rice culture too it has been a common practice to plough the unharvested parts of rice plants into soil in order to return some of the nutrients back into the system. In Taiwan, this pracrice has led to decline in the productivity of rice fields (Chou & Chiou, 1979), particularly in poorly drained areas. Chou & Lin (1976) obtained proof that decomposing, submerged rice- straw produced a series of pytotoxic phenolic acids including p-hydrobenxoic, p-cinnamic, syringic, vanillic and ferulic acids, which could remain in soil for upto 4 months and cause decreased yeilds of subsequent rice crops. Maximum toxicity occurred in the first month of decomposition and declined thereafter.

In southern Taiwan, a crop of rice is often followed by a legume crop. Yields of soybean have been increased greatly by burning the rice straw prior to planting soybean. Rice (1968, 1971) and associates produced evidence that the decreased yields of unburned fields could be due to an inhibition of nodulation and nitrogen-fixation by Rhizobium in the nodules of soybean. The five phenolic acids identified by Chou and Lin and sterile extracts of decaying rice straw in soil markedly inhibited the growth of Rhizobium, nodule numbers, hemoglobin content of nodules of two bean varieties and also N-fixation (acetylene reduction) in other legume spp.

(Rice, 1984; Putnam, 1986). There is also strong evidence that allelochemicals inhibit the nitrification process in soil. The nitrification process converts ammonium which is held tightly in soil to the easily leachable and available nitrate. Since loss of nitrate from arable lands is of concern, both because of pollution and conservation of expensive nitrogen, the impact of allelochemicals on nitrification in agricultural soil is of great interest at the present time (Putnam, 1986).

The cause for low yeilds of monoculture sugarcane ratoon crop in Taiwan was investigated, and the researches (Wang et al. 1967) concluded that phytotoxicty produced by phenolic acids present in decomposing sugarcane leaves in soil under water-logged conditions, was at least one important factor. Wu et al. (1976) reported that the reduction of sugarcane yeilds was at least partly due to the microbial activity of the fungus Fusarium oxysporum which was present in very high numbers in the rizosphere of ratoon cane compared to newly- planted cane. They also found that a phytotoxin, fusaric acid, produced by the fungus significantly affected the growth of cane.

Allelopathic potential has now been suggested for about 90 species of weeds (Table 2.1). Some of the suspected allelochemicals have also been characterized. However, Putnam (1985a) states that the evidence for allelopathy by many of these species remain weak and may not hold true in future testing. On the other hand, for some species, the evidence is quite convincing. It has been strongly suggested that allelopathy may well be one of the mechanisms through which aggressive weed species gain dominance on agricultural sites. For instance, there is a ample evidence to show that the some of aggressive perennial weeds such as quackgrass [Agropyron repens (L.) Beauv.], yellow nutsedge (Cyperus esculentus L.), Canada thistle (Cirsium arvense) and johnsongrass [Sorghum halepense (L.) Pers.] can dominate agricultural sites, and may impose allelopathic influence, particularly through toxin released from their residues (Rice, 1984; Putnam, 1985). Several workers have isolated and identified the toxic chemicals released from rhizomes and decaying residues of quackgrass (Gabor & Veatch, 1981). Recent studies by Weston and Putnam (1986) indicated that living or dead quackgrass released compounds that inhibited nodulation and nitrogen-fixation on a number of legumes.

Purple Nutsedge (Cyperus rotundus), listed by Holm et al (1977) as the World's No.1 ranking weed, has been found to be strongly allelopathic. soil previously infested with the weed for 9 to 12 weeks significantly reduced germination of mustard, barley and cotton seeds; soil infested for only 6 weeks significantly reduced germination of mustard and cotton seeds (Friedman & Horowitz, 1970). Ethanol extracts of previously infested soil inhibited radicle growth of barley also. Decomposing tubers of nutsedge reduced root and top growth of barley, sorghum and soybean (Rice, 1987).

Table 2.1: A summary of some of the known allelopathic species, source of allelochemicals, type of acitivity and allelochemicals thought to be responsible

Allelopathic plant species	Allelochemical(s) known	Source	Type of Activity	Reference
Abutilon theophratsi	Free amino acids	Leaves/ Roots	seed germination inhibition	Gressels & Holm, 1964
Acroptilin repens	Acroptin	Stem/ Leaves	growth regulator	Stevens & Merill, 1985
Agrostemma githago	amino acids, including allantoin, tryptophan	Leaves/ Roots	growth/germination stimulation	Gajic (1972) in Grodzinsky, 1987
Amaranthus palmeri	2,6 dimethoxy-benxoquinone, Phytol, Vanillin Chondrillasterol 3-methoxy-4-hydroxy-nitrobenzene	Stem/ Leaves	seed germination inhibition	Fisher & Quijano, 1985 Bradow, 1985
Ambrosia artemisifolia	- Bisabolene - Guayene - Caryophyllene Bergamotene, Bulnesene, Patchoulin	Roots/ Leaves	seed germination inhibition	Fischer & Quijano, 1985

Table 2.1: Continued

Allelopathic plant species	Allelochemical(s) known	Source	Type of Activity	Reference
Ambrosia cumanensis	Sesquiterpene lactones Cumanin, Cumambrin-A Cumambrin-B, Peruvin Psilostachyin-B Psilostachyin-C	Root/ Leaf leachates	autotoxicity, stimulation/ inhibition of growth of many species	Anaya et al, 1987
Ambrosia psilostachya Ambrosia acanthicarpa	Sesquiterpenes	Leaves/ Roots	growth retardation/ inhibition	Millers et al, 1968 Geissman et al, 1969
Antennaria microphylla	Hydroquinone Caffeic acid Arbutin, Benzoquinone	Leaves/ Roots	root growth inhibition	Mannerrs & Galitz, 1985
Artemisia absinthium	- Caryophyllene - Bisabolene Chamazulene, Quercetin	Leaves/ Roots	seed germination inhibition	Grummer, 1961
Asparagus officinalis	3,4-dihydroxybenzoic 3,4-dimethoxybenzoic 2,5-dihydroxybenzoic 3,4-dihydroxyphenylacetic acids	Whole plants	autotoxicity	Young, 1986 Chou, 1987

Table 2.1: Continued

Allelopathic plant species	Allelochemical(s) known	Source	Type of Activity	Reference
Astragalus gummifer	Xenognosin A, B	Leaves/ Stem	haustorial inducer	Lynn, 1985
Avena sativa (Oat)	Scopoletin	Leaves	seed germination inhibition	Baskin et al, 1967
Baccharis megapotamica	Trichothecenes Roridin A, E Baccharinoide	Leaves/ Roots	growth inhibition	Jarvis et al. 1985
Biden pilosa	Phenylpeptatriene	Leaves	growth inhibition	Rice, 1984
Camellia sinensis (Tea)	Caffeine Theobromine	Seed/ Leaves	seed germination inhibition	Rizvi & Rizvi, 1987 Waller et al, 1986
Coffea arabica (Coffee)	Caffeine 1,3,7-trimethylxanthine	Seed/ Leaves Seeds	Seedling growth/ Seed germination inhibition	Friedman & Waller, 1983; Rizvi et al, 1981, 1987
Convallaria majalis	Stropanthidin	Leaves/ Roots	seed germination inhibition	Robinson, 1983
Centaurea repens	Polyacetylenes Repin	Leaves/ Roots	growth inhibition	Tower & Wat, 1978 Stevens & Merill, 1985

Table 2.1: Continued

Allelopathic plant species	Allelochemical(s) known	Source	Type of Activity	Reference
Centaurea solstitialis	Solstitialin	Leaves/ Stem	growth regulator	Stevens & Merill, 1985
Croton pyramidalis	3,5-dihydroxy-7,4'-dimethoxyflavone Pyramidolactone	Leaves	growth/germination inhibition	Anaya et al, 1987
Datura stramonium	Scopolamine Hyosoyamine	Seed/ Leaves	seed germination inhibition	Lovett et al, 1981
Delonix regia	4-Hydroxybenzoic 3,4-dihydroxybenzoic 3,4-dihydroxycinnamic Gallic, Chlorogenic acids 3,4-dihydroxybenzaldehyde	Seed/ Leaves/ Twigs/ Flowers	growth inhibition of understorey spp.	Chou & Leu, 1992
Digitalis pupurea	Digitoxigenin	Leaves/ Stem	seed germination inhibition	Evenari, 1949
Eleocharis microcarpa	Oxygenated fatty acids	Leaves	seed germination inhibition	Van Aller et al,1985
Eleocharis coloradoensis	Dihydroactinidiolide	Leaves	growth regulation	Ashton et al, 1985

Table 2.1: Continued

Allelopathic plant species	Allelochemical(s) known	Source	Type of Activity	Reference
Eucalyptus camaldulensis	Cineole -Phellandrene -pinene -pinene	Leaves	growth/germination inhibition	Del Moral & Muller, 1970
Festuca arundinacea	Lactic, Succinic, Malic, Citric, Shikimic, Glyceric, Fumaric, Quinic acids	Leaves	growth/seedling inhibition	Peters & Luu, 1985
Galium mollugo	Dismetin trioside	Leaves/ Stem	germination inhibition	Kohlmuenzer (1965) in Rice, 1984
Heracleum lanatum	Xanthotoxin	Leaves/ Stem	growth/ germination inhibition	Camm et al, 1976
Heracleum laciniatum	Pimpinellin, Bergapten Isobergapten,Gelicin	Leaves/ Stem	growth/germination inhibition	Junttila, 1976
Hordeum vulgare (Barley)	Gramine	Roots	growth inhibition	Overland, 1966

Table 2.1: Continued

Allelopathic plant species	Allelochemical(s) known	Source	Type of Activity	Reference
Juglans nigra	Juglone	Leaves	growth/germination	Putnam, 1988a
Kalanchoe daigremontiana	Ferulate esters, Higher fatty alcohols Triacontanol	Roots	growth regulator	Burke, 1987; Nair et al, 1988
Lantana camara	Phenolic acids	Leaves	seed germination inhibition	Singh et al, 1989
Lespedeza sericea	Soyasapogenol B	Leaves	haustorial inducer	Lynn, 1985
Leucaena leucocephala	Mimosine, Quercetin, Gallic, Protocatechuic, P-hydroxybenzoic, p-hydroxyphenylacetic, vanillic, Ferulic, caffeic, and p-coumaric acids	Leaves	seed germination inhibition	Chou and Kuo, 1986
Melitopus alba	Coumarin	Leaves	Seed germination	Winter, 1961
Miscanthus floridulus	phenolic acids	Leaves	Seed germination	Chou, 1987

Table 2.1: Continued

Allelopathic plant species	Allelochemical(s) known	Source	Type of Activity	Reference
Nicotiana tabacum (Tobacco)	Nicotine	Leaf litter	growth regulator	Rizvi & Rizvi, 1987
Oryza sativa (Rice)	p-hydroxybenzoic p-coumaric, vanillic, Ferulic, o- hydroxy phenylacetic acids	Leaves/ Whole plant parts	Seedling growth inhibition, auto-toxicity	Chou & Lin, 1976
Parthenium argentatum (Guayule)	trans-Cinnamic acid	Roots	autotoxicity	Bonner & Galston (1944) in Friedman, 1987
Pathenium hysterophorus	Parthenin	Leaves/ Roots Pollen, Trichomes	seed germination inhibition	Kanchan & Jayachandra 1979a,b;1980
Phyllostachys edulis	Phenolic acids	Leaf litter	seed germination inhibition	Chou, 1987
Pinus densiflora	Benzoic and other Phenolic acids	Leaves/Needles	growth/germination inhibition	Kil & Yim, 1983

Table 2.1: Continued

Allelopathic plant species	Allelochemical(s) known	Source	Type of Activity	Reference
Polygonum aviculare	14-22 Carbon long-chain fatty acids, phenolics	Leaf residues	seed germination inhibition	AlSaadawi et al, 1983
Polygonum sachalinense	Hirsutin, Arabin, w-methyl sulfonylfonyl-alkyl isothiocynate anthraquinones, emodin, physcion	Leaves Roots/ Leaves	seed germination/ seedling growth inhibition	Yamane et al, 1992 Inoue et al, 1992
Prunus persica	Amygdalin	Roots	seed germination inhibition	Patrick, 1955
Psoralea subacaulis	Psoralen	Leaves	growth/germination inhibition	Proebsting et al, 1941
Pyrus malus (Apple)	Quercetin, Phlorizin Phloretin, Phloroglucinol p-hydroxyhydrocinnamic p-hydroxybenzoic, Catechin Kaempferol, Epicatechin	Root residue	growth inhibition	Borner, 1959
Rorippa indica	Hirsutin, Arabin, Camelinin, isothiocyantaes	Leaves	seed germination inhibition	Yamane et al, 1992

Table 2.1: Continued

Allelopathic plant species	Allelochemical(s) known	Source	Type of Activity	Reference
Rorippa sylvestris	Salicylic, p-hydroxy benzoic acids, hirsutin 8-methyl sulfinyloctyl isothiocynate, 4-methoxy indole-3- acetonitril Pyrocatechol	Leaves	seed germination inhibition	Yamane et al, 1992
Salvia leucophylla Salvia apiana Salvia mellifera	Camphane, Camphor Cineole, Dipentene -pinene, -pinene	Leaves	root growth inhibition	Muller & Muller, 1964
Sasa crenua	p-Coumaric, Ferulic Vanillic,p-Hydroxybenzoic acids, p-Hydroxybenzaldehyde	Leaves	growth/germination inhibition	Li et al, 1992
Saccharum officinarum (Sugarcane)	p-Hydroxybenzoic, Ferulic, p-Coumaric Syringic, Vanillic, Formic, Acetic, Oxalic Tartaric, Malonic acids	Leaves/Whole plants	autotoxicity	Chou, 1987
Secale cereale (Rye)	DIBOA, BOA	Leaves/ Whole plants	seed germination inhibition	Barnes et al, 1987

Table 2.1: Continued

Allelopathic plant species	Allelochemical(s) known	Source	Type of Activity	Reference
Sorghum bicolor	Dhurrin p-benzoquinone	Leaves	seed germination inhibition	Putnam, 1988a,1988b
Striga asiatica	Strigol Strigol acetate	Roots	growth/germination stimulation	Pepperman et al, 1985
Tagetes patula Tagetes erecta	Thiophenes,	Leaves/ Roots	seed germination inhibition	Rice,1984; Putnam, 1988a Altieri & Doll, 1978
Triticum aestivum (Wheat)	DIMBOA	Leaves	seed germination inhibition	Zuniga et al, 1983

Weed seeds can lie dormant in the soil for decades and still remain viable. During this period they resist decay by soil microbes, and allelochemicals appear to be responsible for both these phenomena. Weed problems in agricultural systems result mainly from the enormous numbers of seeds which remain viable in soil for long period of time, and also from the lack of uniformity in their germination. Certain lactones, phenolic compounds, flavonoids and tannins, present in high concentrations in seeds appear to be responsible in this regard (Rice, 1984). Rudiger and Lohaus (1987) produced impressive evidence that oat caryopses, particularly their husks, contain germination inhibitors that inhibit the germination of several plants, e.g. Avena sativa, Sorghum spp., Phallaris spp., Raphanus spp., Amaranthus caudatus and Lepidium sativum.

2.6.5 In Horticulture:-

Pickering (1917) provided evidence as early as 1917 that orchard sods interfere with apple trees by release of toxin as well as by competition. Tukey (1969) reviewed the impacts of budding and grafting trees and indicated that these biochemical interactions (often inhibitory) could be considered as allelopathic responses. Molisch's pioneering work with ethylene from pome fruts showed the potential for growth promotion and inhibition with the same compound (Molisch, 1937)

An important horticultural problem that involves allelopathy is the well-known 're-plant syndrome'. Replant problems are complex and often involve build-up of pest such as nematodes or plant pathogens. However, soil toxins have also been held responsible for the difficulty to re-establishing apple, peach, citrus, asparagus (Rice, 1974; Tukey, 1969; Putnam, 1986). Patrick et al (1964) and Borner (1971), presented evidence that the toxins phlorizin and amygdalin were respectively involved in the re-planting problems of apple and peach. It is known that after hydrolysis these two compounds become phytotoxic to plant growth; phlorizin is converted to p- hydrobenzoic acid and its derivatives, plus glucose, and amygdalin is hydrolyzed in to HCN, benzaldehyde and glucose.

2.7 The Nature of Allelochemicals

A myriad of compounds ranging from extremely simple gases and aliphatic compounds to complex polycyclic aromatic compounds are known to be produced and released into the environment by plants and their associated residues. Many of these are considered as secondary plant metabolites; several are also degradation products which occur in the presence of micro-organisms and their enzymes.

(i) **Aliphatic compounds**

Several water-soluble simple organic alcohols and acids are common plant and soil constituents. They include methanol, ethanol, n-propanol and butanol, and crotonic, oxalic, formic, butyric, lactic, acetic and succinic acids (Mandava, 1985) all of which have the potential to inhibit seed germination or plant growth. A variety of tricarboxylic acids are present in sorghum herbage and contribute to toxicity of sorghum residues (Lehle & Putnam, 1983). Simple aliphatic acids are formed upon anaerobic decomposition of plant residues in soil. For instance, acids are released from decaying quackgrass residues and the straw of cereal grains (Lynch et al, 1980; Lynch, 1980). Under aerobic conditions, however, aliphatic acids are metabolized in the soil and therefore, may not be considered a major source of allelopathic activity (Mandava, 1985).

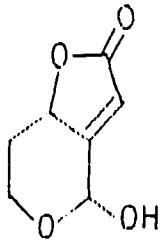
(ii) **Unsaturated lactones**

Several simple lactones (those arising from acetate) are potent inhibitors of seed germination. The most notable lactone identified as a potent allelopathic agent is patulin (8), produced by several Penicillium sp. growing on wheat and other plants. At 10 micrograms per ml, patulin completly inhibits the germination and growth of seedlings of certain cultivated plant species, including corn (Mandava, 1979). Other lactones such as psilotin (9) and psilotinin (10) are also implicated in allelopathy.

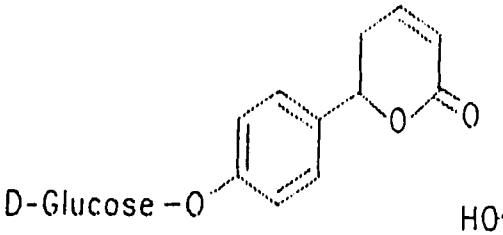
(iii) **Aromatic Compounds**

Various types of aromatics comprise the largest group of compounds implicated with allelopathic activity. Among simple phenols, hydroquinone and its glycoside arbutin, have been identified as allelopathic agents (Muller & Chou, 1972). Among phenolic acids, benzoic, p-hydroxy-benzoic (11), o-hydroxyphenylactic (112), syringic (13), chlorogenic, vanillic (14), and salicylic acid, have all been implicated with soil 'sickness' problems caused by corn, oat, sorghum and wheat residues, in the 'autotoxicity' shown by Saccharum officinarum (Chou, 1987) and also in the allelopathic exclusion of understory vegetation by Leucaena leucocephala (Chou & Kuo, 1986), Phyllostachyl edulis (Chou, 1987), and Delonix regia (Chou & Leu, 1992).

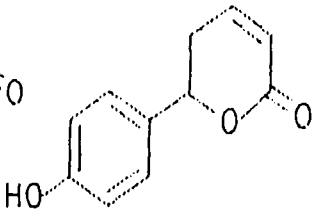
According to Kil and Yim (1983) benzoic acid as the key factor, and 11 other phenolic acids are implicated in allelopathic exclusion of undergrowth in Pinus densiflora forests. Cinnamic acids and its derivatives p- coumaric (15), ferulic (16), o-hydrocoumaric, syringic, vanillic, chlorogenic and caffeic acids are known to be produced and released into soil during decay of cereal residues, causing 'soil sickness' syndromes (McCalla & Haskins, 1964; Guenzi & McCalla, 1962; Rice, 1984). Recently, Li



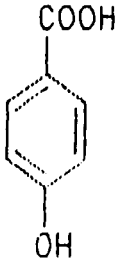
Patulin (8)



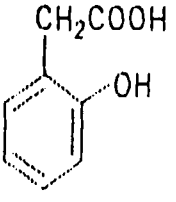
Psilotin (9)



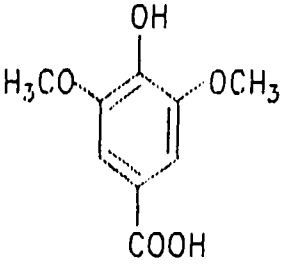
Psilotinin (10)



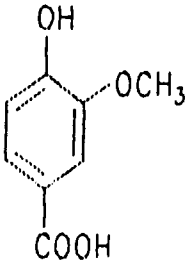
P - Hydroxybenzoic acid (11)



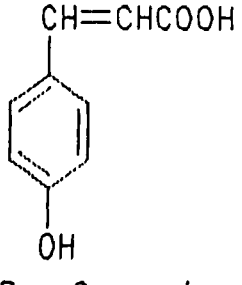
O - Hydroxyphenylactic acid (12)



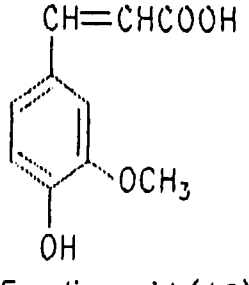
Syringic acid (13)



Vanillic acid (14)



P - Coumaric acid (15)



Ferulic acid (16)

et al (1992) reported the involvement of phenolic acids in the allelopathic interactions of Sasa crenua.

Various quinones such as 'juglone' (5-hydroxy-naphthoquinone) (17) from walnut trees are known to be associated with allelopathic phenomena. Quinones, formed upon oxidation of phenolics have been implicated in resistance of plants to diseases. An extremely bioactive compound, 2,6-dimethoxy-p-benzoquinone (18) has been found as a most active haustorial inducer from sorghum roots for the parasitic weed Striga (Putnam, 1988a).

(iv) Fatty acids

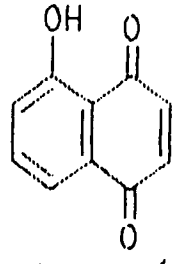
Though the role of fatty acids in allelopathy is not fully understood, they are known to be toxic to plant growth (Rice 1974). Fatty acids including myristic, palmitic, oleic, linoleic, and stearic acid have been found in decomposing residues of Polygonum aviculare which were alleiopathic towards bermudagrass (Cynodon dactylon) (AlSaadawi & Rice, 1982a; AlSaddawi et al, 1983). Dihydroxy stearic acid is also known exhibit allelopathic activity (Mandava, 1979).

(v) Tannins

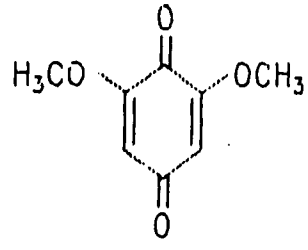
Tannins, and tannic acids, viz. gallic (19), digallic (20), egallic (21) acids, are water-soluble polyphenolic compounds with molecular weights between 300 and 500. They are divided into hydrolysable and condensed tannins and are usually composed of complex combinations of phenolic acids. They have been reported to inhibit plant growth and seed germination (Rice, 1984). They appear to be important phytotoxins affecting forest regeneration (Lodhi & Johnson, 1989). Many have been found to be growth retarders of nitrifying and nitrogen fixing bacteria (Rice, 1984).

(vi) Cyanogenic compounds

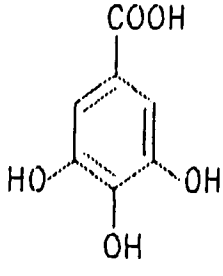
Amygdalin (22) and Dhurrin (23), two compounds implicated in allelopathy are cyanogenic glucosides (cyanohydrins) which upon hydrolysis, release other moieties besides the cynaide, that also contribute toxicity. Dhurrin is isolated from member of genus Sorghum and other grasses. Hydrolysis of dhurrin results in HCN, glucose and benzaldehyde or p-hydroxy-benzaldehide. The latter compounds, when oxidized to form benzoic acid, produce toxicity to numerous plant species (Putnam, 1988a). Amygdalin has been implicated with the peach replant problem and has been isolated as a potent allelochemical from peach roots (Prunus persica).



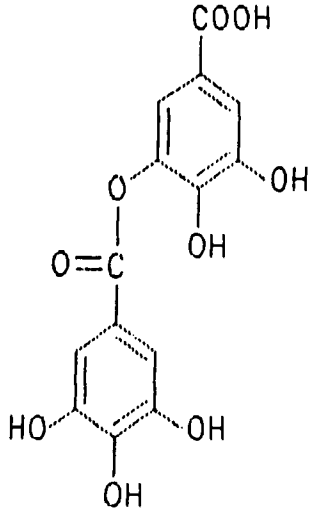
Juglone (17)



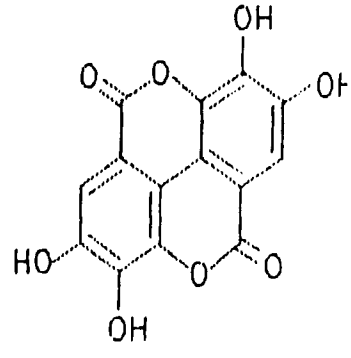
2,6 - dimethoxy - p - benzoquinone (18)



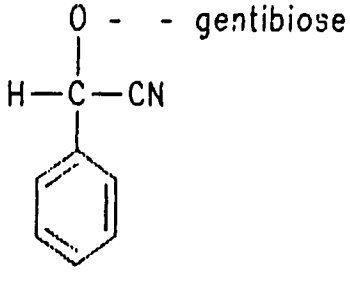
Gallic acid (19)



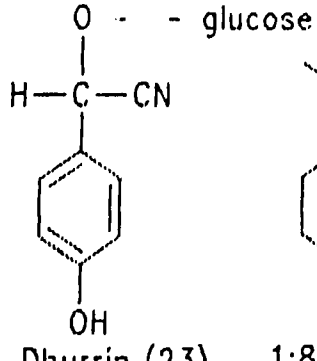
m - Digalic acid (20)



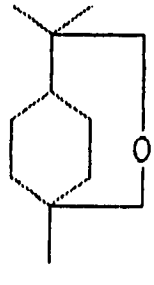
Ellagic acid (21)



Amygdalin (22)



Dhurrin (23)



1:8 Cineole (24)

(vii) Terpenoids

Terpenoids are ubiquitous in the higher plant kingdom. The role of these compounds is still being debated, but considerable evidences are accumulating to prove their function as defence against of plants. Basically, terpenes are built from 5-carbon "isoprene" structural units linked together in a variety of configurations that differ in ring closure, functional group, and degree of saturation.

Monoterpenes, 1:8-cineole (24), camphor (25), α-pinine (26), β-pinine (27), camphene (28), all of which are volatile compounds, are known to be responsible for the allelopathic activity of Salvia bushes near which many other species do not grow in arid regions (Muller & Muller, 1964; Muller, 1965). Volatile monoterpenes may also contribute to allelopathy in Eucalyptus and Artemisia (Muller, 1966; 1969; Muller & Chou, 1972).

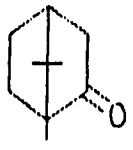
A large number of sesquiterpenes and their lactones have been implicated in allelopathic potential of a variety of plants and allelopathic phenomena. Fischer and Quijano (1985) isolated a mixture of phytoactive sesquiterpenes from Ambrosia artemisifolia L. that were highly inhibitory to the germination of crop seeds. Among these were bergamotene (29), caryophyllene (30), and β-bisabolene (31). The sesquiterpene lactones acroptilin, solstitiolide (32), repin (33) acroptilin (34) and centaurepensin (35) were isolated from the allelopathic weeds Centaurea repens L. and Centaurea solstitialis (Stevens, & Merrill, 1985).

Sesquiterpene lactones of the pseudoguanoloide type, viz. Ambrosin (36), Damsin (37) and Psylostachyn, isolated from Ambrosia peruviana have been found to be active in preventing seed germination and growth of root and shoots in lettuce and cress (Lee et al, 1982). Parthenin (38) was found in allelopathic root exudates, leaf washings, pollen and trichome leachates of the aggressive weed Parthenium hysterophorous L. (Kanchan & Jayachandra, 1979a, 1979b, 1980).

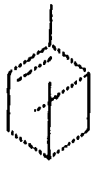
The allelopathic activity of triterpenes has been rarely reported probably due to the challenging isolation and characterization involved with them (Fischer et al, 1988, 1989). A few triterpenes however, have been reported as allelochemicals. The triterpene soyasapogenol-B (39) was reported as a haustorial inducer from Lespedesa sericea (Leguminosae) foliage (Lynn, 1985). Erythrodiol (40) and urasolic acid (41) from Ceratiola ericoides, have been reported as potent seed germination inhibitors (Mandava, 1979).

(viii) Alkaloids

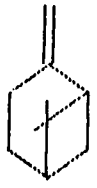
Alkaloids cannot be easily defined because there are innumerable exceptions. Most alkaloids are alkaline, always contain at least one ring,



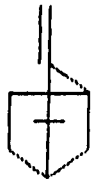
Camphor (25)



α- Pinene (26)

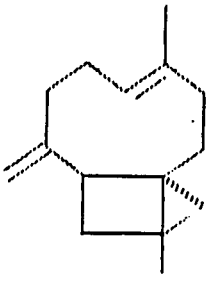


β- Pinene (27)

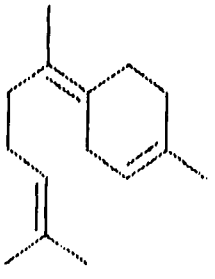


Campnene (28)

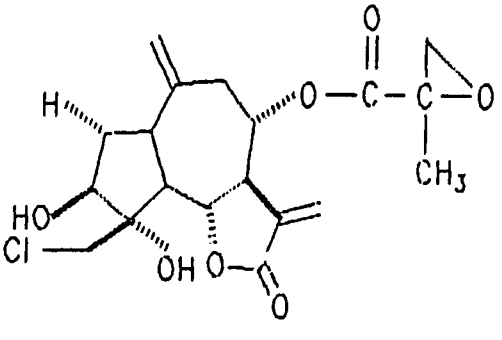
Bergamotene (29)



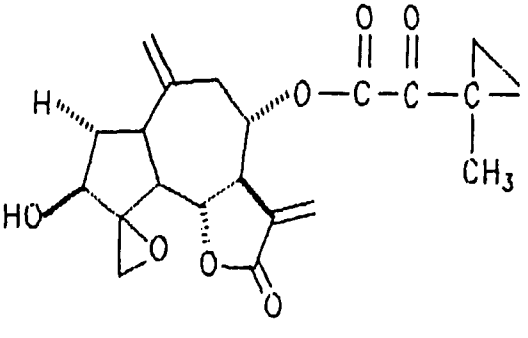
Caryophyllene (30)



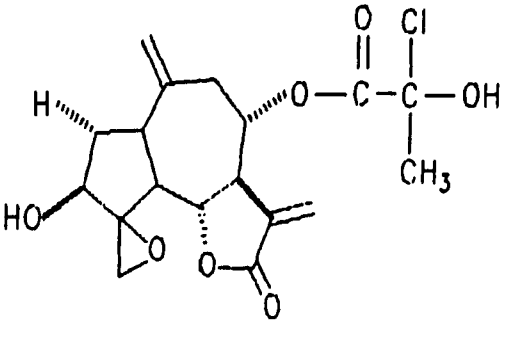
β- Bisabolene (31)



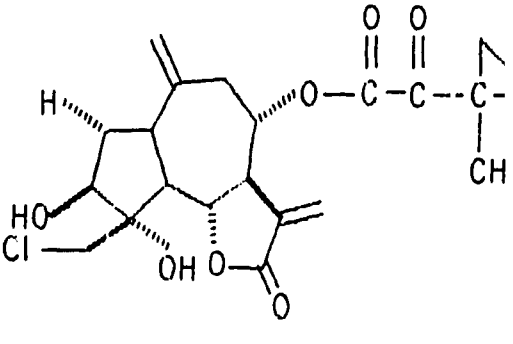
Solstitiolide (32)



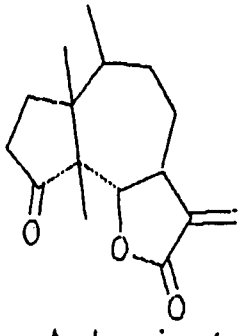
Repin (33)



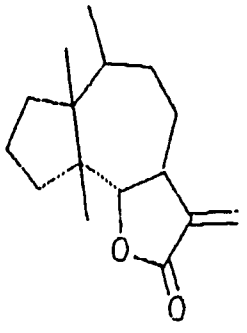
Acroptilin (34)



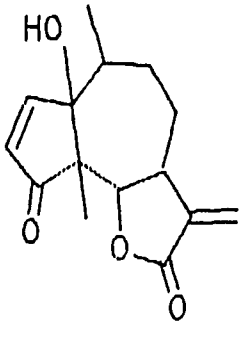
Centaurepensin (35)



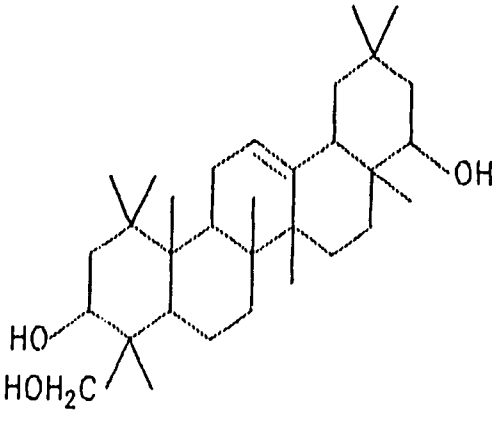
Ambrosin (36)



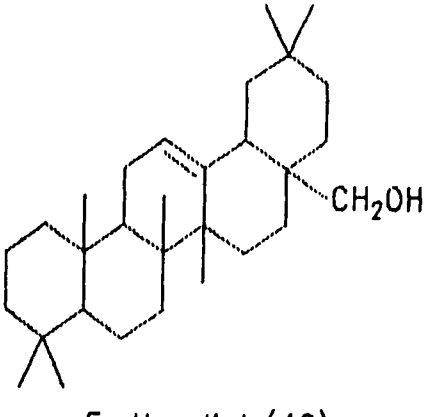
Damsin (37)



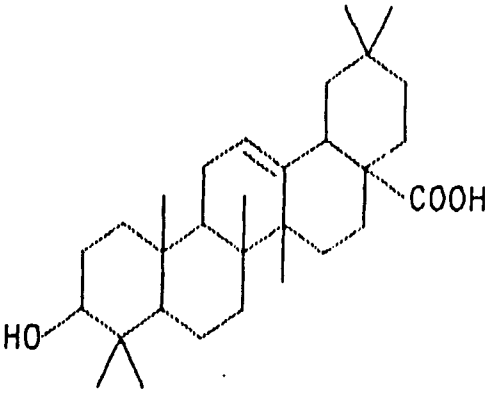
Parthenin (38)



Soyasapogenol-B (39)



Erythrodiol (40)



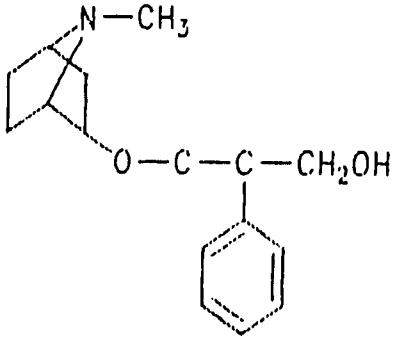
Urasolic acid (41)

nitrogen as part of the ring structure and are usually heterocyclic (Duke, 1986). Because nitrogen is first assimilated into plants in amino acids, all alkaloids can be traced back to an amino acid. Several amino acids including non-aromatic ones, have been implicated as precursors of alkaloids, although many are derived from tyrosine. Some plant species are known to produce relatively high levels (> 1% dry weight) of alkaloids, but they are minor constituents in most species. Their physiological and/or ecological role is largely unknown, although they are generally believed to be involved in chemical defense against pathogens and herbivores (Duke, 1986). Furthermore, the medicinal, narcotic, pharmacological properties of many alkaloids have been known to man for thousands of years; yet, relatively little is known about their effects on plants.

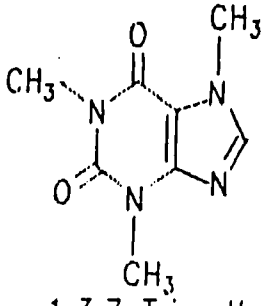
Several alkaloids have recently been identified as allelochemicals by Lovett and his associates (Lovett et al, 1981; Lovett & Potts, 1987). The tropane alkaloids Scopolamine and Hyosyamine (42) were found in aqueous leachates of both seed and foliage of Datura stramonium L. and from field soils at a farm where reduced crop emergence and vigour had been reported (Levitt & Lovett, 1984). Phytotoxicity of these alkaloids remained in soil for 5 to 8 months depending on the type of soil. It was revealed that these alkaloids were highly toxic to sunflower (Helianthus annus L.) and several cereals (Levitt et al, 1984).

Several toxic purine alkaloids belonging to the methylated xanthine group, i.e. caffeine (43), theophylline (44), and paraxanthine (45), have been identified and their possible allelopathic autotoxic influences reported from coffee (Coffea arabica L.) and tea [Camellina sinensis (L.) O. Kuntze] (Rizvi et al, 1981; Waller et al, 1986). It was found that caffeine might be used as a selective herbicide, because it inhibited the weed spiny amaranth (Amaranthus spinosus L.) without adverse effects on Phaseolus mungo (black gram) at very high concentrations (Rizvi et al, 1981). Shettel and Balke (1983) also found caffeine to be selective, being much more phytotoxic to certain weeds than to corn or soybean at 11.2 kg/ha post-emergence in field tests.

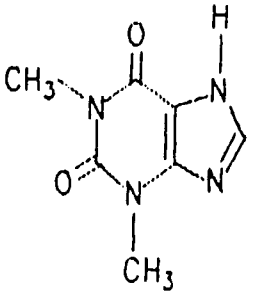
Recent research by Putnam and his associates (Barnes & Putnam, 1983, 1986, 1987; Barnes et al, 1986) proved the strong interference ability of living rye and its residues applied as surface mulches against the germination and growth of several weed species. They noted that while weeds were killed, several large-seeded crop plants which are planted deeper in soil, and several transplanted vegetable species tolerated tha effects of rye. Aqueous extracts of rye herbage, when fractionated and their activities followed by bioassay, the two most active compounds isolated were 2,4-dihydroxy-1,4(2H)-benzoxazin-3-one (DIBOA) (46) and a breakdown product, 2(3H)-benzoxazolinone (BOA) (47), both belonging to the group of compounds called benzoxazinones, which are related to alkoloids. Two other potent allelochemicals identified as 2,4-dihydroxy- 7 -methoxy -1,4- benzoxazin-3-



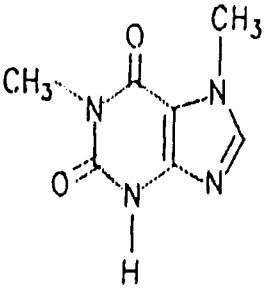
Hyoscyamine (42)



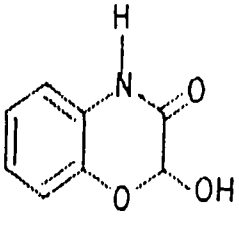
1,3,7-Trimethylxanthine (43)
(caffeine)



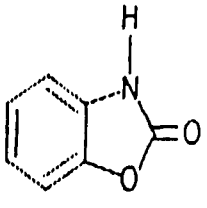
1,3-Dimethyl xanthine (44)
(Theophylline)



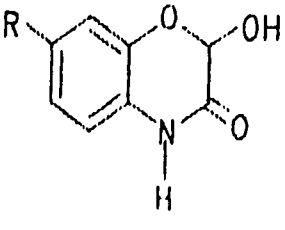
1,7-Dimethyl xanthine (45)
(Para xanthine)



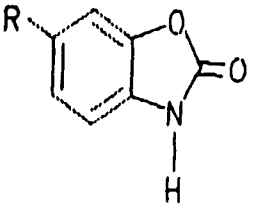
DIBOA (46)



BOA (47)



DIMBOA (48)



MBOA (49)

one (DIMBOA) (48), the methylated product of DIBOA and 6- methoxy - benzoxazolin -2-one (MBOA) (49), appear to be the key compounds in other cereals including corn and wheat. These compounds have been found to be strongly antifungal or insect antifeedant (Putnam, 1988a).

(ix) Coumarins

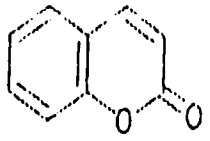
Coumarins are lactones of o-hydroxycinnamic acid. Members of coumarin family such as coumarin (50), scopoletin (51), psoralen (52), umbelliferone (53), and esculetin (54), which occur most commonly in grasses legumes and citrus fruits, exhibit high allelopathic activity on seed germination and seedling growth of many species (Rice, 1974.) Often they occur as glucosides and get leached out into the environment. This group of compounds contain some of the most phytotoxic naturally occurring chemicals produced by higher plants and are among the most studied. Of major interest is the fact that certain coumarins are powerful anticoagulants of blood and are common rat-poison ingredients (Whitlon et al, 1978).

Nicollier and Thompson (1982) found coumarin to be a more potent growth inhibitor than most aromatic acids and all flavonoids tested. Exposure to only 30 uM coumarin inhibited wheat root growth by almost 50% within 4 days. Coumarin is about two orders of magnitude more potent as a seed germination inhibitor than ferulic or caffeic acid (Duke et al, 1984), a more potent growth inhibitor than other relatives such as umbelliferone, and scopoletin (Moreland et al, 1966). Van Sumere et al (1972) reported that lettuce seed germination was inhibited 100, 53, 28, 11 and 0% by 100 nmoles per seed of coumarin, herniarin, umbelliferone, esculetin and scopoletin, respectively, after 120 h.

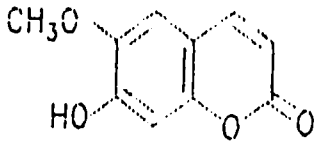
(x) Flavanoids

In plants, flavonoids generally occur as sugar conjugates. The basic flavonoid structure without sugars (the aglycone) contains 15 carbons arranged in a C_6-C_3-C_6 configuration (two aromatic rings connected by three carbons). From this basic structure, a large number of compounds grouped into several classes, are formed, depending on the various modifications of hydroxyl groups of the flavonoid nucleus.

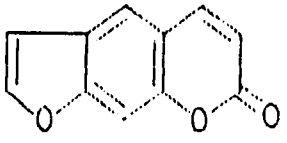
Several flavanoids are also known to exhibit allelopathic properties, including inhibition of seed germination and seedling growth. Tricin (55) and several closely related flavonoid compounds appear to be responsible for the allelopathic activity of quackgrass (Weston & Putnam, 1986; Weston et al, 1987). Seedling root growth of many plants and nodulation on seedling roots of several legumes are known to be inhibited by extracts and residues of quackgrass. The herbage of quackgrass has been



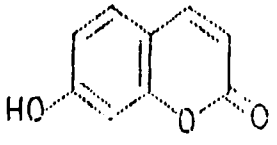
Coumarin (50)



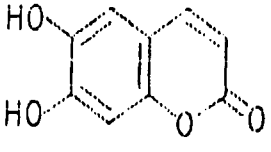
Scopoletin (51)



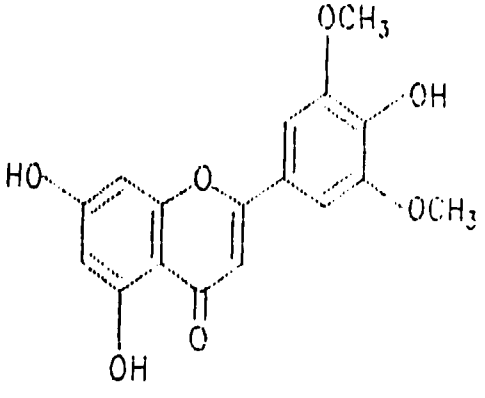
Psoralen (52)



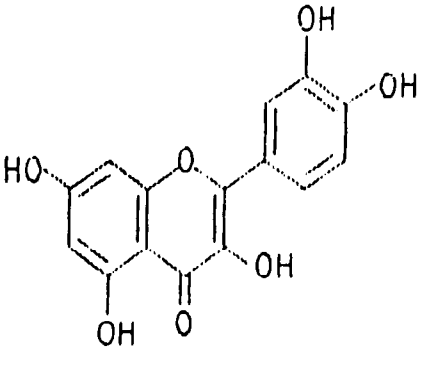
Umbeliferone (53)



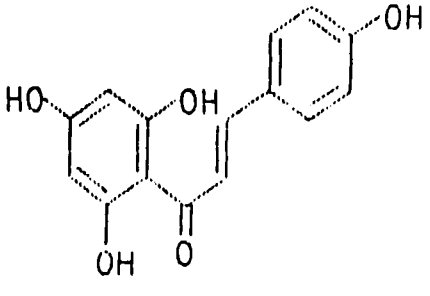
Esculetin (54)



Tricin (55)
(5,7,4-trihydroxy-3'5'-dimethoxy flavone



Quercetin (56)



Phloretin (57)

found to contain more of these flavones than rhizomes, but the latter also contained inhibitory compounds (Weston & Putnam, 1986). Quercetin (56) and Phloretin (57) have been implicated with the 'apple replant problem' (Borner, 1971).

Nine isoflavones isolated from Lupinus angustifolius roots, have been found to be strong insect feeding deterrents. These compounds which included Licoisoflavone-B (58) and Licoisoflavone-A (59), were also found to be potent anti-fungal compounds (Putnam, 1988b), and play specific roles in the resistance of various lupines to insects and disease pests. Mung bean seed coats have been found to contain large amounts of glucosyl-flavonoids that may leach from the germinating seed and inhibit both germination and growth of adjecent seed and seedlings (Tang & Zhang, 1986). Vitexin (60) and Isovitexin (61) were the two major flavonoids implicated.

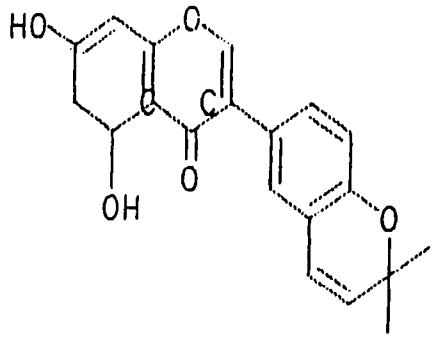
(xi) Polyacetylene compounds

Polyacetylenes are widespread in the Asteraceae family and many of these compounds have been reported as allelochemicals (Putnam, 1988b), which influence a variety of organisms. Of the several polyacetylene compounds isolated from Centaurea repens, one of which proved strongly phytotoxic to other species. The compound which contained a thienyl group (62) was found from the soil around the root system of Centaurea also, throughout the growing season (Putnam, 1988b).

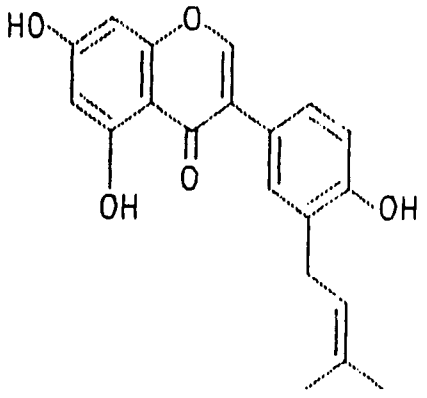
Four inhibitory thiophenes were found from the marigold (Tagetes erecta) plant's rizosphere. These compounds (63) - (66) along with two benzofurans, are thought to account for strong allelochemical effects shown by this plant (Putnam, 1988b). Other Asteraceae species Solidago and Erigeron species released cis-dehydromatricaria ester and cis- and trans-matricaria esters, respectively ((Kobayashi et al, 1980), which were present in soil also in concentrations inhibitory to test plants, raising the poosibility that these allelochemicals are significant contributors to several of the Asteraceous weeds such as Solidago, Centaurea species, which aggressively and readily form monocultures (Putnam, 1988a; 1988b).

(x) Naturally occuring Carbon- Phosphorus compounds

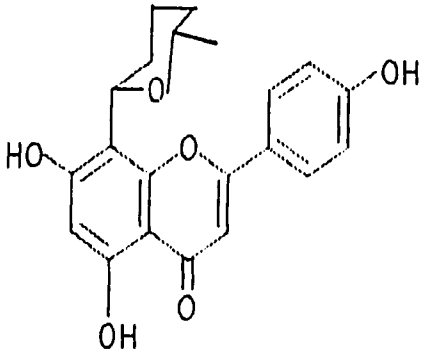
The isolation of a bioactive compound, 2- amino ethyl phosphonic acid (AEP) opened a new avenue for allelopathic chemistry studies in phosphorus-containing compounds. Usually phosphonates are known to possess strong biological activities including herbicidal, plant growth regulatory and even antibacterial properties. The most successful synthetic phosphonate herbicide in the world is 'glyphosate' [N-(Phosphonomethyl) glycine], which is still a top-ranking systemic, non-selective herbicide.



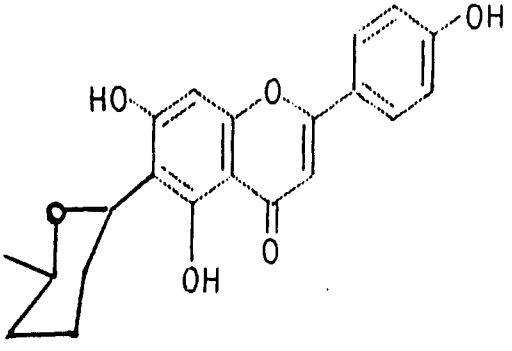
Licoisoflavone B (58)



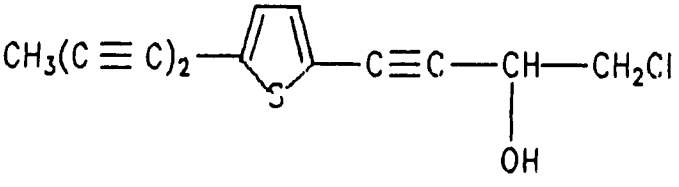
Licoisoflavone A (59)



Vitexin (60)



Isovitexin (61)



Polyacetylene (62)

Several phosphorus-containing, potentially allelopathic compounds have been isolated from higher plants, but these have not proven to be sucsesful herbicides. However, some compounds isolated from microorganisms have provided excellent herbicidal activity. Bialaphos [L- 2 - amino -4 ((hydroxy)(methyl)Phosphonyl) butyryl -1-alanyl -2- alanine), isolated from Streptomyces viridochromogenes is the first naturally occuring phosphonate compound that has been produced by fermentation technology to become a commercially successful herbicide (Duke, 1986). Like glyphosate, bialophos is also non-selecive and controls both dicotyledon and monocotyledon weeds. These examples show how allelopathic studies may lead to the discovery of novel herbicide chemistries.

(xi) Non-Protein Amino Acids

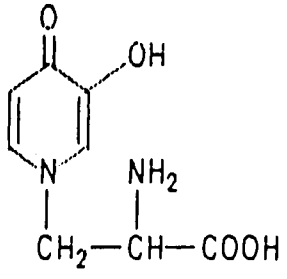
Plants synthesize over 200 known non-protein amino acids (Fowden, 1974), some of which are phytotoxic to other plants. Some are known to be the toxic constituents of certain higher plants to animals. Mimosine (67), a compound positively implicated in the allelopathic exclusion of understorey vegetation by Leucaena leucocephala (Chou & Kuo, 1986; Chou, 1987) is one such compound. Another amino acid, α-aminolevunilic acid (ALA) (68) from higher plants is suggested as a promosing herbicide, with a photodynamic mechanism (Duke, 1986)

2.8 Biosysnthesis of Allelochemicals

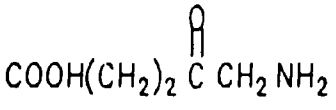
The majority of known allelochemicals are secondray plant metabolites because they are sporadic in occurrence and thus, do not appear to play a role in the basic metabolism of organisms (Rice, 1974; 1984). Most of the known phytoactive allelochemicals which range from simple hydrocarbons to complex polycyclic compounds of molecular weights of several hundreds, originate mainly from the acetate and shikimic acid pathways (Robinson, 1980; Rice, 1984). The probable major biosynthetic pathways leading to the production of the various categories of allelopathic agents are given in Figure 2.2.

2.9 Mode of Action of Allelochemicals

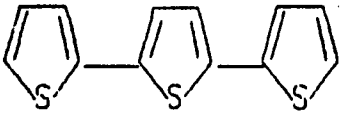
Allelochemicals may stimulate or inhibit plant growth processes depending on concentration. Osvald (1950) showed that high extract concentrations of dried stolons and roots of quackgrass strongly retarded the germination of rape (Brassica napus L.) and oat seeds, while lower concentrations stimulated germination. It has been found that a wide range of processes are affected by allelochemicals. Some appear to interfere with



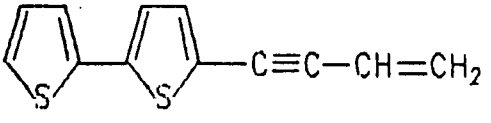
mimosine (67)



levulinic acid (68)



(63)



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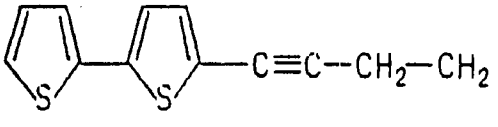
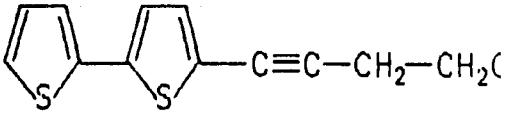


(65)



(66)

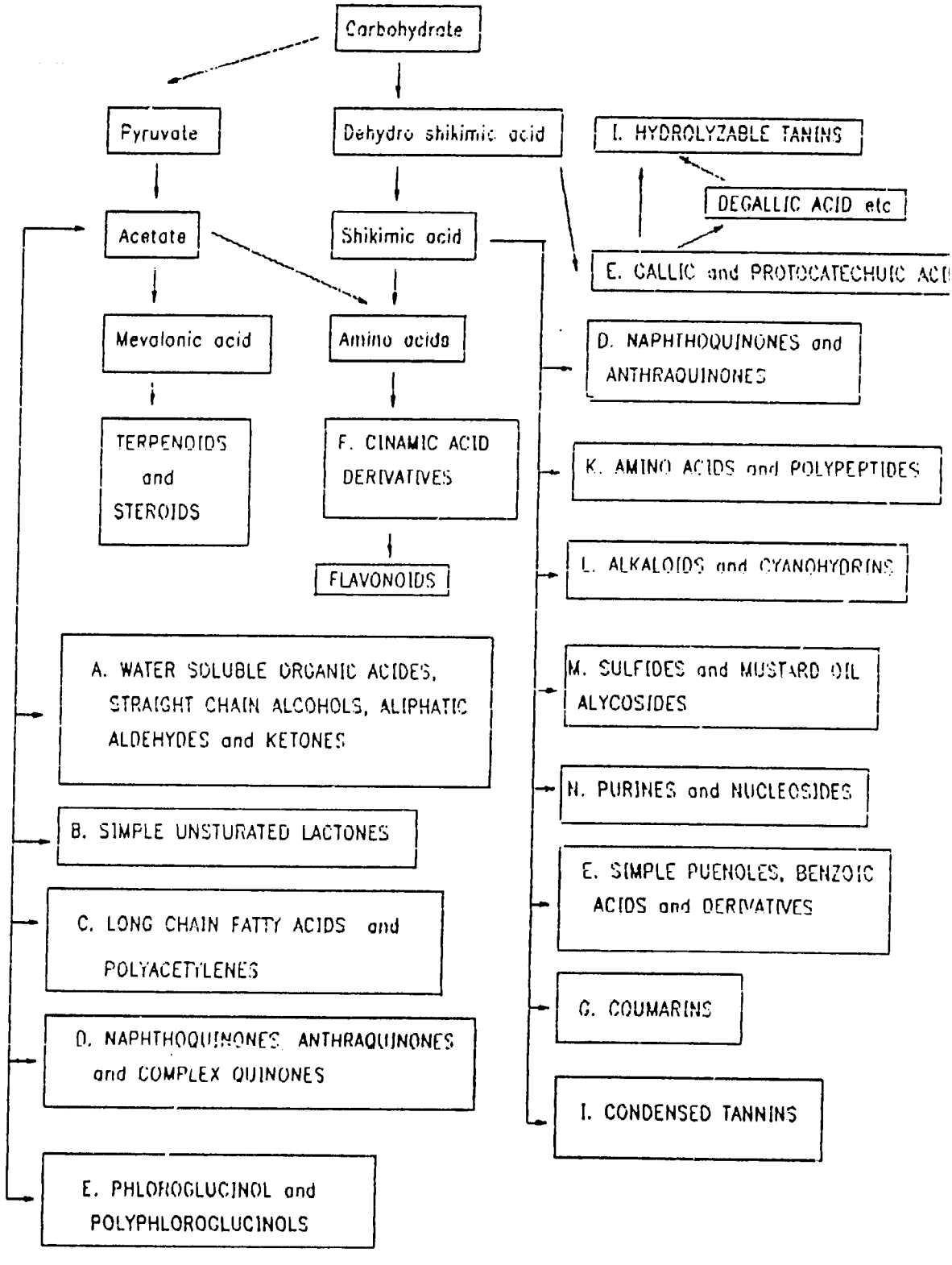


Fig. 2.2: Probable Biosynthetic pathways involved in the production of Allelochemicals (adapted from Rice, 1974,1984)

ontogenic processes, some with energy transfer processes and still others with nutrient uptake or transport. Rice (1974; 1984) and Einhellig (1984) have extensively reviewed the possible modes of action of allelochemicals.

(a) Interference with Ontogenic Processes:

A variety of chemicals have been shown to inhibit mitosis in plant roots (Rice, 1984). Coumarin has been known to completely block mitosis in onion roots within hours of treatment (Cornman, 1946). Volatile monoterpenes from Salvia inhibited mitosis and root cell elongation in several plants including cucumber seedlings. These compounds were also inhibitory to cell division of a large number of bacterial species isolated from soil (Muller, 1965). Corcoran et al (1972) tested a wide variety of identical allelopathic chemicals and found that several tannins, benzoic acids and cinnamic acids inhibited gibberellic acid-induced growth. They found that some allelochemicals inhibited, while others stimulated IAA-induced growth.

(b) Interference with Energy transfer processes:

Energy transfer processes such as respiration, oxidative phosphorylation, photosynthesis and enzyme activity may also be inhibited by allelochemicals. Chemicals may stimulate or inhibit respiration, both of which may be harmful to energy producing processes (Putnam, 1985a). In the case of stimulation (enhanced O_2 uptake) the oxidative phosphorylation sequence may be uncoupled, resulting in a lack of ATP formation. Juglone has been shown to uncouple oxidative phosphorylation, causing more than a 90% reduction in the respiration of corn roots after 1 hour exposure (Koeppe, 1972). A variety of aromatic acids, phenolics, aldehydes, flavonoids and coumarin compounds have also been implicated in similar mechanisms (Rice, 1984). Several compounds isolated from soils have been shown to inhibit the respiration of plant roots.

There are only limited reports concerning the influence of allelopathic agents on photosynthetic reactions. One study demonstrated a reduction in photosynthesis of sunflower, tobacco and pigweed soon after treatment with scopoletin, a coumarin compound (Einhellig et al, 1970). Two other studies indicated that several of the phenolic acids may reduce the chlorophyll content and photosynthetic rates in soybean plants (Einhellig & Ramussen, 1979; Patterson, 1981).

The functioning of certain enzymes involved in energy-transfer processes appear to be inhibited by allelochemicals. For instance, the phosphorylase of potato is inhibited by chlorogenic acid, caffeic acid, and catechol. Concentrations of these inhibitors in potato peels are probably sufficient to completely inhibit the activity of the enzynes (Schwimmer,

1958). Root secretions of crops have been reported to inhibit catalase and peroxidase activities in common lambsquater and redroot pigweed (Amaranthus retroflexus) (Dzubenko & Petrenko, 1971 quoted by Rice, 1984).

(c) Interference with Nutrient Uptake and/or Transport:

Many studies have shown that allelopathically affected plants have much lower nutrient contents than normal plants, but few of these studies have actually shown how this may occur. There is considerable evidence to suggest that allelopathic chemicals can alter the rate of ion uptake by plants. In fact, this may be the underlying mechanism behind many plant interference responses that have been labelled 'competition'. For instance, salicylic acid, a phenolic compound has been shown to inhibit the uptake of potassium by plants. Both salicylic acid and ferulic acid were inhibitory to K^+ uptake by oat roots, particularly at lower pHs (Harper & Balke, 1980). Glass and Dunlop (1974) have shown that several phenolic acids that inhibit ion uptake depolarize root membranes. These studies give the best insight into how nutrient uptake may be influenced by phenolic acids, but as Rice (1974) points out, knowledge of how allelochemicals affect nutrient uptake is far from complete.

Buchholtz (1971) observed that the presence of quackgrass produced N and K deficiency symptoms on corn, and that heavy fertilization with these elements could not eliminate the symptoms.

2.10 Exploiting Allelopathy as a Weed Control Strategy

Having seen the dominance achieved by many of the strong allelopathic weed species on agricultural sites, Putram and Duke (1974) explored the posibility of achieving similar dominance of crops over weed using allelopathic mechanisms, thus opening up new possibilities for weed contol based on biological/ biochemical interactions. Their original approach was to search for allelopathic charastaristics among the germplasm of crops, in the hope that these might be incorporated genetically in to cultivars, either by conventional breeding or other genetic transfer techniques. With this approach, superior weed suppressing crop germplasms have been alredy found in cucumber (Putnam & Duke, 1974), oat (Fay & Duke, 1977), and sunflower (Helianthus annus) (Leather, 1983).

Another approach is the manipulation of allelopathic crops or their residues either sequentially (as rotational crops) or as companion plants (intercrops) in annual or perenial cropping systems (Putnam & DeFrank, 1983). This may be done by planting strips of species which actively release substances toxic to weeds, but not to the crops being grown (Altieri & Doll,

1978). Several cereals in particular, have strong interference ability and are known to be strong allelopathic. For decades growers have used barley (Hordeum valgare) and rye as smother crops. Overland (1966) reported that as at least a portion of barley's suppressive effect can be attributed to allelopathy. Dead or decaying plant material may provide a major source of allelochemicals.

Putnam and co-workers have conclusively proved that allelopathy plays an important role in both rye (Putnam et al, 1983) and sorgum (Lehle & Putnam, 1983) and have proposed that living plants and or residues of barley, rye, sorghum, wheat or oat can provide exceptional suppression of certain weed species. Putnam (1985a) pointed out that not only can surface residues have detrimental influences on crop emergence, growth and productivity, but they may influence similar aspects of weed growth. There is little doubt that managment of cover crops and surface residues can greatly reduce germination and growth of numerous annual weed species, in addition to being benificial for conservation of soil and water. Much research is currently undertaken around the world to develop crop managment systems that employ allelopathic cover crops prior to planting and sequential intercropping to supress weeds between rows.

According to Putnam (1985a; 1986), the probable involvement of allelochemicals in regulating weed-seed germination of seed- decay opens up the posibility of attacking the weed -seed bank in agricultural soils, through a better understanding of the allelochemicals involved. He suggests that research should be directed towards finding ways to increase weed-seed decay or methods to stimulate or inhibit weed seed germination. Overcoming the problem of long-term weed-seed viability so that seed banks could be reduced on agricultural land may become an important weed managment stratergy for future.

It has become evident that research on allelochemicals (i.e. both higher plant and microbial products) could lead to a new generation of pesticides, including herbicides, and plant growth regulators. Several of the major chemical companies in U.S.A., Japan and Europe are actively pursuing this idea and some promising leads have alredy been found. A good example is the production of herbicides with properties similar to the popular herbicide glyphosate ('Round up'), from broth cultures of Streptomyces, an actinomycete organism (Putnam, 1986). A cineole derivative is also now being developed as a herbicide (Putnam, 1986).

As Rice (1984) and Putnam (1985a, 1986, 1988a, 1988b) have suggested much of this knolwedge about chemical warefare between plants, can be put into profitable forestry, agricultural or horticultural production systems, with a cooperative effort of scientists from several disciplines. The progress made on allelopathy research in the last two decades has provided a strong foundation from which to launch these efforts

of exploiting the phenomenon for our benefit.

2.11 Allelochemicals, as Herbicides

As shown previously, allelochemicals may provide clues to new herbicide chemistry. By modifying these allelochemicals the end product could be made more active, selective, or persistent. The precursor for the end product might be obtained from plant sources, if the economy of the latter approach is superior to that of chemical synthesis. An example of a commercial herbicide that contains a natural product moiety is cinmethylin. A portion of the molecule is cineole, a terpene contained in high concentrations in certain desert plants such as Salvia, as previously discussed. If the economics favoured the extraction of precursor from natural sources rather than through synthesis, production of herbicides would be greatly revolutionized. In the future, plants also may be genetically manipulated to increase their yeilds of useful secondary metabolites (Putnam 1988a)

CHAPTER 3

BIOLOGICAL STUDIES WITH GLIRICIDIA AND TITHONIA LEAF MATERIALS

SECTION 3.1

MATERIALS AND METHODS

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3.1.1 Laboratory Trials with Water Extracts of Leaf Materials of Gliricidia and Tithonia

3.1.1.1 Preparation of Extracts

Studies were carried out with aqueous extracts of both (a) fresh leaf materials and (b) dried leaf 'litter'. The latter was prepared by sun-drying freshly collected leaf materials for 2-3 days.

Aqueous extracts were made by taking a known weight of fresh or dried leaves and (a) blending the leaves with the appropriate volume of water in an electrical blender (blended extract), or (b) soaking the in water (100 ml) with continuous shaking for 24 hrs (leachate extract). The term 'leachate' was used to designate a mild form of leaching such as could

occur in natural conditions, as opposed to blending or chemical extraction. Extracts were filtered first through cheese-cloth, and later under suction through Whatman No. 2 filter paper. Extracts were then diluted as appropriate to produce a dilution series in the range corresponding to concentrations of 2.5 to 250 mg/ml of leaf material.

3.1.1.2 Petri-dish Studies

The effects of the fresh leaf or dried leaf extracts on seed germination and seedling growth of several common weed and crop species were examined using standard petri-dish assays. Seeds of the test species were either collected from the field (weed species) or purchased from seed outlets of the Department of Agriculture (crop species). Seeds were surface sterilized by dipping in 1% Mercuric chloride for 1 minute just before use. Twenty-five seeds of each test species were placed in each petri-dish lined with Whatman No.1 filter paper. Four ml of each extract was added to each of the dishes. The petri dishes were kept on the laboratory bench under continuous light at 29 + 1 ^{0}C and 70 + 5 % relative humidity. Three replicate dishes were used for each treatment and the dishes arranged on the bench in a completely randomized design. Percent seed germination was taken at 7 or 10 days. At 21 to 28 days after the initiation of each experiment, the growth of the seedlings was also measured by taking their shoot length, radicle length and/or seedling fresh weight. These values were compared with the results of the untreated controls.

3.1.2. Greenhouse Studies with Leaf Residues of Gliricidia and Tithonia

3.1.2.1 Preparation of Residues

Residues of Gliricidia or Tithonia were prepared by harvesting fresh foliage and sun-drying the leaves for 48-72 hrs. Once dried, the leaf material was ground to a uniform size in a toothed-disk mill, and stored in large glass bottles until required for experiments.

3.1.2.2 Greenhouse Conditions and Soil

Experiments with residues were carried out in a greenhouse, under natural daylight only, which was received generally between 8.00 and 17.00 hrs. The day temperature within the greenhouse ranged between 29 and 31^{0}C, while at night it was between 27 and 29^{0}C. The relative humidity was 75% or above. A sandy-loam soil which contained 40-45% sand, 24-25% each of silt and clay and 2-2.5% organic matter, was used in the studies.

3.1.2.3 General

Each of the following experiments was carried out. There were a minimum of three replicate pots per treatment and all pots were completely randomized, or arranged in randomized complete block designs on the glasshouse bench. Each experiment was repeated at least once more.

3.1.2.4 Studies with Soil-Incorporated Residues

The purpose of this study was to determine whether incorporated residues of Gliricidia or Tithonia produced any inhibitory (or stimulatory) effect on the growth of selected crop and weed indicator species. Either Gliricidia or Tithonia Residues were incorporated into soil at concentrations of 0, 1, 2, 4 or 8 % (w/w). The plant material was uniformly mixed with separate batches of 25 kgs of soil, and the incorporated soil taken up in an appropriate number of 20 cm diameter clay pots. Ten seeds of each of the test species (Crop- tomato, chilli; Weed- Tridax procumbens, Hyptis capitata, Achyranthes aspera) were planted one cm deep in each pot. No extra fertilizer was provided. Water was added daily in equal volumes across the pots, to keep the soil just under field capacity. Percent seed germination was obtained at 14 days, after which plants were allowed to grow until a final harvest at 28-30 days.

3.1.2.5 Studies with Residues applied as 'Surface mulch'

This study investigated the effect of the residues of the two species, applied as 'surface mulch' only, on the germination and growth of indicator species. As in the case of incorporated residues (Section 3.1.2.4), the rates of residues for mulch treatments used were 0, 1, 2, 4 or 8 % (w/w). The appropiate amount of residue material per pot, was placed on the soil surface as a surface mulch. Seeds of crop and weed indicator species were planted one cm deep in the soil and the pots watered daily to a level just under the field capacity. Growing conditions were the same as in the previous experiment. The growth of test species was followed up to 28-30 days.

3.1.2.6 Studies with Soil-incorporated and decomposing Residues

To examine the effect of decomposing residues of Gliricidia or Tithonia on the germination and seedling growth of the selected indicator species, dried leaf residues were incorporated into batches of soil at rates of 1, 2, and 4% w/w. Control soil was free of any residues. Batches of soil 8 or 10 kg each, incorporated with residues, were placed in double layered poly-ethylene bags and kept in the glass house for decomposition of residues

to occur. One hundred ml of water was provided per bag per week to replace the water that may have been lost through evaporation. After allowing for decomposition of residues to take place for 1, 2, 4, and 6 weeks, samples of soil were taken out from each treated batch into small plastic pots (10 cm diam) and assays carried out as in the previous cases with suitable test species. Ten seeds each of the indicator species were planted in each pot and allowed to grow for 28-30 days after which plants were harvested. Percent seed germination was recorded at 14 days. At final harvest, the mean dry weight of indicator species was measured per pot.

3.1.3 Field Trials to determine weed-suppression ability of Gliricidia and Tithonia Leaf Residues

A number of field experiments were carried out over a three year period, in order to determine mainly the weed suppression ability as well as other general beneficial (stimulatory) effects of Gliricidia and Tithonia leaf residues in upland cropping. Given below are the details of four such field trials.

3.1.3.1. General

The sites used for field trials were cleared of all weeds, large rocks and rubble, and levelled using manual labour. The soil was tilled with mamoties and turned up to a depth of about 12 inches. Plots, one square meter (1 m^2) in size were prepared, surrounded by ditches on all sides. Due to the possibility of the soil being deficient in organic matter, about 10 kgs of semi-dried cow-dung was added to each 1 m^2 plot, allowed to dry completely and well-mixed with the soil. No additional inorganic fertilizer was added to any of the plots.

The trials were conducted with three crop species, chilli, brinjal and tomato, used in various combinations, depending on the availability of seed. All three species were first raised in a nursery up to about three-weeks of age, after which they were transplanted into the field plots. Usually up to about three seedlings were initially transplanted, from which only one healthy plant was allowed to grow after establishment. Tomato plants were always in the centre of each plot, brinjal and/or chilli, always on the four corners. The plots were well-watered until the establishment of the crop seedlings, for about two-weeks after trans- planting.

Gliricidia and Tithonia leaf materials were collected in large amounts from usual collection sites and transported to the field sites, normally one day before applications to the field. Fresh leaf materials were applied on the plots at rates given below for each trial.

At the time of harvesting, the crop plants were uprooted washed free of soil, and their total dry weight (without fruits) taken after drying in an oven (80^0C) to a constant weight. The yield of tomato, brinjal or chilli fruits was always taken as their fresh weight. In addition to the crop plants, all the weeds that were growing in each plot were also uprooted, and their total dry weight obtained. The weeds present were also identified and recorded.

3.1.3.2 Field Trial I:

A first field trial was conducted in a field site in Borupana, Ratmalana. The effect of Gliricidia leaf residues was tested, at two rates of 1.0 kg/m^2 or 2.0 kg/m^2. These two rates were equivalent to applications of 10 and 20 metric tons per hectrare, respectively, of 'mulch' material in a field. Two applications of the leaf material were made; the first application was at one week after transplanting of seedlings, and the second, at four weeks after transplanting. Leaf material was equally distributed over the whole plot. Control plots were left without any added residues and unweeded. The field trial was terminated 3-months after transplanting the crop plants in the field.

3.1.3.3 Field Trial II

The second field trial, also with Gliricidia leaf residues, was conducted in a field site within the University of Colombo premises. A similar plot arrangement as in Trial 1 was used. The rates of residue application were 2 kg/m^2 or 4 kg/m^2 (corresponding to field appli-cations of 20 or 40 Metric Tons per ha, of leaf material, respectively). There were three application times in this trial, at one-week, one month and two months after transplanting of test species. Control plots were left without any plant residues and also unweeded. The field trial was terminated 4-months after tranplanting the crop plants in the field.

3.1.3.4 Field Trial III

The third field trial, studied the effects of Tithonia leaf residues, and was conducted in the field site within the University of Colombo premises. A similar plot arrangement as in Trials I and II was used. The rates of residue application were 2 kg/m^2, 4 kg/m^2 or 6 kg/m^2 (corresponding to field applications of 20, 40 or 60 Metric Tons per ha, of leaf material, respectively). There were two application times in this trial, at one-week, and one month after transplanting of test species. Two

types of control plots were established without any plant residues: one was a weeded-control, kept weed-free by manual weeding, and the other was an unweeded-control. The field trial was terminated 3-months after transplanting of the crop plants in the field.

3.1.3.5. Field Trial IV

A fourth field trial, compared the effects of applying Gliricidia and Tithonia leaf residues as mulches, with that of several other popular leaf mulch materials, viz. leaf residues of Chromalaena odorata L., Croton lacciferus, and Salvinia molesta D.S. Mitchell. This trial was also conducted in the field site within the University of Colombo premises. The rates of residue application were 0 (control), 2 kg/m^2 or 4 kg/m^2, of each type of mulch. There were two mulch application times in this trial, at one-week, and one month after transplanting of test species. Two types of control plots were established without any plant residues: one was kept weed-free by manual weeding, and the other was left unweeded. The field trial was terminated 3-months after tranplanting of the crop seedlings in the field.

3.1.4 Statistical Analyses

Statistical analyses were done using the Computer statistical package INSTAT (Computer Applications Centre, Reading University, U.K.). All data generated in the studies (seedlingh lengths, fresh or dry weights; whole plant dryweights) were analysed by standard Analyses of Variance to determine the significance of treatment effects. Percent seed germination data were transformed to the Arcsine and analysed. Significant meand were separated using the L.S.D. procedure or the Duncan's Multiple Range test.

SECTION 3.2

RESULTS

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3.2.1 Laboratory trials with Water Extracts of Leaf materials of Gliricidia and Tithonia

(a) Gliricidia:

Both the blended and leachate extracts of Gliricidia leaves caused a significant inhibition of seed germination of tomato, brinjal, chilli, Tridax and Achyranthes, at the highest extract concentration of 250 mg/ml of leaf material (Table 3.1). The blended extract was more active and inhibitory than the leached extract possibly due to greater extraction of phytotoxic compounds during blending. The aqueous fresh leaf extract of Gliricidia at 250 mg/ml was also inhibitory to the seedling growth of the four test species as indicated by the reduction in seedling length (Table 3.2). However, it was evident that at the extract concentration of 25 mg/ml, the fresh leaf extract was somewhat stimulatory to the seedling growth of chilli and Tridax.

Similarly, blended and leached extracts of Gliricidia dried leaves were inhibitory to the seed germination of tomato, chilli, Tridax and Achyranthes at the extract concentration of 250 mg/ml of leaf material. Chilli seeds were noticeably less sensitive to the leached extract, compared with the blended extract (Table 3.3). Extracts of Gliricidia dried leaf materials were also found to be both stimulatory and inhibitory of seedling

Table 3.1: The Effect of Aqueous Extract of Fresh Leaves of Gliricidia on the % Germination of selected Crop and Weed species (at 10 days)

Extract Type/Species	Extract Concentration (mg/ml) 0	2.5	25	250
(a) Blended Extract				
Tomato	69a	73a	65a	11b
Brinjal	69a	61a	53a	8b
Chilli	41a	36a	26b	8c
Tridax	45a	33a	42a	0b
Hyptis	74a	77a	65a	0b
Achyranthes	53a	52a	45a	0b
(b) Leachate Extract				
Tomato	69a	68a	76a	64a
Chilli	41a	39a	44a	19b
Tridax	49a	55a	49a	5b
Achyranthes	68a	68a	59a	32b

In this and all Tables to follow Mean values in each row followed by the same letter are not significantly different at 5% level of probability according to the Duncan's Multiple range test.

Table 3.2: The Effect of aqueous Fresh Leaf extract of Gliricidia on the Mean Seedling Length (cm) of selected Crop and Weed Species (at 28 days)

Species	Extract Concentration (mg/ml) 0	2.5	25	250
Brinjal	7.9b	8.9a	8.1b	7.2c
Chilli	8.0a	7.9a	8.4a	6.0b
Tridax	5.1a	5.4a	5.2a	2.6b
Eupatorium	3.9a	3.5ab	2.9b	1.3c

growth, depending on species and concentration. In particular, chilli and Tridax seedling growth was very greatly stimulated by the Gliricidia dried leaf extract at 25 mg/ml, as with the fresh leaf extract (Table 3.4).

(b) Tithonia:

In general, the blended and leachate extracts of fresh Tithonia leaves were inhibitory to the test species at the 200 mg/ml concentration. Significant inhibition of germination of all test species occurred at this concentration, while the response brought about in different species varied slightly, being dependent on concentration (Table 3.5). The aqueous fresh leaf blended extract of Tithonia was also inhibitory to seedling growth of the four test species as indicated by the reduction in seedling fresh weight (Table 3.6). The leachate extract was comparatively less effective.

When compared with the above results, the blended and leachate extracts of Tithonia dried leaves were highly inhibitory to the seed germination of tomato, chilli, Tridax and Achyranthes (Table 3.7). Seed germination of tomato, chilli, and Achyranthes were significantly and adversely affectly by the extract concentrations of 100 mg/ml and above. Seeds of Tridax and Achyranthes were significantly inhibited from germination by the extract even at the concentration of 50 mg/ml. The aqueous dried leaf extracts were inhibitory to the seedling growth of all species at 100 mg/ml (Table 3.8). The two weed species Tridax and Achyranthes were affected even by the lower concentration of 50 mg/ml, as indicated by the reduction in seedling fresh weight (Table 3.8). Chilli was relatively less sensitive to both types of extracts as shown by seed germination, as well as seedling growth. Tomato on the other hand, was very sensitive to Tithonia dried leaf extracts.

3.2.2 Greenhouse studies with Leaf Residues of Gliricidia and Tithonia:

3.2.2.1. Studies with Soil-incorporated Residues

(a) Gliricidia

Incorporation of Gliricidia residues into soil did not affect the germination of tomato, brinjal, Tridax and Achyranthes (Table 3.9). However, the residues significantly reduced the germination of Hyptis at all three rates of incorporation.

On the other hand, the residues had a remarkable and significant stimulatory effect on the growth of some indicator seedlings. A marked

Table 3.3: The Effect of Aqueous Extract of Dried Leaves of Gliricidia on the % Germination (at 14 days) of selected Crop and Weed species

Extract Type/Species	Extract Concentration (mg/ml) 0	2.5	25	250
(a) Blended Extract				
Tomato	73a	78a	68a	0b
Chilli	85a	80a	80a	0b
Tridax	70a	84a	74a	0b
Achyranthes	90a	78b	32c	0d
(b) Leachate Extract				
Tomato	70a	66a	68a	0b
Chilli	85a	84a	89a	48b
Tridax	76a	73a	73a	0b

Table 3.4: The Effect of Aqueous Extract of Dried Leaves of Gliricidia on the Mean Seedling Fresh Weight (mg) of selected Crop and Weed Species (at 14 days)

Extract Type/ Species	Extract Concentration (mg/ml) 0	2.5	25	250
(a) Blended Extract				
Tomato	5.6a	3.5b	2.01c	0d
Chilli	11.7c	12.5b	20.5a	0d
Tridax	1.8b	4.1b	3.6a	0c
Achyranthes	4.1b	6.2a	2.4c	0d
(b) Leachate Extract				
Chilli	11.5b	12.3b	18.1a	5.4c
Tridax	2.1b	4.4b	3.4a	0c

Table 3.5: The Effect of Aqueous Extract of Fresh Leaves of *Tithonia* on the % Germination of selected Crop and Weed species (at 14 days)

Species	Extract Concentration (plant material mg/ml)				
	0	10	50	100	200
(a) Blended Extract					
Tomato	93a	80b	83b	78b	36c
Chilli	78a	89a	96a	89a	79a
Achyranthes	80a	76a	71a	67a	20b
Tridax	96a	92a	88a	79b	40c
(b) Leachate Extract					
Tomato	65a	75a	65a	77a	51a
Chilli	80a	87a	89a	89a	75a
Achyranthes	47a	37a	77b	51a	23a
Tridax	89a	88a	83a	47a	60a

Table 3.6: The effect of Aqueous Extract of Fresh Leaves of *Tithonia* o the Mean Seedling Fresh Weight (mg) of selected Crop and Wee Species (at 14 days)

	Extract Concentration (plant material mg/ml)				
Species	0	10	50	100	200
(a) Blended Extract					
Tomato	12.3a	12.3a	8.2a	11.9a	7.4b
Chilli	16.7ab	25.4a	27.4a	21.0a	14.3b
Achyranthes	7.7a	9.2a	8.3a	7.9a	1.5b
Tridax	6.3a	7.4a	6.8a	4.2b	1.7b
(b) Leacnate Extract					
Tomato	6.5a	10.0a	7.8a	6.2a	6.2a
Chilli	27.3a	31.7a	44.3a	33.2a	22.8a
Achyranthes	2.8b	2.7b	6.2a	3.8b	2.8b
Tridax	4.5a	5.4a	5.5a	2.2b	2.7b

Table 3.7: The Effect of Aqueous Extract of Dried Leaves of *Tithonia* on the % Germination of selected Crop and Weed species (at 14 days)

Species	Extract Concentration (mg/ml)				
	0	10	50	100	200
(a) Blended Extract					
Tomato	68a	80a	64a	9b	0c
Chilli	85a	81a	93a	77a	25b
Achyranthes	73a	76a	73a	29b	0c
Tridax	76b	96a	51c	0d	0d
(b) Leachate Extract					
Tomato	76a	77a	55a	13b	0b
Chilli	93a	76a	73a	71a	25b
Achyranthes	79a	73a	8b	4b	0c
Tridax	90a	80a	1b	0b	0b

Table 3.8: The effect of Aqueous Extract of Dried Leaves of Tithonia on the Mean Seedling Fresh Weight (mg) of selected Crop and Weed Species (at 14 days)

Species	Extract Concentration (mg/ml) 0	10	50	100	200
(a) Blended Extract					
Tomato	10.4a	11.0a	13.4a	1.6b	0c
Chilli	24.4a	26.9a	27.6a	13.7b	2.9c
Achyranthes	7.1a	10.3a	8.9a	0.7b	0c
Tridax	4.8b	6.8a	2.3c	0d	0d
(b) Leachate Extract					
Tomato	17.8a	16.4a	11.7a	1.5b	0b
Chilli	20.9a	10.7b	12.3b	9.3b	0.5c
Achyranthes	5.8a	6.9a	0.6b	0.3b	0b
Tridax	5.0a	4.2a	0.1b	0b	0b

Table 3.9: The Effect of Gliricidia Leaf Residues incorporated into soil on the Germination and Growth of indicator Species.

Species	Residue Concentration (% w/w)							
	0	1	2	4	0	1	2	4
	Germination (%)				Fresh Weight (g)			
Tomato	63a	30a	40a	67a	1.0b	1.1b (+10)	0.9b (+10)	1.9a (+90)
Brinjal	73a	40a	37a	30a	0.6b	0.6b	1.1a (+83)	1.4a (+133)
Tridax	47a	20a	27a	40a	0.8a	1.1a (+38)	0.8a	1.3a (+63)
Hyptis	67a	31b	30b	23b	0.7a	0.2a (-71)	0.5a (-29)	0.7a
Achyranthes	37a	27a	20a	27a	0.6a	1.0a (+67)	0.5a (-17)	0.5a (-17)

% Germination after 14 days; Fresh Weight after 28 days.
Figures within parenthesis refer to percent stimulation or inhibition compared with controls

promotion of growth of crop species tomato (by 90%) and brinjal (by 133%), and the weed Tridax (62.5%) was recorded at the rate of 4% (w/w) incorporated residues, compared with no residue controls (Table 3.9). Even the 2% incorporated residues had stimulated chilli growth by 83%, although tomato growth was not increased.

The residues at higher rates, were inhibitory to growth or had little or no effect on the weed species Hyptis and Achyranthes. However, at the low rate of 1%, the growth of Tridax and Achyranthes was stimulated by 37.5 and 66.7%, respectively.

(b) Tithonia

Soil-incorporated residues of Tithonia significantly reduced the germination of tomato and chilli seeds at residue levels 4 and 8% (w/w). However, the residue rates 1 and 2% (w/w) somewhat stimulated the germination of tomato and chilli seed compared to controls (Table 3.10). Residues incorporated at 1% or above were inhibitory to seed germination of Tridax, but in this experiment the seeds of Tridax in untreated controls also showed poor germination. Achyranthes seed germination was significantly reduced by the Tithonia residues at 2% (w/w) or above.

When the seedling fresh weight was measured at 28 days, the incorporated residues had significantly promoted the growth of tomato at the rate of 1 and 2% (w/w) (Table 3.10). However, tomato seedling growth was adversely affected at 4 and 8% (w/w) levels of residues. Growth of chilli seedlings was not promoted by Tithonia residues, but inhibited at residue rates 2 % and above. The seedling growth of weed species Tridax and Achyranthes was significantly reduced by all residue rates. In general, incorporated Tithonia residues appeared to cause more inhibition and less stimulation of seedling growth of the indicator species.

3.2.2.2 Studies with Residues applied as 'Surface Mulch'

(a) Gliricidia

Gliricidia leaf residues placed on the soil surface as mulch at 4% (w/w) significantly reduced the % germination of Achyranthes and inhibited the germination of Tridax and Hyptis (Table 3.11). Germination of Tridax was prevented even by the 2% (w/w) mulch rate. The mulch did not inhibit the germination of the large-seedad crop species tomato and brinjal at any of the application rates, and in fact promoted their seed germination at the 2% (w/w) mulch rate.

Table 3.10: The Effect of *Tithonia* leaf residues incorporated into soil on the Germination and Growth of indicator Species.

Species	Residue Concentration (% w/w)									
	0	1	2	4	8	0	1	2	4	8
	Germination (%)					Fresh Weight (mg)				
Tomato	35b	35b	53a	27b	33c	1.4b	2.0a (+42.9)	1.7ab (+21.3)	0.7c (-50)	0.3c (-78.6)
Chilli	35b	53a	47a	20c	8d	0.6a	0.4a (-33.3)	0.2b (-66.6)	0.2b (-66.6)	0.02c (-96.7)
Tridax	20a	10b	15ab	0c	0c	0.6a	0.08b (-86.7)	0.1b (-83.3)	0c (-100)	0c (-100)
Achyranthes	75a	70a	35b	25b	13c	1.0a	0.9ab (-10)	1.2a (+20)	0.7b (-30)	0.03c (-97)

% Germination after 14 days; Fresh Weight after 28 days.

Table 3.11: The Effect of Gliricidia Leaf Residues applied as a Surface Mulch on the Germination and Growth of indicator Species.

	Residue Concentration (% w/w)							
Species	0	1	2	4	0	1	2	4
	Germination (%)				Fresh Weight (g)			
Tomato	53b	90a	67b	50b	0.4c	2.1b (+425)	2.7a (+575)	3.0a (+650)
Brinjal	40bc	87a	80ab	20c	0.3c	1.4a (+367)	1.4a (+367)	1.1b (+267)
Tridax	33a	23a	0b	0b	0.3ab	0.6a (+100)	0.1b (-67)	0b
Hyptis	63a	17b	10bc	0b	0.6a	0.5a (-17)	0.3a (-100)	0a
Achyranthes	47a	37a	42a	13b	0.4c	1.0ab (+150)	1.4a (+250)	0.8bc (+100)

% Germination after 14 days; Fresh Weight after 28 days.
Figures within parenthesis refer to percent stimulation or inhibition compared with controls

Subsequent growth of tomato and brinjal was very significantly and spectacularly promoted by the mulch. The increments in growth in the case of tomato, compared to no residue control plants were 650, 575 and 425 %, respectively, for the rates 4, 2 and 1% (w/w) residues. For brinjal, the increases were 366% for each of 1 or 2% mulch rates and 266% for the 4% rate. The growth of Achyranthes was also increased by mulch at all rates, the highest increase being at 2% residues. The growth of weed species Tridax and Hyptis was severely inhibited by the 2% mulch. Furthermore, the low rate of 1% mulch caused stimulation of Tridax growth, but caused a slight inhibition of Hyptis growth.

(b) Tithonia

Tithonia leaf residues applied as surface mulch at the high rate of 8% (w/w) were totally inhibitory to the germination and seedling growth of all indicator species (Table 3.12). The 4% (w/w) rate of the mulch was also generally inhibitory to seed germination, but did not affect seedling growth significantly, compared to no residue controls.

As in the case of Gliricidia mulches, Tithonia mulch treatments at 1 or 2% (w/w) caused a very significant stimulation of seed germination and seedling growth of both tomato and chilli. Compared to no residue controls, tomato growth was stimulated by as much as 133% and 583.3%, respectively, by these two mulch rates; the same figures for brinjal were 117% and 84%. As was observed with Gliricidia residues, there was some stimulation of Achyranthes growth (49%) by the Tithonia mulch at 2% rate.

3.2.2.3 Studies with Soil-Incorporated and Decomposing Residues

(a) Gliricidia

The results of this study produced a somewhat variable response, sometimes indicating stimulation of growth and at other times, phytotoxicity and rate-dependent growth inhibition of indicator species. A close examination of the response of the four indicator species however, indicates that as residues decomposed there was a tendency to lose phytotoxicity, resulting mainly in stimulation of growth of the indicator species. For instance, the decomposing Gliricidia residues at 4% (w/w) significantly promoted the growth of tomato even after 6-weeks of decomposition (Fig. 3.1). After 4- and 6-weeks of decomposition, the lesser rates of Gliricidia residues (i.e. 1 or 2% w/w) also, generally stimulate tomato growth.

Table 3.12: The Effect of *Tithonia* leaf residues applied as a Surface Mulch on the Germination and Growth of indicator Species

Species	Residue Concentration (% w/w)									
	0	1	2	4	8	0	1	2	4	8
	Germination (%)					Fresh Weight (g)				
Tomato	18b	43a	58a	10b	3c	0.6b	1.4b (+133)	4.1a (+583.3)	0.6b	0c (-100)
Chilli	45b	70a	58ab	15c	0c	0.6b	1.3a (+117)	1.1a (+83.3)	0.5b (-16.7)	0c (-100)
Tridax	15a	28a	5b	0b	0b	0.4a	0.5a (+25)	0.2a (-50)	0b (-100)	0b (-100)
Achyranthes	80a	78a	75a	43b	0c	1.0a	0.3b (-70)	1.4a (+40)	0.9a (-10)	0c (-100)

% Germination after 14 days; Fresh Weight after 28 days.

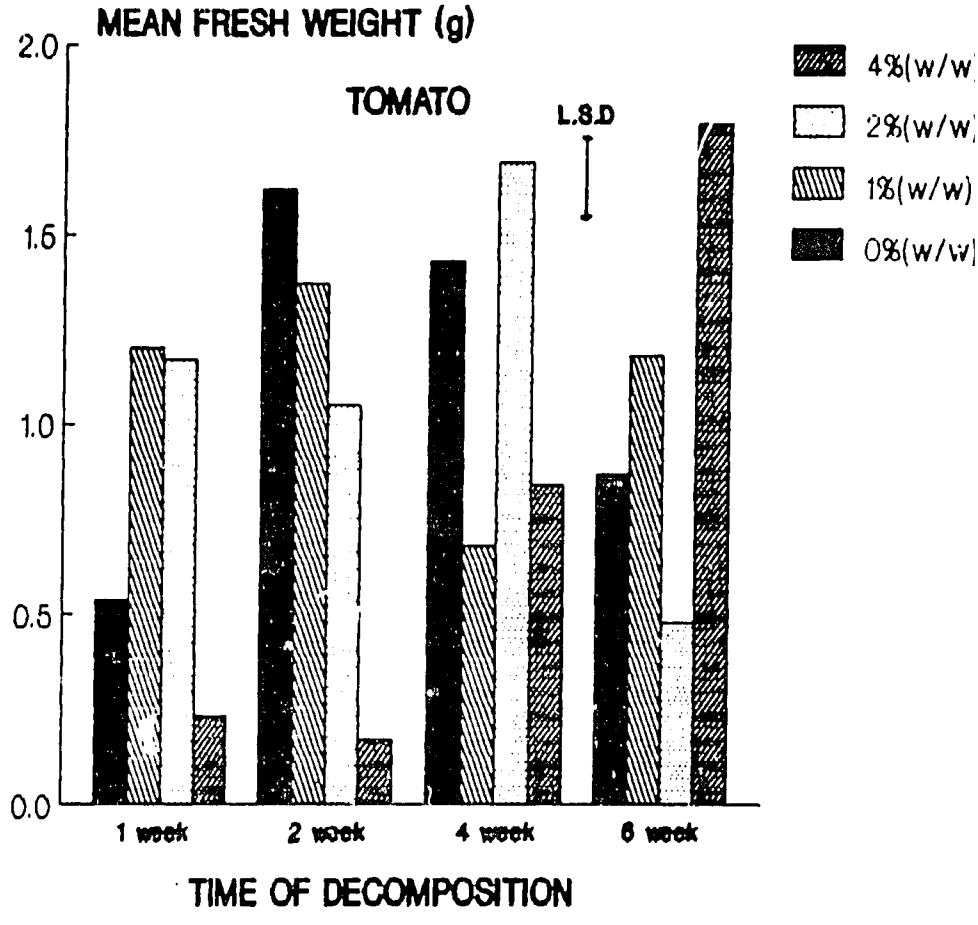

Figure 3.1: Mean Fresh Weight (at 28 days after planting) of Tomato in soil in which incorporated Gliricidia residues had decomposed for 1, 2, 4 and 6-weeks

The growth of brinjal also produced evidence of the decomposing residues ammending the soil to cause growth stimulation even after 6-weeks of decomposition (Fig. 3.2). The response of Hyptis (Fig. 3.3) was also not too different. Pronounced growth stimulation was evident in Hyptis after Gliricidia residues (1 or 2% w/w) had decomposed for 1-, 4- or 6-weeks.

The growth of Achyranthes was stimulated by Gliricidia residues at all rates of incorporation, irrespective of length of decomposition time (Fig. 3.4), providing conclusive evidence of species-dependent growth stimulation by Gliricidia, as the breakdown of residues takes place.

(b) Tithonia

The incorporation and subsequent decomposition of 8 % (w/w), a high rate, Tithonia residues altered the nature of soil and caused a profound inhibition of the growth of all indicator plants. However, at lesser rates of incorporation followed by decomposition, the residues caused a somewhat variable response.

Residues incorporated at 1, 2 or 4% (w/w) and which had decomposed for 1-week, significantly stimulated the growth of tomato (Fig. 3.5). A tendency for growth stimulation was observed when residues had decomposed for 2-weeks, but the effect was much less and not significant. After 4-weeks of decomposition, only the 1% residues caused a growth stimulation, while the other rates were generally inhibitory. After 6-weeks of residue decomposition, the ammended soil was definitely toxic to the growth of tomato.

Tha response of chilli to the decomposed Tithonia residues (Fig. 3.6) produced evidence of growth stimulation relative to no residue controls, with 1, 2 or 4% (w/w) residue rates which had decomposed for 1, or 2-weeks. Although the results were variable, even after the residues had decomposed for 4 or 6-weeks, the soil ammended by the decomposed, incorporated residues (1, 2 or 4% w/w) caused stimulation of chilli growth.

The growth of Iridax was very sensitive to and was also stimulated after Tithonia residues (1 or 2%) had decomposed for one-week (Fig. 3.7). However, as decomposition progressed with time, all residue incorporations apparently made the soil more toxic to the growth of Iridax, compared to the respective no residue controls.

The response of Achyranthes was also highly variable, particularly after 4- or 6-weeks of residue decomposition (Fig. 3.8). There was evidence of phytotoxicity in soil as well as an ability to stimulate Achyranthes. However, after the 1 % (w/w) incorporated residues had decomposed for 1, 2, 4 or 6-weeks, growth stimulation still occurred. In general, the higher rates of residues appear to have ammended the soil to be phytotoxic.

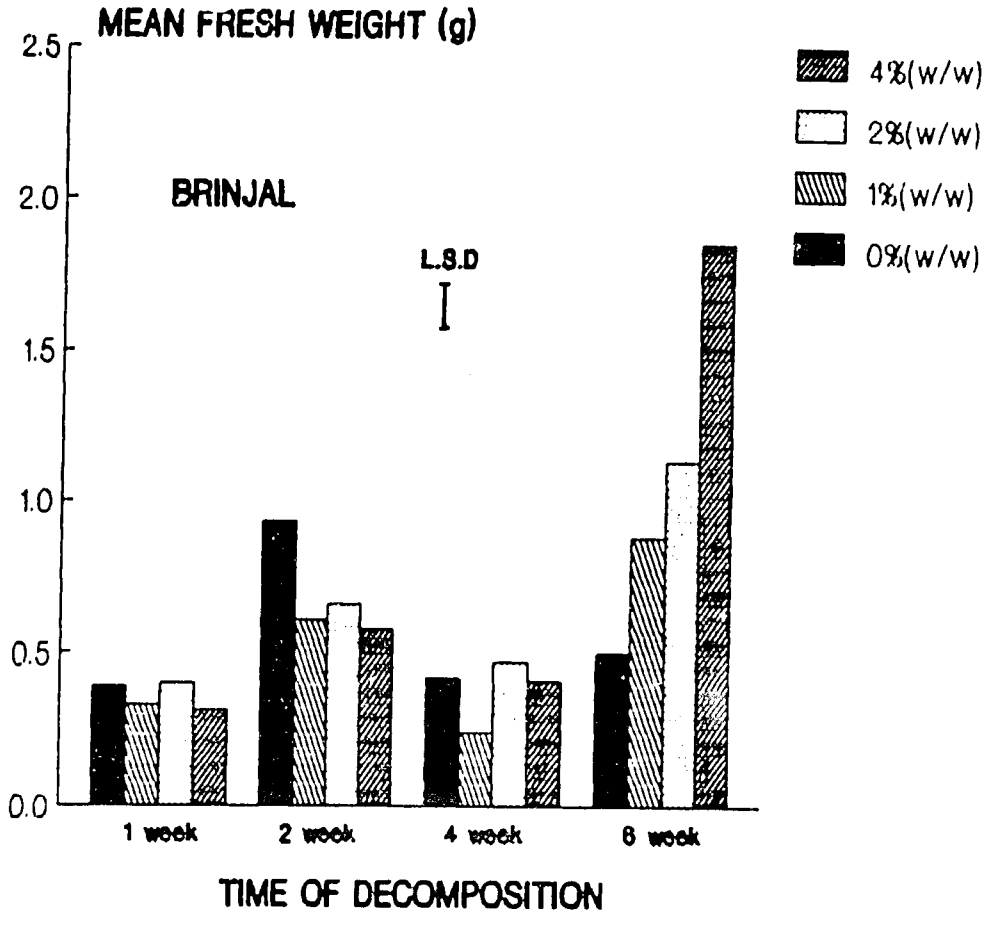

Figure 3.2: Mean Fresh Weight (at 28 days after planting) of Brinjal in soil in which incorporated Gliricidia residues had decomposed for 1, 2, 4 and 6-weeks

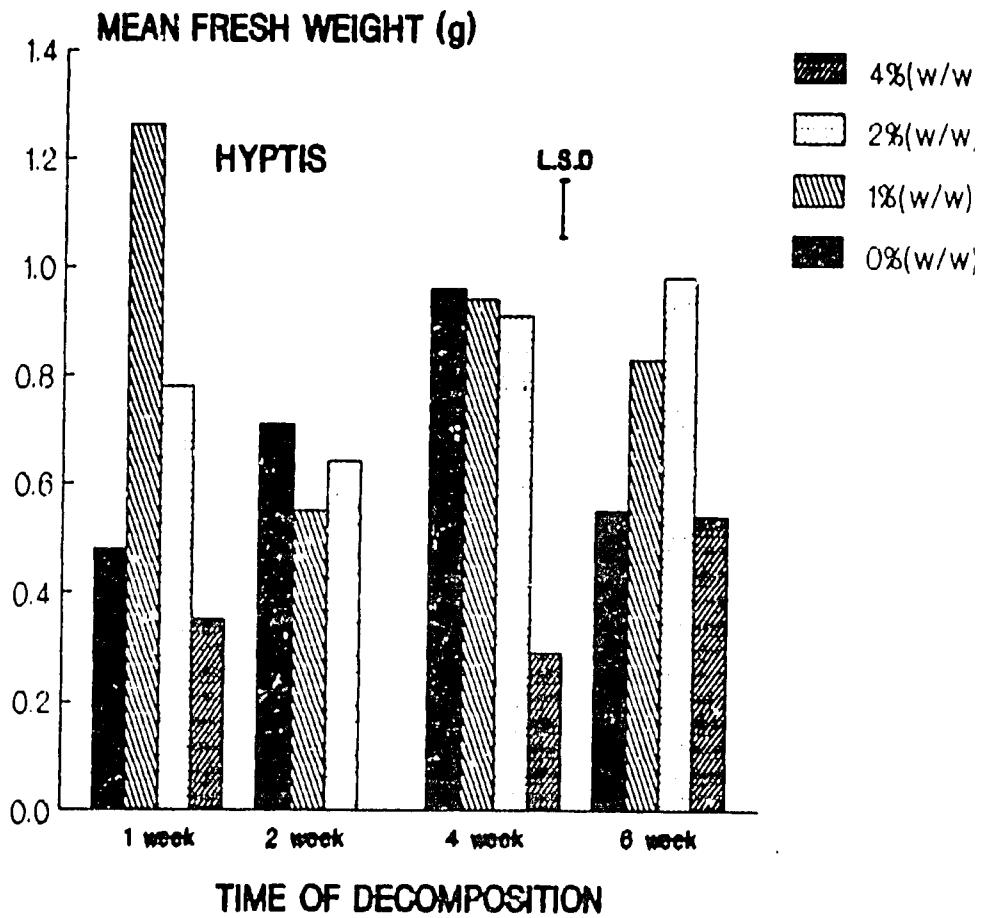

Figure 3.3: Mean Fresh Weight (at 28 days after planting) of Hyptis in soil in which incorporated Gliricidia residues had decomposed for 1, 2, 4 and 6-weeks

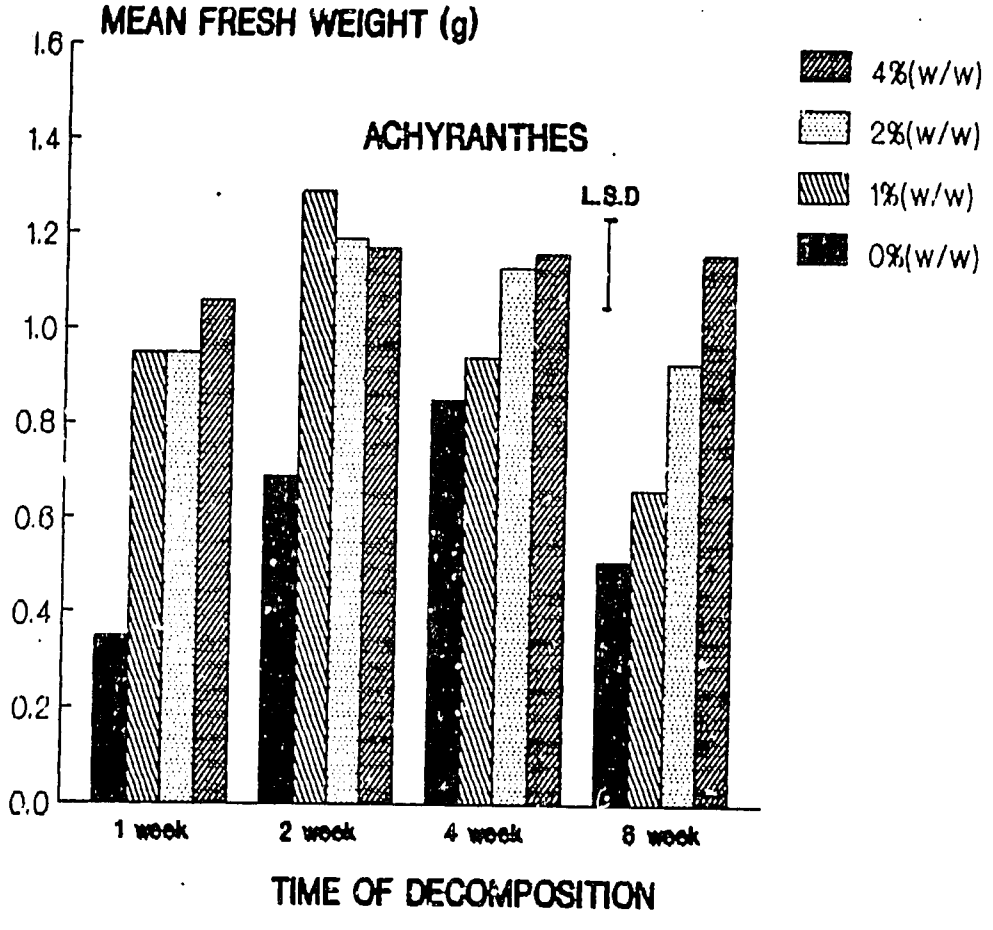

Figure 3.4: Mean Fresh Weight (at 28 days after planting) of Achyranthes in soil in which incorporated Gliricidia residues had decomposed for 1, 2, 4 and 6-weeks

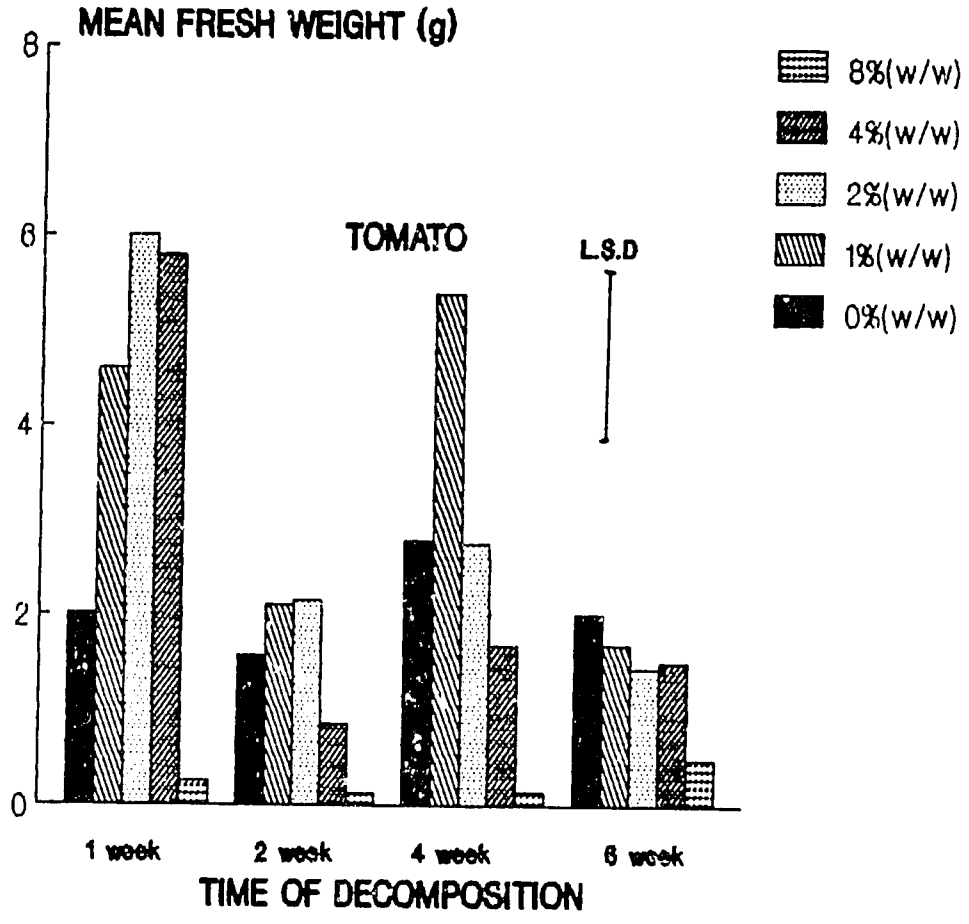

Figure 3.5: Mean Fresh Weight (at 28 days after planting) of Tomato in soil in which incorporated Tithonia residues had decomposed for 1, 2, 4 and 6-weeks

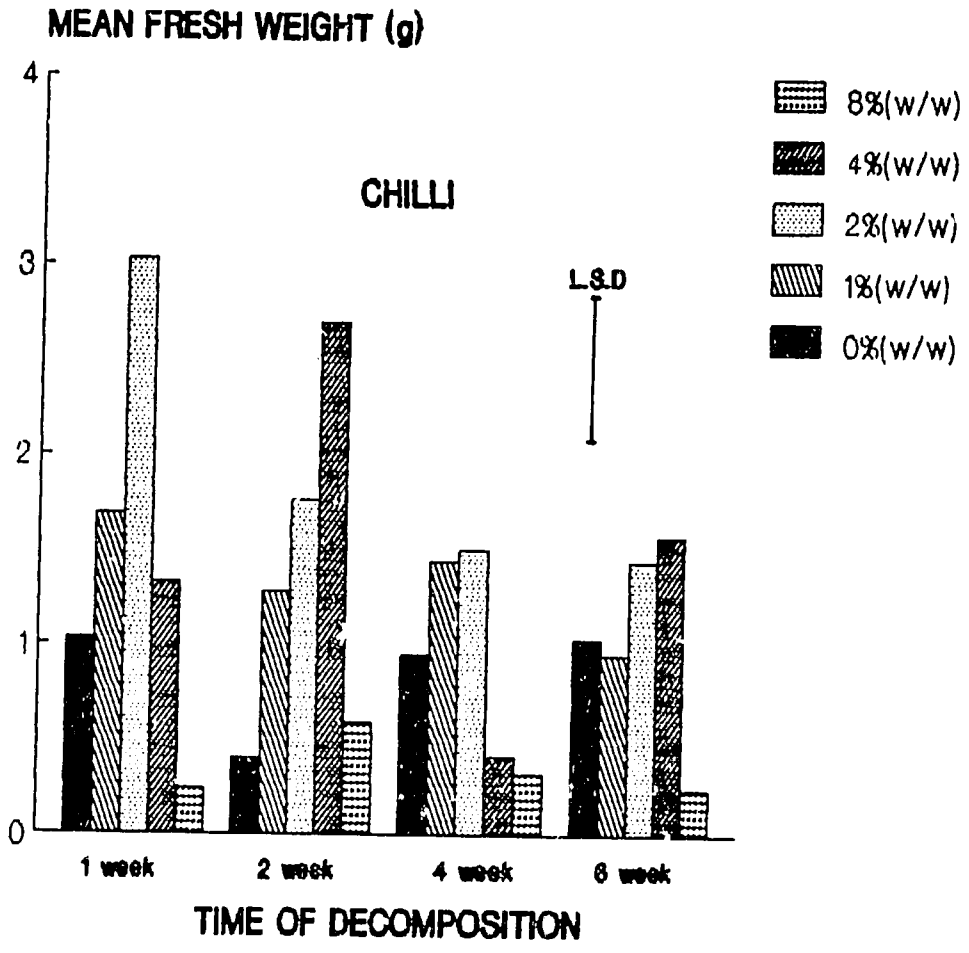

Figure 3.6: Mean Fresh Weight (at 28 days after planting) of Chilli in soil in which incorporated Tithonia residues had decomposed for 1, 2, 4 and 6-weeks

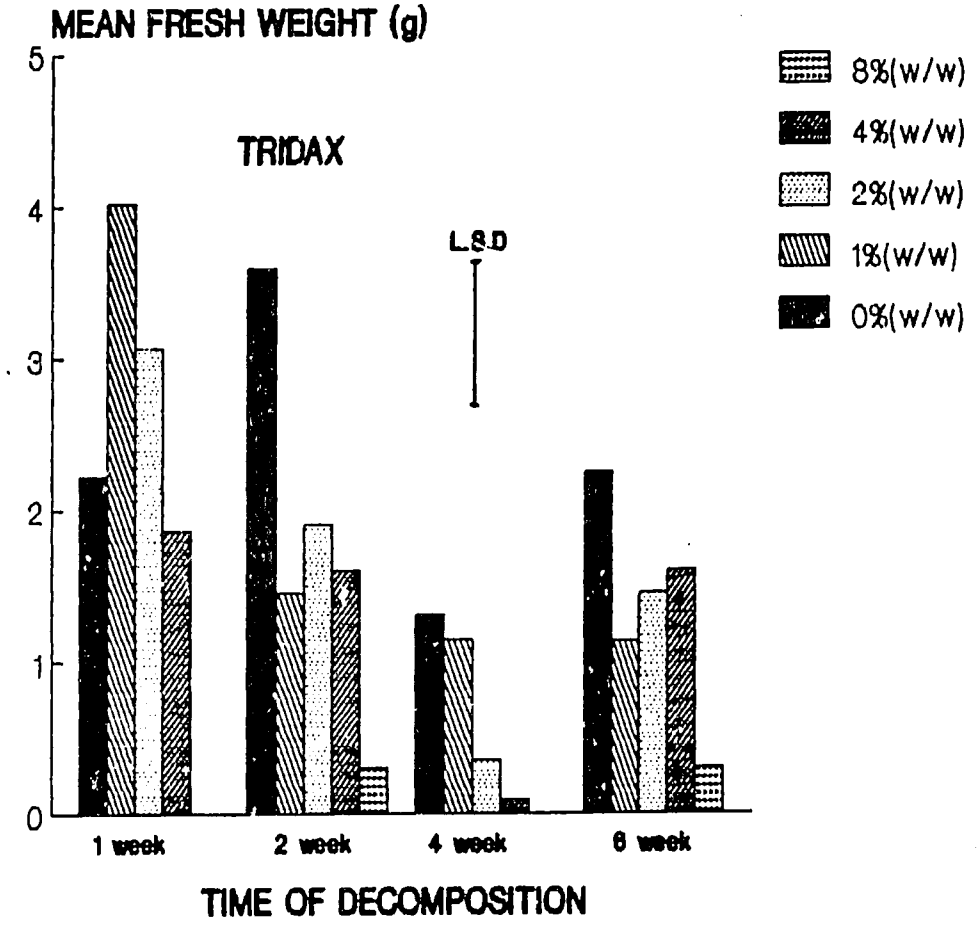

Figure 3.7: Mean Fresh Weight (at 28 days after planting) of Tridax in soil in which incorporated Tithonia residues had decomposed for 1, 2, 4 and 6-weeks

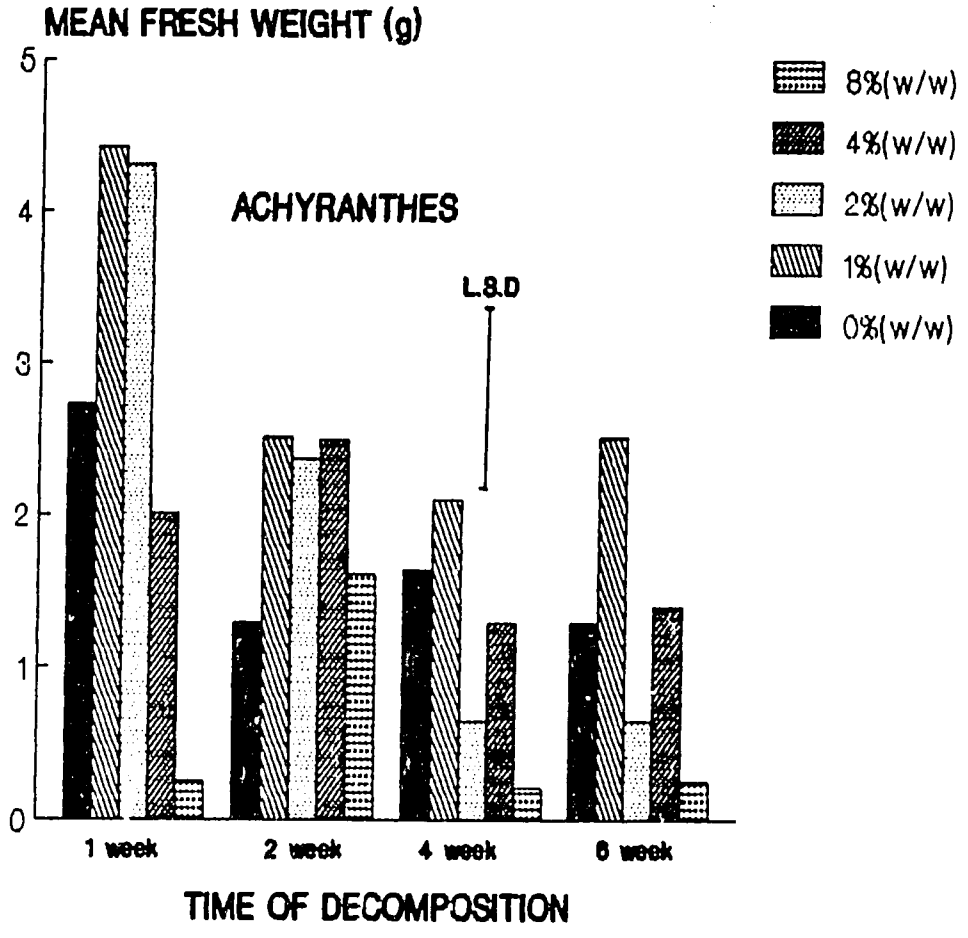

Figure 3.8: Mean Fresh Weight (at 28 days after planting) of Achyranthes in soil in which incorporated Tithonia residues had decomposed for 1, 2, 4 and 6-weeks

3.2.3 Field Studies with Gliricidia and Tithonia Leaf Residues

3.2.3.1 Field Trial I- Gliricidia Residues:

The results of the first field trial in which either 1 or 2 kg of Gliricidia leaf material was added to field plots twice during the experimental period, indicated a very significant growth stimulation effect of Gliricidia residues on the three crop species. at both rates of the mulch (Table 3.13). The effects were somewhat species-specific. The growth of both tomato and chilli in particular, was spectacularly enhanced by the end of three months, while the increase of brinjal growth was less. At the higher mulch treatment of 2 kg/m^2, the growth of tomato, chilli and brinjal was enhanced by 1516, 235 and 55 %, respectively, compared with controls.

At the same time, Gliricidia residues at this higher mulch rate, reduced the weed biomass of the plots by 61.3% compared to the weed biomass in the unweeded, no residue controls. However, the residue rate of 1 kg/m2 did not reduce the weed biomass in this trial, and in fact caused a slight stimulation of weed growth as well (Table 3.13). The weed population in the plots was dominated by Vernonia cinerea, Amaranthus viridis L., Phyllanthus debilis, Acalypha indica L., Cynodon dactylon (L.) Pers., and Eleusine indica (L.) Gaertn.

3.3.3.2 Field Trial II- Gliricidia Residues:

The second field trial conducted on a different field location from the first with Gliricidia residues, in which either 2 or 4 kg leaf material was applied per m^2 three times during the experimental period, also indicated spectacular stimulation of the growth of the three crop species (Table 3.14), in much the same way as in the first trial. Enhancement of tomato and chilli growth by the 2 kg/m^2 rate was less than in trial I. However, at this location, the the growth of brinjal was also very significantly enhanced by both rates of application. This was nearly eight times more than the increment of brinjal growth at the previous site. The higher mulch rate of 4 kg/m^2 stimulated the growth of tomato, chilli and brinjal by as much as 1390, 1780 and 1590 %, respectively.

The weed flora at this site was totally different from the one at the first field site. The dominant weeds at the second site were: Boerharvia erecta, Boerharvia diffusa, Cleome viscosa, Aerua lanata, Mimosa pudica, Euphorbia geniculata, Tridax procumbens, Amaranthus viridis, Alys-sicarpus vaginalis Achyranthes aspera, Eleusine indica, Dactyloctenium aegyptium and

Table 3.13: The effect of field-applied Gliricidia residues on the growth of selected Crop spp. and the weed biomass in field-plots (Field-Trial I)

Rates of Residue application (kg/m2)	Crop spp.			
	Tomato	Chilli	Brinjal	Weeds
	Mean Dry Weight of Plant (g) [Percent Stimulation (+) or Inhibition (-)]			
0	7.3b	5.7a	26.6a	301.6a
1	74.3a (+917.9%)	15.3a (+168.4%)	34.3a (+28.9%)	303.3a (+0.6%)
2	118.0a (+1516.4%)	19.1a (+235.5%)	41.3a (+55.3%)	122.7b (-61.4%)

*First application- 1-week after transplanting (WAP); Second application- 4 WAP; Third application- 12 WAP

Mean values along columns followed by the same letter are not significantly different at 0.05 level of probability, according to DMRT.

Table 3.14: The effect of field-applied *Gliricidia* residues on the growth of selected Crop spp. and the Weed Biomass in field-plots (Field-Trial II)

Rates of Residue application (kg/m2)	Crop spp.			
	Tomato	Chilli	Brinjal	Weeds
	Mean Dry Weight of Plant (g) [Percent Stimulation (+) or Inhibition (-)]			
0	5.3b	2.5a	6.8a	366.7a
2	35.6ab (+571.7%)	4.7b (+88.0%)	34.8b (+411.8%)	267.3a (-23.2%)
4	79.0a (+1390.6%)	47.0a (+1780.0%)	115. 0a (+1591.2%)	43.8b (-88.0%)

*First application- 1-week after transplanting (WAP); Second application- 4 WAP; Third application- 12 WAP

Mean values along columns followed by the same letter are no significantly different at 0.05 level of probability, according to DMRT.

Digitaria spp. The weed growth in the mulch treated plots was also significantly less than in the control plots. The degree of weed control achieved by the residues was less (23.2%) on this site with 2 kg/m^2, compared to the first trial (61%). However, Gliricidia residues at 4 kg/m^2 achieved 88% weed control in the treated plots. In this trial it was observed that the effects of weed suppression particularly by the 4 kg/m^2 rate, persisted for a period in excess of 3-months after the end of the experiment. These plots remained totally free of weeds during this period, an observation which is probably of high significance.

3.2.3.3 Field Trial III- Tithonia Residues:

The third field trial which examined the effect of applying Tithonia leaf residues three times during the experimental period, at either 2, 4 or 6 kg/m2 (Plates 3.1, 3.2, 3.3). The reason for making a third application was the fact that Tithonia residues decomposed very rapidly in the field under warm, wet favourable conditions which prevailed at the time. These residues also produced significant growth promotion of the three crop species, relative to the unweeded, no residue control plots (Table 3.15). However, the growth increments in each case was less than what was obtained with Gliricidia, despite the field plots receiving three residue applications. In fact with the 2 kg/m^2 treatment on tomato, there was a slight reduction in the final mean total plant dry weight.

Results of the fruit yields of the crop plants also indicated very significant stimulation of plant growth with resultant higher yields, particularly at 4 and 6 kg/m^2 residue rates, but only with tomato and brinjal. Surprisingly, the yield of chilli fruits/pods was not significantly altered by the Tithonia residues, despite a significant increase on overall plant growth.

The two control treatments indicated the effect weeds had on the crop plants. Crop growth in no residue-weeded control plots was very high compared to that of no residue-unweeded treatments. For instance, the total removal of weeds resulted in yield increases of 143.6%, 916% and 370% for tomato, chilli and brinjal, respectively. In general, as the amount of residue increased, greater weed suppression was achieved in the field plots. The three mulch rates 2, 4 and 6 kg/m^2 reduced the growth of weeds by 47, 73 and 82 %, respectively, compared to unweeded controls, indicating the considerable weed control ability of Tithonia residues (Table 3.15), combined with the ability to stimulate crop growth. In general, the weed flora of the plots in this trial was the same as in trial II, which was carried out on the same location.

Table 3.15: The effect of field-applied Tithonia residues on the growth of selected Crop spp., their fruit yields and Weed Biomass in field-plots (Field-Trial III)

Rates of Residue application (kg/m2)	Crop spp.			
	Tomato	Chilli	Brinjal	Weeds
(A) Mean Dry Weight of Plant (g) and [Percent Stimulation (+) or Inhibition (-)] :-				
0 (unweeded)	101.2c	2.6c	32.3d	293.2a
0 (weeded)	246.5b	25.5a	151.9ab	-
2	76.3c (-24.6%)	13.9b (+431.6%)	67.3cd (+108.4%)	154.3b (-47.4%)
4	242.9b (+140.0%)	17.4b (+569.2%)	118.1bc (+265.6%)	79.6c (-72.9%)
6	346.8a (+242.7%)	27.8a (+969.2)	183.3a (+467.5)	50.9c (-82.6%)
(B) Yield of Fruits (Fresh Weight) (g):-				
0 (unweeded)	102.2b	40.5	79.7c	-
0 (weeded)	491.3a	43.9	764.9a	-
2	363.3ab	21.5	331.2b	-
4	606.8a	34.6	723.4a	-
6	375.8a	50.6	804.8a	-

*First application- 1-week after transplanting (WAP); Second application- 4 WAP; Third application- 12 WAP
Mean values along columns, followed by the same letter are not significantly different at 0.05 level of probability, according to DMRT

3.2.3.4 Field Trial IV

This field experiment which was conducted in a different, but nearby location from both trials I, II and III, compared the effects of several other types of plant residues frequently added to field plots, with the effects produced by Gliricidia and Tithonia residues. The overall effects were highly species-dependent and also rate-dependent.

(a) Response of Tomato:

At this partcular experimental site, the greatest stimulation of tomato growth compared to unweeded controls was caused by Chromalaena (176-39/ %) and Tithonia (115-186 %) residues, at both mulch rates (Table 3.16). The response brought about by Gliricidia was somewhat variable. Gliricidia residues at 4 kg/m^2, stimulated tomato growth by 49%, but failed to cause a significant effect at 2.0 kg/m^2, unlike in previous trials. Croton and Salvinia residues were slightly inhibitory to tomato growth at 2 kg/m^2, but only Salvinia caused a significant increase of growth (38 %) at the higher treatment. When the weeds were manually removed, however, in the weed-free plots tomato showed a 67% growth increase, compared to unweeded controls.

(b) Response of Chilli:

In the case of chilli, removal of weeds by manual means resulted in a 30 % increase in the growth of chilli in weeded-plots, compared with inweeded-controls (Table 3.16). However, all five types of residues failed to cause an increase in overall growth of chilli, compared to the weeded control plots. The increase in growth in comparison with the unweeded control in which weeds interfered with chilli growth, was also not high and ranged between 30-60 %). Again, although not statistically significant ($P>0.05$), Tithonia residues appeared to make a greater stimulatory impact on chilli than other residues.

(c) Response of Brinjal:

The previously noted ability for growth stimulation by Gliricidia and Tithonia residues, was further confirmed by the response of brinjal (Table 3.16). As expected the higher rate of 4 kg/m^2 of residues caused the greater growth stimulation. Tithonia residues caused a spectacular stimulation of growth (265 %) over the plants in the unweeded control. The promotion of growth caused by Croton (205 %) or Gliricidia (203 %) was also highly significant and staistically not different from that caused by

Table 3.16: The effect of a range of plant residues applied as 'mulch' on crop and weed growth in field-plots

Treatments	Mean D.W. of Plant (g) and [% Stimulation (+) or Inhibition (-)]			D.W. of Weeds (g) & [% Inhibition of Weed Biomass]
	Tomato	Chilli	Brinjal	
No Residue/unweeded	25.7f	18.6b	20.0d	329.8a
No Residue/weeded	42.9d (+66.9%)	24.2a (+30.1%)	32.9c (+64.5%)	--
Gliricidia:				
2.0 kg/m2	27.5ef (+7.0%)	16.9b (-9.0%)	38.1a (+90.0%)	118.8e (-63.9%)
4.0 kg/m2	38.3de (+49.0%)	24.7a (+32.7%)	60.5a (+202.5%)	84.5f (-74.3%)
Tithonia:				
2.0 kg/m2	55.3c (+115.2%)	18.9b (+1.6%)	35.4c (+77.0%)	144.0d (-56.2%)
4.0 kg/m2	73.5b (+186.0%)	29.7a (+59.7%)	72.9a (+264.5%)	96.9e (-70.5%)
Croton:				
2.0 kg/m2	18.3f (-28.8%)	20.9b (+12.4%)	51.3b (+156.5%)	186.1c (-43.4%)
4.0 kg/m2	27.8e (+8.2%)	25.8a (+38.7%)	61.0a (+205.0%)	102.6e (-68.9%)
Chromalaena:				
2.0 kg/m2	70.9b (+175.9%)	17.7b (-4.8%)	45.7b (+128.5%)	159.2d (-51.6%)
4.0 kg/m2	127.7a (+396.9%)	25.5a (+37.1%)	50.5b (+152.5%)	290.3b (-12.0%)
Salvinia:				
2.0 kg/m2	23.9f (-7.0%)	21.3ab (+14.5%)	34.2c (+71.0%)	195.9c (-40.4%)
4.0 kg/m2	35.4d (+37.7%)	24.9a (+33.9%)	45.7b (+128.5%)	149.2d (-54.6%)

Tithonia. Less, but notable promotion of brinjal growth was caused by Chromalaena (153 %) and Salvinia (129 %) residues as well.

The weed flora of the experimental site was dominated by Euphorbia geniculata, Tridax procumbens, Acalypha indica, Cleome viscosa, Achyranthes aspera, Boerharvia erecta, Boerharvia diffusa, Hedyotis corymbosa, Borreria ocymoides, Alyssicarpus vaginalis, Amaranthus viridis, Amaranthus spinosus, Dactyloctenium aegyptium and Eleusine indica. In general, these represented a flora that was capable of flourishing under conditions which were not altogether wet, as the experiment was done during November- March, the driest period of the year.

When the overall weed suppression ability was quantified based on weed biomass reduction, all plant residues caused a significant reduction in the weed biomass in the plots, compared to no residue-unweeded controls. The most effective reduction however, was caused by the Gliricidia residues (i.e. 74% at 4.0 kg/m^2, 64% at 2.0 kg/m^2), closely followed by Tithonia and Croton residues ranked second and third in weed suppression ability with either rate of residues. Chromalaena residues were the least effective for weed control and in fact appears to have promoted the growth of weeds as well, in addition to promoting the growth of crops. Salvinia residues caused 40-55 % inhibition of growth of weeds from the 2.0 and 4.0 kg/m^2 residue applications.

SECTION 3.3

DISCUSSION

Contents:

3.3.1 Activity of Aqueous Extracts
3.3.2 Studies with Soil-Incorporated Residues and Surface-applied Mulches
3.3.3 Studies with Soil-Incorporated and Decomposing Residues
3.3.4 Field Trials on Weed suppression ability of Residues

3.3.1 Activity of Aqueous Extracts

Data of the laboratory studies described in Section 3.2 amply demonstrated that the fresh or dried leaves of Gliricidia and dried leaves of Tithonia contain water-extractable phytotoxic compounds. Generally the blended extracts were more active than leachate extracts, possibly due to the fact that blending causes greater extraction of phytoactive materials. Of particular significance was the presence of phytotoxicity in the extracts of dried leaf residues ('litter'), in both Gliricidia and Tithonia, since both types of plant residues are heavily used in Sri Lanka as 'green manure' which dry up rapidly and also decompose fairly quickly in the field. In general, the dried leaf extracts were more inhibitory than the extracts from fresh leaves.

The fact that many residues of plant parts, including stems, leaves and hay, contain water-extractable phytotoxic compounds is well recorded in the literature and extensively reviewed (Rice, 1974, 1984; Chou, 1987). Only a few relevant examples are quoted below. Nielson et al (1960) investigated the effects of aqueous extracts of alfalfa (Medicago sativa L.) hay, timothy (Phleum pratense L.) hay, and mature corn, oat straw, and potato vines on seed germination and seedling growth of corn, soybeans, peas (Pisum sativum L.), oats, alfalfa, and timothy. Alfalfa, a legume, caused the greatest delay in germination of all test species except one; the germination of timothy was delayed the longest with timothy extract. Thus Nielson et al (1960) demonstrated that certain crop plants (especially, alfalfa) contain water-soluble materials that inhibit seed germination and seedling growth of other crop plants.

Leather (1983b) examining the phytoactivity of aqueous extracts of dried sunflower and rape (Brassica napus) tissue, reported that the extracts inhibited or stimulated germination and growth of weeds, and that

the response depended upon the source of extract, the extract concentration and the weed species tested. Undiluted (4 g of ground tissue shaken with 100 ml of water for 24 h) leaf extracts of two varieties of sunflower and native sunflower, inhibited seed germination of wild mustard [Brassica kaber (DC.) L.C. Wheeler var. pinnatifida (Stokes) L.C. Wheeler], but stimulated the same at 1:10 or 1:100 dilutions. Stem tissue extracts stimulated germination at all levels of dilution. The altered germination response over the range of concentrations suggested that the major chemical constituents extracted from the tissues were growth regulators.

Seedling growth of velvetleaf (Abutilon theophratsii Medic.), jimsonweed (Datura stramonium L.), tall morning glory [Ipomoea purpurea (L.) Roth] and wild mustard, was inhibited by leachates of sunflower Hybrid 201 leaf and stem tissue (Leather, 1983b). Rape tissue leachates inhibited the growth of velvetleaf and morning glory but did not affect wild mustard. As in the germination tests, inhibition of seedling growth depended upon weed species and source of allelochemicals. No correlation was observed between stimulation or inhibition of seed germination and the growth of seedlings. Leather (1983) explained that his data indicated the variable nature of the results obtained in bioassays for allelopathic activity, which are concentration dependent and highly species specific. In our results, a close parallel can be seen between stimulation or inhibition of seed germination and seedling growth by both Gliricidia and Tithonia aqueous extracts, despite variability in results.

In the investigations of reason(s) for the lack of an understorey vegetation in Leucaena plantations in Taiwan, it was found that aqueous extracts of Leucaena fresh leaves, dried leaf litter, soil and seed extracts caused significant phytotoxic effects on many test species, including rice, lettuce, and Acacia. Wu et al (1975) as quoted by Chou (1987) had compared the phytotoxic effects of aqueous extracts of some cover crops, namely Centrosema sp. (Leguminosae), Indigofera sp. (Leguminosae) and Paspalum notatum (Gramineae) on the growth of pea, mustard, cucumber, cauliflower, rape, Chinese cabbage, mung beans, watermelon, tomato and rice. They found that rape was the most sensitive crop to the extracts of the cover crops, and that Centrosema and Indigofera exhibited the greater phytotoxic effect, demonstrating strong species-specific responses.

Nicollier et al (1985) screened the phytoactivity of 90 weed and crop species by dissolving the residues from water and methanol extracts of whole plants in distilled water and making a dilution range 3, 30 or 300 ppm. Extracts of six species significantly stimulated root growth of purple top turnip; extracts of 18 species significantly reduced root growth, indicating further the species-dependent effects of phytoactive extracts.

These and numerous other studies demonstrate that extracts of fresh or dried leaves of plants contain phytoactive compounds, which often display rate-dependent, species-specific, stimulation or inhibition of seed germination and/or seedling growth of other species. Often such demonstrations provide the first evidence for allelopathic activity worthwhile pursuing further. One of the precautionary steps taken in this type of bioactivity testing is the use of extracts containing extractable material at concentrations, which do not bring about high osmotic potential effects on the seeds of the tested species. In our studies it was assumed that the range of concentrations of extractable materials used did not cause osmotic effects on the test seeds.

3.3.2 Studies with Soil-Incorporated Residues and Surface-applied Mulches

Many types of plant residues have been reported to have either inhibited or stimulated the growth of other plants, the degree of response being dependent on such factors as test plant species, quantity, placement and duration of weathering/decomposition of residues, nutrient status of soil, microbial activity and other soil parameters. Patrick and Koch (1958) noted that substances possessing phytotoxic properties may be formed during decomposition of plant residues in soil, either abiotically, or more commonly through the action of microorganisms.

As pointed out by Patrick (1971), the decomposition of residues or any organic substrate is a continuing process which requires rapid and sensitive assay methods to detect phytotoxic compounds during the short interval of their production and disappearence. This is evident from the various conflicting results often obtained during decomposition of plant residues. The ephemeral nature of such products and their rapid transition from one type of physiological activity to another may explain why their occurrence is often missed. Plant injury is dependent on the frequency of a chance encounter with a growing root system, or with fragments of a decomposing plant residue, at the time when decomposition is favourable for toxin production.

The present studies aimed to determine and compare the overall effects of soil incorporation of Gliricidia or Tithonia residues, or their placement on soil surface, on the germination, seedling emergence and growth of several test species. Results showed that the incorporated Gliricidia residues were not inhibitory to the germinating seeds of most test species, although the germination of one weed species, i.e. Hyptis, was significantly reduced. Soil-incorporated Tithonia leaf residues on the other hand, were both inhibitory and stimulatory to the germination of test plant species. In general, whilist there was a mixed response at

lower residue rates, Tithonia residues incorporated at 4 and 8% (w/w) were strongly inhibitory to all test species.

The present studies also indicated that the surface-placed 'mulch' treatments of Gliricidia residues were strongly inhibitory towards the germination of the generally smaller weed seeds, although, again, the Gliricidia 'mulch' did not inhibit the germination of the relatively larger-seeded crop species tomato and brinjal even at high rates. Tithonia residue 'mulch', on the other hand was found to be strongly inhibitory to the germination of all species at the higher rates of 4 or 8 % (w/w). However, at the low application rate of 1 %, Tithonia mulch appeared to have caused a fairly consistent stimulation of seed germination of chilli and tomato. A review of published literature on phytoactivity of leaf residues, either incorporated or surface-placed, also indicates similarly variable, species-dependent and strongly rate-dependent, stimulatory or inhibitory responses.

The effect of several plant residues on the germination of seeds of many weed species was studied by Altieri and Doll (1978). In general, they found that bean, cassava, Amaranthus dubius and Tagetes patula residues had the most significant inhibitory effects on a wide range of weed species. Their studies demonstrated the highly significant allelopathic nature of Tagetes patula, in particular. Tagetes residues incorporated 10 days after planting exerted a considerable adverse effect on the germination of a number of weed seeds and reduced seedling emergence by more than 50%. Residues, however, had no effect on the germination and seedling growth of corn, beans, Bidens pilosa L., and Euphorbia heterophylla L. Altieri & Doll (1978) also reported that incorporation of Tagetes residues immediately before sowing enhanced inhibitory effects and all species except corn and Bidens imressively reduced. They suggested that Tagetes foliage contained toxic compounds (or substances which stimulate toxin production by soil microbes), which have a wide allelopathic spectrum, but which lose their effectiveness when the material decomposes in soil. The loss of bioactivity of the residues with time, they argued, could be due to microbial inactivation, colloidal adsorption, volatilization, percolation in rain water or biochemical degradation. It was also suggested that corn and Bidens were more tolerant to these compounds.

Guenzi and McCalla (1962) in their studies on the possibility that plant residues used for 'mulching' inhibit the growth and development of other plants. All residues (oat, wheat straw, soybean and sweet-clover hay, corn and sorghum stalks, bromegrass and sweetclover stems) extracted with cold or hot water (1:15) gave extracts that inhibited the germination and growth of indicator species, corn, wheat and soybean. Cold water extracts of the residues were more toxic than hot extracts, indicating the thermolabile nature of some of the extracted compounds.

In other related studies, Schreiner and Williams (1967) reported that decaying root residues of giant foxtail (Setaria faberi Hermm.) inhibited corn root growth. Common lambsquater (Chenopodium album L.), redroot-pigweed (Amaranthus retroflexus L.) and yellow foxtail [Setaria glauca (L.) Beauv.] residues demonstrated allelopathic activity to corn and soybean, according to Bhowmik and Doll (1982), which resulted in significant growth inhibition of the crop species and their yields.

The effect of soil-incorporated or surface-applied decomposing Leucaena leaves on the growth of Leucaena itself and other plants, was studied by Chou and Kuo (1986). Their results indicated that Leucaena seedlings survived 100% and grew in the residues, while seedlings of Alnus formosana and Acacia confusa showed a survival rate of 50% and also did not grow well. Two other species, Casuarina glauca and Mimosa pudica were totally killed by the decomposing Leucaena in soil, and also by mulch applications. In general, 'mulch' treatments were somewhat less phytotoxic than incorporated residues. Mimosine, the toxic non-protein amino acid, present in nearly all parts of the plant, quercetin and eight other phenolics were implicated in this allelopathic phenomenon (Chou & Kuo, 1986). Bhowmik and Doll (1982) reported that incorporated weed residues were more phytotoxic to corn and soybean growth, compared to surface placement of residues. They argued that roots of receiver plants were in direct contact with incorporated residues, and were more exposed to allelochemicals that may be released through leaching or microbial breakdown. They also pointed out that incorporation may increase the rate of decomposition of residues by micro-organisms, which may or may not increase phytotoxicity through allelochemicals produced from residues. In complete contrast to this, Putnam & De Frank (1983) reported that sorghum residues were inhibitory to other plant species only when they were placed on the soil surface. Inhibition was lost when the residues were mixed throughout soil. They suggested that an apparent zone of inhibition develops near the soil surface with surface mulch placements, where weed seeds germinate.

Menges (1987) reported under warm, moist, saline soil conditions and intense sunlight following spring plantings, soil-incorporated residues of Palmer amaranath (Amaranthus palmeri S. Watts.) at biomasses of 5.1 and 8.5 kg/m2, inhibited the growth of carrot (Daucus carota L.) 49 % and onion (Allium cepa L.) 68 % during trials in 1980-1983. He also noted that phytotoxicity persisted 11-weeks in cabbage and carrot plantings, and in 1985 trials, phytotoxicity was dissipated 16-weeks after soil-incorporation of the weed residues. Furthermore, soil-incorporated johnsongrass residues (biomass of 3.6 kg/m2) decreased the growth of onions 71 %, in 1982; residues from 5.4 kg/m2, decreased the growth of cabbage 26 %, onion 62 % and sunflower 10%, in 1983, while carrot was unaffected (Menges, 1987).

Incorporated dried shoot or root residues of lantana (Lantana camara L.) into soil, significantly reduced milkweedvine growth compared with growth in control soil (Achhireddy & Singh, 1984). Irons and Burnsioe (1983) reported that 2 % or greater amounts of ground, mature sunflower (Helianthus annus) leaves mixed into soil significantly reduced emergence, height fresh and dry weights of treated plants.

Johnson and Coble (1986) examined the possibility of allelopathy being involved in an observed population shift from previously dominant grasses fall panicum (Panicum dichotomiflorum Michx.) and large crabgrass [Digitaria sanguinalis (L.) Scop,] to broadleaf signalgrass [Brachiaria platyphylla (Griesb.) Nash] in certain fields of North Carolina. They reasoned that production of inhibitors or stimulators by one species could affect the population dynamics of the other species, causing a population shift. In their studies with the residues of the three grasses, applied as mulch or soil-incorporated, a variable response was obtained for five indicator species, the three weeds themselves, corn, and soybean. Of the five species evaluated, only the germination and dry weight yields of soybean was inhibited by soil-incorporated large crabgrass residues. The germination or growth of other species were unaffected by large crabgrass residues. None of the species were significantly affected by these residues applied as mulch, indicating that it was unlikely that large crabgrass residues contributed to the observed population shift. The germination and dry weight yield of corn were reduced by soil-incorporated fall panicum residues. However, these residues, at 0.5% (w/w) level, stimulated the germination (by 26.3%) and the biomass yield (by 250%) of broadleaf signalgrass, whilst making no impact on any other species. Only the germination of large crabgrass was significantly inhibited (by 34.3%) by fall panicum mulch. But this mulch did not affect the biomass of any indicator species, including large crabgrass. Johnson and Coble (1986) reasoned that in a field heavily infested with fall panicum, a population shift to broadleaf signalgrass could possibly happen due to the apparent stimulation of its germination and growth by fall panicum residues. In addition, the inhibition of large crabgrass by fall panicum mulch could also be a contributory factor in the population shift from crabgrass to fall panicum. All of these studies demonstrate the species-specific, often inhibitory or stimulatory effects plant residues may have on other species.

The overall result of our studies indicated that Gliricidia leaf residues mixed with soil up to 4 % (w/w) could cause highly significant growth promotion of some receiver plant species. It is probable that incorporated residues were decomposed by micro-organisms, releasing much of the nutrients including N- compounds directly and rapidly into soil, thereby promoting the growth of other plant species. Stimulation of growth by plant residues, incorporated or surface-applied, is not uncommon in literature, and is dealt with later on in this discussion.

Phytotoxic effects of straw had been a major concern even in U.K. Straw residues from preceding crops are usually burnt because poor crop establishment and yield could result, particularly on heavy soils in wet years (Lynch, 1987). The olderview point had been that this is due to the straw having a high C:N ratio (ca. 100:1) compared with decomposer micro-organisms (ca. 5:1), causing N otherwise available to plants, immobilized into microbial biomass. Symptoms of N-defficiency had been easy to demonstrate in the presence of straw. However, it is now accepted that temporarily immobilized N could subsequently become available to the crop during a growing season. Also, N immobilized in microbial biomass dry matter would prevent leaching losses of N and therefore, could even be beneficial to the N-cycle (Lynch, 1987). There was also evidence that the N tie up was much smaller when residues were left on soil surface, as opposed to being mixed into soil. Allelopathy and microbially produced steam-volatile fatty acids, particularly acetic acid, produced by the decomposing straw, mostly by bacteria, are believed to be the responsible cause of the observed effects. Evidence of toxin production only in the presence of straw tissue, and an exponential concentration decrease with distance from straw, have been presented (Lynch, 1987). In our present studies, it was not determined whether phytotoxic substances were synthesized by soil microorganisms using plant material as a substitute, or were the breakdown products present in the plant tissue. Both situations may have occurred.

3.3.3 Soil-Incorporated and Decomposing Residues

Available published literature provide ample evidence that decomposing plant residues may be toxic at the initial stages of breakdown (through direct release of already formed phytotoxins) or could become more toxic with passage of time, probably with the involvement of the microbial population. Micro-organisms are known to be able to produce toxic metabolites anew, or convert already existing compounds to more phytotoxic ones. A few relevant examples are quoted below.

Increase in growth inhibition of test plants with incubation time was reported by Yun and Kil (1992) with wormwood (Artemesia princeps var. orientalis) residues. These researchers argued that this may have been due to a release of inhibitory substances from decomposing plant tissues or due to the formation of toxic substances by microbes associated with the decomposing leaves, as had been previously suggested by Barnes and Putnam (1983) and Harrison and Peterson (1986).

According to Chou and Lin (1976) and Chou and Chiou (1979), aqueous extracts of decomposing rice residues in soil caused inhibition of radicle growth of lettuce and rice seeds and the growth of rice seedlings.

The phytotoxicity was found mostly in the extracts obtained from the early stages of decomposition (1-week) and gradually declined thereafter. However, some toxicity was persistent even for 4-months. Patrick et al (1963) had earlier reported of severe phytotoxicity to lettuce and broccoli after several small-grain and vegetable crops had decomposed 10-25 days in soil. Toussoun et al (1968) reported that phytotoxicity from bariey residues occurred 7 to 10 days after incorpora-tion in field soil and reached a maximum in 3 weeks. Phytotoxicity from barley and several other small-grain residues declined after 6-8 weeks in soil (Guenzi et al, 1967; Kimber, 1973; Patrick et al, 1963; Toussoun et al, 1968). Menges (1987) reported that the phytotoxicity of Palmer amaranth residues followed a decomposition period of 6 to 11 weeks. He reccommended that commercial plantings of crops sensitive to allelopathic inhibition from Palmer amaranth should be delayed beyond 11-weeks of decomposition.

Shoots extracts of a range of species of grasses were toxic to other grasses and clover (Trifolium sp.) when decomposed anaerobically. The phytotoxicity was caused by organic acids and was less after 20 days of decomposition than after 10 days. Extracts of Festuca rubra, Agrostis stolonifera and Alopercurus pratensis residues were most toxic, consistent with the field observation that residues of the former two species are difficult to seed into. When shoots decomposed aerobically, some were toxic after 10 days, but toxicity disappeared after 20 days when some residues could actually stimulate growth (Lynch, 1987).

In the present studies, although the overall results are somewhat variable and highly species-specific, conclusive evidence of the ability of Gliricidia residues to promote the growth of crop plants tomato, chilli and brinjal, has been obtained. Gliricidia residues are obviously phytotoxic to small-seeded annual weeds such as Tridax and Achyranthes, but phytotoxicity clearly disappears from soil as decomposition proceeds with time, allowing the indicator species to respond positively with growth enhancement. Even at the early stages of decomposition or immediately after an application of Gliricidia residues in to soil, the phytoactive compounds released may not harm the growth of crop species, confirming the possibility of using Gliricidia foliage in upland farming.

The response brought about in indicator species by the decomposing residues of Tithonia is obviously different from that of Gliricidia. Pronounced stimulation of growth of all indicator species was obvious at the early stages of residue incorporation and decomposition; however, as time passed, the soil became increasingly ammended to be phytotoxic. It is probable that micro-organisms are involved in the production of toxins, as evidenced by numerous references already quoted, and as highlighted by Patrick and Koch (1958).

The variability of results in studies of this nature is probably due to the fact that the developing roots of indicator species may not come into direct contact with decomposing residues or their phytotoxins, in a uniform manner. As stated previously, plant injury or stimulation depends on the frequency of a chance encounter of a phytotoxin with a growing root system, when decomposition favours toxin production.

3.3.4 Field Trials on Weed suppression ability of Residues

The presence of crop residues has been reported to both increase and decrease crop yields in the field. However, from the time Overland (1966) first suggested and presented scientific evidence for the possibility of manipulating residues of plant species for weed control in the field, many reports have supported this view (Barnes & Putnam, 1983, 1986; Putnam & De Frank, 1983; Putnam et al, 1983; Liebl & Worsham, 1983; Shilling et al, 1985). In most cases, considerable success has been recorded in the control of certain troublesome weeds, with simultaneous increases in crop yields. Published literature also indicates that in some the well-known cases, even the allelochemicals implicated have also been identified. It is not too difficult to accept the view that under certain conditions, plant residues, either incorporated or surfac-applied as mulches can suppress weeds, but determining the reason(s) present many logistical problems under field conditions. To determine the cause(s), the physical and chemical (i.e. allelochemical) effects of the mulch must be separated. One of the aims of our research also was to establish beyond doubt that Gliricidia and Tithonia foliage applied as mulches, or incorporated, could be used for weed suppression in the field, without undue harm to upland crops.

To demonstrate that mulches suppress weeds allelopathically, Putnam and associates (Putnam & De Frank, 1983; Barnes & Putnam, 1983, 1986; Putnam et al, 1983) used Populus wood shavings to separate chemical and physical effects of mulches. These studies clearly established that plant residues do possess allelocpathic potential. The work of Shilling et al (1985) also supported the view that at least part of the weed suppression by wheat and rye mulches is allelopathic, and that such mulches contribute to significant weed suppression in no-till systems. The studies of Cheema et al (1990) also indicated that wheat residues, surface-applied or incorporated, suppressed weeds in cotton fields, from which cotton benefitted significantly.

Putnam and De Frank (1979) screened numerous covercrops for weed suppression activity. They found that fall-planted cover crops could reduce both weed populations and biomass into the next growing season. Fall-killed

rye residues reduced the weed biomass by 84% over non-residue controls; spring-killed rye residues were less toxic. Additional field trials indicated that a variety of annual weed spp. could be suppressed consistently with residues of immature cereals (including rye, corn, barley, wheat, sorghum and oats). They also found that, whereas larger-seeded crops and vegetables such as corn, cucumber, pea and snapbean, grew quite well or sometimes vigourously without being affected adversely by the residues, smaller-seeded vegetable crops were severely damaged by the residues (Putnam & De Frank, 1983). This could partly be explained on the basis that they are sown deeper (2-2.5 cm) and may be physically separated from the residues. The smaller and more shallowly-seeded crops varied in their tolerance to the residues. Whilst lettuce and cabbage seed germination and growth were severely reduced with all residues, carrot and tomato tolerated some residues. The same explanations may hold true for our results in the current field experiments which confirmed the highly growth promotional nature of Gliricidia and Tithonia residues towards larger-seeded crop plants, combined with the ability to suppress certain small-seeded annual broad-leaf and grass species.

In these same studies, residues of 40-day old green-house grown rye reduced the total weed biomass by 80 %, as compared to a non-toxic control mulch which reduced the weed biomass by 41 % over the no residue controls. Weed biomass was reduced an additional 35 % when compared to the control mulch, indicating that allelopathy was one component of the interference by rye residues on other plant species. Mulches of sorghum and sudangrass applied to apple orchards in early spring reduced weed biomass by 90 and 83 %, respectively. In a 3-year series of field trials, sorghum residues reduced field populations of common purslane by 70 % and of smooth crabgrass by 98% (Putnam & De Frank, 1983). A general conclusion that can be drawn from these studies is that cover crops or crop residues may selectively toxify weed species.

Various fall-planted cover crops were evaluated for their influence on weeds and yield of strawberries by Smeda and Putnam (1988). Spring and mid-summer weed biomass was reduced significantly by all cover crop treatments compared to controls; however, the weed species density data were too diverse and could not be correlated to any treatment effect. Winter barley provided early spring weed suppression, but by mid-summer, weed growth was similar to unweeded controls. Both rye and wheat exhibited similar levels of weed suppression throughout the growing season and also provided early season weed suppression than barley. Weed growth was reduced by 80% to 95% in early-spring evaluations, but decreased to 55% to 85% of control by mid-summer. Weed suppression increased with cover crop residue and was consistently high with increased cover crop seeding rates (Smeda & Putnam, 1988). Strawberry yields however, were not significantly different between treatments, and actually showed a tendency to be reduced where

cover crops were present. These workers suggested that winter annual and early summer annual weed suppression by rye and wheat cover crops beyond a critical weed-free period could delay or reduce the need for chemical weed control practices until post-harvest renovations. They also recommended that additional studies be done on the interaction of cover crop residues and strawberries, in order to ascertain if cover crops or their residues will interfere with strawberry growth or yield, and on other types of potential cover crops for strawberries.

Leather (1983a, 1983bb, 1987), examining the concept that some crop plants may be allelopathic to common weeds of agricultural land, produced evidence that several sunflower varieties possessed to a greater extent an ability to inhibit the germination and growth of associated weeds, compared to that of several native sunflower biotypes. Average density of natural weed populations in cultivated plots increased over a 5-year sampling period. Weed density in plots with a sunflower-oat roration also increased, but the rate of increase was less than in the natural populations. The inhibition of weed density increase in sunflower plots was probably due to direct allelochemical action on weed germination and growth, rather than due to a nutritional factor, because fertilizer had been applied to the plots each year according to recommendations and soil analysis (Leather, 1983).

Lovett and associates (Lovett, 1987) also reported that in field experiments, dry weight of grass weeds excluding wild oats, were reduced by residues of rapeseed, soybean, pea, sunflower and wheat. The germination, growth and yield of wild oats tended to be slightly stimulated by most residues, but significantly so by residues of wheat, a crop with which wild oats is closely associated. According to Lovett this proves that although some products of residue/mulch decomposition and their effects are common to many species, there remains the possibility that a specific component will exert a singular effect.

Shilling et al (1985) examined the feasibility of using a rye mulch for weed suppression in no-till cropping and found that fall-planted, spring-killed rye reduced the above-ground biomass of several weed spp. including redroot pigweed (Amaranthus retroflexus L.), common lambsquater (Chenopodium album L.) and common ragweed (Ambrosia artemisiifolia L.). In no-till tobacco, elimination of tillage and presence of a rye mulch reduced the biomass of Amaranthus retroflexus, Chenopodium album and Ambrosia artemisiifolia by 51, 41 and 75 %, respectively. Rye residues reduced chenopodium album in both tilled and no-till systems by 60 %. In soybean and sunflower crops planted into desiccated green rye residues as mulch, with no-till, the biomass of C. album, A. artemisiifolia and A. retroflexus was reduced by 99, 92 and 96 %, respectively, by the mulch treatments, indicating the very distinct possibility of manipulation of a selected

residue type for weed control. Subsequent isolation of compounds with toxicity in aqueous extracts of rye resulted in the identification of B-phenylactic acid (PLA) and B-hydroxybutyric acid (HBA), which they implicated with allelopathic activity of rye residues. The research of Barnes and Putnam (1987), Barnes et al (1987) also indicated 2,4-dihydroxy-1,4 (2H)-benzoxazin-3-one (DIBOA) and 2(3H)-benzoxazolinone (BOA) as the major allelochemicals in rye residues. When tested with several weed and crop spp., overall, DIBOA ad BOA were consistently more inhibitory to seed germination and seedling growth of the tested species, although monocotyledons and dicotyledons varied in their response and sensitivity.

In our field studies the most spectacular aspect observed was the vigour with which the crop species, tomato, chilli and brinjal grew in the presence of Gliricidia and Tithonia residues, applied as mulches, whilst achieving very significant weed control. Since the crops were not directly seeded into the residues, but were transplanted after been raised for two-weeks in a nursery, adverse effects were probably not encountered by the crop plants. It is possible that the root system of the crop seedlings were not in contact with the residues or the toxins produced in their immediate vicinity. Another possibility why crop plants were not affected while the weed seeds were inhibited from germination and establishment, could be the explanation Putnam and De Frank (1983) gave, viz. that the smaller and more shallow-buried seeds (such as those of most annual weeds) are most likely to be advesely affected by the allelopathic residues. Some degree of tolerance to the phytotoxins produced inbuilt into crop plants and not present in the annual weeds encountered, may also explain the result.

It is probable that allelopathic effects of plant residue 'mulches' are due to inhibitory compounds being released directly from plant residues or being leached out into soil by rain water, and also by decomposition of residues by micro-organisms. The role that micro-organisms play in the production and persistence of phytotoxins from plant residues has also been amply demonstrated (Guenzi et al, 1977; Chou & Patrick, 1976; Liebl & Worsham, 1983; Kimber, 1973; Toussoun et al, 1968). It has been postulated that the inhibitory effects of crop residues might be due to a combination of toxins released from plant residues and from microorganisms that are more prolific wherever plant residues are present. The same postulation may be made for our result of inhibition of weeds by the Gliricidia and Tithonia mulches, whilst promoting the growth of crop plants. A compound identified from alkali extracts of wheat residues having the greatest toxicity against several weed species was Ferulic acid (4-hydroxy-3-methoxy-cinnamic acid). Shilling et al (1985) found that Ferulic acid was decarboxylated by a bacterium living on carpels of the seeds of Sida spinosa L., a weed severely inhibited by the wheat mulch, to a more phytotoxic styrene derivative, 4-hydroxy-3-methoxy styrene. These

compounds plus other unidentified phytotoxins could, in part, explain suppression of certain weeds by rye and wheat mulches in no-till systems.

It is unlikely that any one particular compound could be responsible for reduced weed growth in the mulch treatments. Without any doubt, micro-organisms using the plant residues as carbon sources, produce a myriad of phytotoxic compounds. If these compounds are present in the right combination and concentration, phytotoxic effects may be observed in sensitive plants. Many allelochemicals are decomposed in soil, either abiotically or by micro-organisms. Obviously, the attainment of active concentrations of allelochemicals in soil depends on the relative rates of addition and inactivation. It is also possible that microbial decomposition of allelochemicals does not result in a decrease in allelopathic activity. In fact, the reverse may be often true, as evidenced by published reports. Hydrojuglone is oxidized in soil to juglone, the quinone that is inhibitory to several plant species at a concentration of 10^{-6} M. Isoflavonoids produced by red clover are decomposed to even more toxic phenolic compounds. Amygdalin from peach roots is changed to HCN and Benzaldehyde which cause the 'peach-replant' problem (Patrick, 1955), and phlorizin from apple roots is decomposed to several phenolic compounds that appear to be responsible for the 'apple-replant' problem (Borner, 1971).

It is also known that most allelopathic effects are the result of the combined action of several allelochemicals, often with each below a threshold concentration for an impact. In allelopathy phenomena which implicate phenolic acids, soil concentrations have ranged from below 10 to above 1000 ppm for each compound. The lower end of the spectrum is below a concentration required for an effect in current bioassay (Leather & Einhellig, 1988; Einhellig, 1986). Additive or synergistic effects have been demonstrated however, for combinations of cinnamic acids, benzoic acids, benzoic and cinnamic acids and p-hydroxybenzaldehyde with coumarin. It appears that such combined interactions may be very important under field conditions.

In the present studies, one of the most important aspects has been the spectacular growth stimulation caused by both Gliricidia and Tithonia leaf materials, incorporated or placed on the soil surface. It is therefore interesting and relevant to review some of the reported cases of growth stimulation and draw comparisons with our results.

Stimulation of growth of one plant by another plant is very much within the context with with Molisch (1937) introduced the novel word "allelopathy", although the exact meaning of the word was inappropriate for the phenomenon, as discussed by Rice (1984). Rice (1986) points out that few scientists involved in allelopathy research have reported examples of stimulatory effects, although numerous instances of stimulatory effects of

microorganisms on other microorganisms, and of plants on microorgani have been reported by numerous scientists who were not consciously work on allelopathy, but on N-fixing organisms or plant diseases.

With regard to stimulatory interactions of higher plants, R (1986) recognizes two categories: (a) stimulation of growth of parasi plants, and (b) stimulation of growth of non-parasitic plants. Parasi angiosperms usually germinate in the field only in the presence of a ho and this appears to happen after a chemical recognition of a host. developmental feature uniquely common to parasitic angiosperms is haustorium (Lynn, 1985) which forms the physiological and morphologi attachment between host and parasite. Haustoria of the parasite do not f when the plants are grown axenically, but are rapidly induced in presence of host roots or host root exudates (Lynn, 1985). Seve haustorial-inducing compounds have been now characterized. Xenognosin-A Xenognosin-B were identified from gum tragacanth, an exudate f Astragalus gummifer, and Soyasapogenol-B was identified in roots Lespedeza sericea. In addition, Johnson et al (1976) as quoted by R (1986), reported the synthesis and testing of several Strigol analogu some of which were powerful seed germination stimulants for the parasi species Striga and Orobranche. Cotton roots were found to exude Stri powerfully stimulating the seed germination of witchweed (Striga), altho cotton was not a host for Striga (Lynn, 1985).

On growth stimulation of non-parasitic angiosperms, Neill and R (1971) quoted by Rice (1986) found that rhizosphere soil from weste ragweed (Ambrosia psilostachya) markedly stimulated the growth of seve plant species which occurred in the same field, indicating that sc stimulatory factor was present in the rhizosphere of this major weed. same factor was not found in soil at least 1 m away from ragwee Subsequent experiments had indicated that the growth of two out of 10 t species was stimulated by root exudates of Ambrosia, four of the same t species were stimulated by leaf leachates, and four species stimulated dried leaves of western ragweed (2 g/kg, i.e. 0.2% w/w) incorporated i soil. In addition, Gajic and co-workers (Gajic, 1976; Gajic & Vrbaski, 1 as quoted by Rice, 1986) studying allelopathy of corncockle (Agroste githago) on wheat, reported that wheat yields increased when grown mixture with corncockle, as compared with pure stands of wheat. Ster seedlings of the weed stimulated the growth of sterile wheat seedlings agar, thus demonstrating that an excreted compound was involved. One of t stimulatory compounds isolated from Agrostemma was "Agrostemmin", t application of which at 1.2 g/ha to wheat fields increased the grain yie on both fertilized and non-fertilized fields. Moreover, the free tryptop content of wheat increased and the quality of the resulting flour and bre improved. Thus, substantial evidence had been presented on a stimulatc allelochemical and its potential use in agriculture.

Chopped alfalfa (Medicago sativa), when applied to soil as a sidedressing in a band below and to one side of the crop seeds or seedlings, stimulated the growth of tomato, cucumber, lettuce and several other plants (Ries et al, 1977). An application of 117 kg of dry alfalfa per hectare increased tomato yields by 10 metric tons per hectare. Cucumber and lettuce yields were also significantly increased in the field. Nitrogen analysis of lettuce leaves showed that alfalfa applications also produced an increase in the concentration of total N, but that N-content was lower than in lettuce receiving ammonium nitrate. The average nitrate levels were similar in lettuce receiving alfalfa treatments and controls, while nitrate levels in plants receiving ammonium nitrate were 54% higher than those in lettuce treated with alfalfa, indicating that the increase in yield of lettuce was not solely due to nitrogen in alfalfa. Many additional factors present in, or associated with alfalfa such as N, other nutrients, microorganisms, organic matter and pest control, were shown not to be responsible for increased yields (Ries et al, 1977), which led to a postulation that a stimulating factor which operated at very low concentrations was present in alfalfa. Alfalfa extracts applied to rice seedlings grown in nutrient culture had stimulated the growth of seedlings. The stimulatory allelochemical had been subsequently identified as triacontanol (TRIA), as stated previously.

In another study Rice (1985) reported that ground-ivy (Glechoma hederacea) is strongly allelopathic towards certain other species. Both root exudates and decaying leaves siginificantly stimulated the growth of radish (Raphanus sativus) plants and decaying leaves significantly stimulated the growth of downy brome (Bromus tectorum). A most important raised by Rice was the fact that despite numerous changes in experimental conditions, stimulation of growth was consistently recorded, the greatest stimulations in growth being always in the presence of additionally added N, P, and K. Rice (1986) argued that the large differences between control and test treatments could not have been caused by added minerals in the laef materials of ground-ivy, because only 2 g of this material was added per kg of soil (0.002% w/w), and only a very small % of leaf material consisted of minerals. This suggested that a chemical(s) in ground-ivy may have increased the uptake of minerals by test plants, because a well-known mechanism of action of certain allelochemicals is on the uptake of minerals. Decaying ground-ivy leaves (2g/kg of soil) stimulated shoot growth of downy brome by 770% and of radish by 1004%; root growth of downy brome was similarly stimulated by 251% and of radish by 1354%.

In conclusion, it may therefore be said that the type of spectacular crop growth stimulation, with simultaneous weed suppression, observed with Gliricidia and Tithonia leaf residues is not unique to these plants. The studies of Jayawickrema and Thenabadu (1989), reviewed earlier in Chapter 1 (Tables 1.1 and 1.2), clearly indicated that incorporation of

Gliricidia and Tithonia as green manures significantly increased the water-soluble and exchangeable-N in soil, with the legume, Gliricidia, which contained more leaf-N (4.24% as compared to 3.9% in Tithonia), releasing more N into soil. It was also reported that the incorporation of green manure caused a significant increase in the microbial activity of soil. These studies also showed that Gliricidia and Tithonia did not differ much in their rate of breakdown and release of N, with both residues achieving the maximum release of N on day 24, after incorporation (Table 1.2).

The results of our studies very strongly support the view held by Wijewardene (personal communications) and many of his associates (Wijewardene & Waidyanatha, 1984), that Gliricidia loppings/leaves used in "Alley-cropping" provide an excellent means for weed control possibly through allelopathic activities, with which the use of certain other herbicides can be suitably combined. The studies in Sri Lanka on Gliricidia alley-cropping, reviewed by Handawela and Kendaragama (1992) (Tables 1.3, 1.4), also provide ample evidence of the weed control abilities of Gliricidia leaves, the cause of which requires elucidation. Having established potential weed control abilities and other benefits crop production may have through the use of Gliricidia and also Tithonia, the next endeavour of our research was to pursue this activity through appropriate activity-directed, bioassay-based fractionation methods.

With the proper choice and management of certain plant residues and/or cover ccrops or companion crops, it is probably possible to supplement, if not reduce, the amount and number of herbicides used in crop production, particularly in no-till systems, by utilising natural phytotoxic substances. The implication of all these studies are, firstly, that agroecosystems could be manipulated to biologically reduce certain weed pressures; and secondly, chemicals present in mulch/residues could be of practical significance in terms of new herbicide chemistries.

CHAPTER 4

ISOLATION AND FRACTIONATION OF BIOACTIVE COMPOUNDS FROM GLIRICIDIA AND TITHONIA LEAF MATERIALS

SECTION 4.1

GENERAL METHODS AND APPARATUS

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4.1.1 Bioassay

Inhibitory activity of the initial aqueous extracts, organic extracts and/or fractions obtained by chromatographic separation of active extracts, was monitored by seed germination and seedling growth bioassays involving very sensitive species. In order to reduce variability between replicates and different experiments, it was found that bioassays using seeds of crop species Tomato and/or chilli, were better. Both species had been previously found to be highly sensitive to Gliricidia and Tithonia extracts.

Four ml aliquots of a crude or partially purified extract or a purified fraction under investigation were pipetted out into 9 cm diameter petridishes lined with a single, 9 cm diameter filter paper (Whatman No. 1). When extracts contained organic solvents, these were allowed to evaporate under vacuum (ca. 2 h at room temparature). Four ml of water were then added along with twenty five seeds of each test species, which had been previously surface sterilised by dipping in 1% $HgCl_2$ solution for one minute. The petri dishes were kept on laboratory benches under continous light in growth rooms or growth cabinets, which had 26-28 ^{0}C

temperature, 65-70% relative humidity and light which was measured at 380 $uE/m^2/sec$ of photon flux density (PPFD) light intensity. Seed germination was monitored during this period and it was found that the best time to record germination and seedling fresh weights was after 10, 12 or 14 days. All bioassays were carried out in triplicate with appropriate solvent controls and the arrangement of petridishes was always completely random. A seed that had produced a radicle in excess of 1 mm was considered to have germinated. Ungerminated seeds were not included in the determination of seedling fresh weights. Where germinated seedlings were weighed for fresh weight, the remnants of the seed coats were removed without damaging the seedling with forceps.

All data on bioassays were subjected to standard analyses of variance using % germination (arcsine transformed) and mean seedling fresh weight (mg) as variables. Column fraction bioassays were also analysed in a similar way. All mean separations were based on Duncan's Multiple Range Test.

4.1.2 Calculation of the concentration of Fractions used in the bioassay

The concentration of a fraction used in the bioassay was calculated according to the following equation.

$$\text{Concentration of the fraction} = \frac{a}{A} \; 2C$$

A= Weight of the mother fraction subjected to the separation procedure.

a= Weight of the fraction obtained from the separation

C= Concentration of the mother fraction used in the bioassay.

4.1.3 Chromatography

Thin-Layer Chromatography (TLC) systems were extensively used to monitor the progress of column chromatograpic separations. Vanillin - Conc. H_2SO_4 was used as the spray reagent to visualise the components on TLC. Silica gel of TLC-grade was used in vacuum liquid chromatography (VLC), and silica of mesh size (60-120) was used in gravity column chromatography. Sephadex LH 20 in dichloromethane-methanol (1:1) was used in gel-filtration chromatography.

The High Performance Liquid Chromatogarph (HPLC) (Waters) fitted with a Silica semipreparative column was used for normal phase separations. A C_{18}-column was used for reversed phase separations. A Refractive Index (RI) detector was used to detect the components. All solvents used in chromatography were purchased from the Petroleum Corporation, Sri Lanka, and were distilled before use.

4.1.4 Chemical Extraction

The plant material of *Gliricidia* for the studies was collected from several trees found in the Colombo University premises. *Tithonia* leaf material was always collected from two nearby sites in Kandy, ca. 72 miles from Colombo in the mid-country. The collected plant materials, either in fresh state or in sun-dried state were dipped in methanol (2 kg in 5 litres of solvent) for one week, after which the solvent was decanted off and dried under vacuo at 30 ^{0}C.

4.1.5 Solvent partitioning of the crude extract

A modified Kupchen scheme was adopted for the solvent partitioning of the crude extract. The crude extract was redissolved in 10% aqueous methanol and partitioned with hexane. The water content of the aqueous layer was increased up to 30% and partitioned again with dichloromethane. Ethyl acetate solubles were also separated after increasing the precentage of the aqueous layer to 60%. Each fraction was then concentrated under reduced pressure. Appropriate amounts of each residue was redissolved in the same respective solvent to yield the concentration ranges required for bioassays.

4.1.6 Apparatus

4.1.6.1 Determination of melting points

The melting points of active chemicals obtained were recorded by using glass capillaries on Gallenkamp melting point apparatus.

4.1.6.2 Optical Rotation

The optical rotations of optically active compounds were measured by using Schmidt & Haensch polartronic-D polarimeter at 27 ^{0}C.

4.1.6.3 Spectrometry

(a) Mass Spectrometry

Electron Impact (EI) mass spectra were recorded on Finnigan MAT-112 and MAT-113 spectrometers coupled with PDP 11/34 computer system. High Resolution (HR) experiments were carried out on JEOL ---- spectrometer. Field Desorption (FD) mass spectrometry was conducted on MAT-312 mass spectrometer.

(b) NMR Spectrometry

1D and 2D ^{1}H-NMR spectra were recorded at 300 and 400 MHz on Bruker AM-300 and AM-400 spectrometers with Aspect 300 data system. The ^{13}C- NMR experiments were performed on the same instruments at 75.4 and 100 MHz. For NOE difference measurements, each sample was frozen under liquid nitrogen and degassed. A lower decoupler power of 0.2 watts with 35 attenuation in dbs was used. The pre- irradiation time was 11 sec which is the sum of three delays as used in the NOE difference programme of Bruker. The impulse length of ten micro-seconds was maintained to avoid saturation.

The two-dimentional COSY-45^{0} experiments were acquired at 300 MHz with a sweep width of 4000 Hz (256 t_1 values zero-filled to 1k) in w1. The heteronuclear 2D ^{1}H and ^{13}C chemical shift correlation experiments were carried out at 300 MHz with a sweep width of 12820 Hz (2K data points) in w2 and 1024 Hz (256 t1 values zero filled to 2K) in w_1. In both 2d experiments a sec relaxation delay was used and 16 transients were performed for each t_1 value.

SECTION 4.2

ISOLATION OF ALLELOCHEMICALS FROM GLIRICIDIA

4.2.1. Isolation of allelochemicals from Gliricidia fresh leaves

Solvent partitioning of the methanolic extract from Gliricidia fresh leaves, by the modified Kupchen scheme indicated that inhibitory activity was present in the polar dichloromethane and ethyl acetate fractions (Table 4.1) and not in the non-polar hexane fraction. The two polar fractions were then subjected to intensive bioassay-guided fractionation and chromatographic separation, with a view to isolating the allelochemicals. The following procedure was adopted (Flow-Chart, Fig 4.1):

4.2.1.1 Chromatographic separation of Dichloromethane Fraction

(a) Vacuum-liquid chromatography on silica gel

Separation of the dichloromethane solubles was carried out by vacuum-liquid chromatography on silica gel in dichloromethane containing gradiently increasing concentrations of methanol from 0% to 50%. Six fractions (code named A to F) were collected and bioassayed (Table 4.2). Fraction A only was found to contain inhibitory bioactivity. Fractions B, C, D, E, and F did not possess bioactivity.

(b) Column-chromotography of Fraction A on silica gel

The active fraction A, obtained above was subjected to further chromotographic separation on silica gel in hexane, containing gradiently increasing concentrations of dichloromethane from 0% to 100%. Five fractions (code named AA1 to AA5) were obtained from which, only fraction AA1 was found to be highly bioactive (Table 4.2).

The compound in fraction AA1 recrystalized in aqueous methanol as a needle-shaped, white, crystalline solid with a yeild of 0.01%. m.p. 68 ^{o}C ; MS m/z (rel. int. %) 146 (M^{+}, 100), 118 (52.86), 89 (25.49); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.01 (d, J= 9.5 Hz) 6.9 (m), 7.2 (d, J= 9.5 Hz), 7.4 (d, J= 9.5 Hz).

Table 4.1: Bioassay of the hexane, methylene chloride and ethyl acetate fractions after partitioning of aqueous methanolic extract of *Gliricidia* according to the modified Kupchen scheme

Fraction	Seed Germination			Mean Seedling Fresh Weight		
	Extract concentration (mg/ml)			Extract concentration (mg/ml)		
	0	0.25	25	0	0.25	25
	(%)			(mg)		
(a) Hexane solubles:						
Tomato	67a	60a	51a	6.0a	5.0a	5.0a
Chilli	60a	51a	51a	6.0a	4.0a	5.0a
(b) Methylene chloride solubles:						
Tomato	73a	75a	3b	11.0a	11.0a	0b
Chilli	72a	15b	1c	5.0a	6.0a	2.0b
(c) Ethyl acetate:						
Tomato	59a	57a	1b	9.0a	9.0a	0.2b
Chilli	33a	32a	5b	3.0a	5.0a	0.3b

Germination and seedling fresh weight measured 10 days after treatment. In this and all other Tables which follow, Mean values along rows followed by the same letter are not significantly different at 5% level of probability, according to the Duncan's Multiple range test.

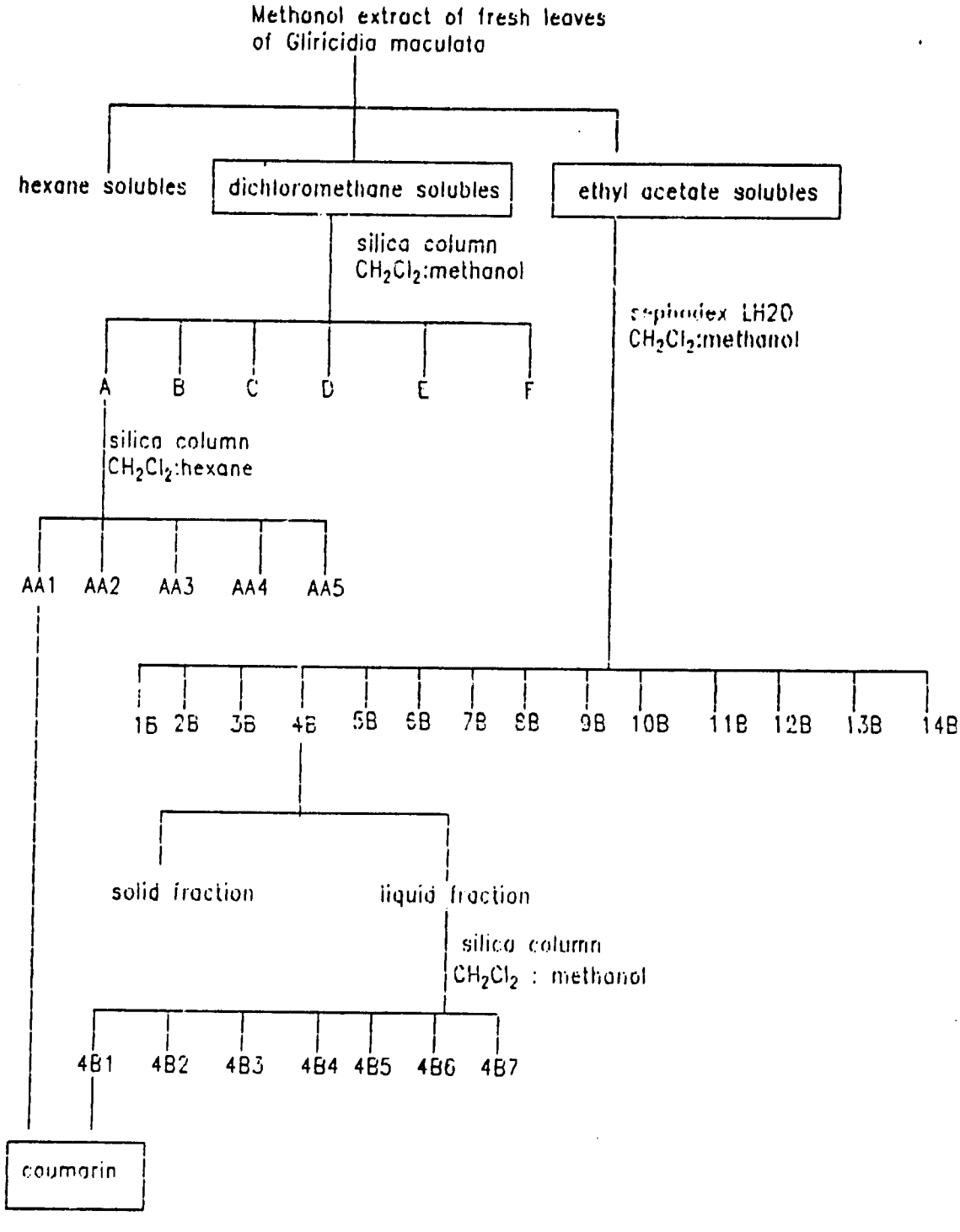

Figure 4.1: Extraction procedure for fresh Gliricidia leaves

Table 4.2: Effect of fractions obtained from methylene chloride solubles of Gliricidia leaves on tomato seed germination and seedling fresh weight

Fraction	Concentration (mg/ml)	% Seed Germination	Mean fresh weight (mg) of seedlings
(a) Fractions from Methylene chloride extract (Kupchen scheme):			
A	9.50	0	0
B	1.32	90	17
C	0.23	85	18
D	1.00	93	19
E	6.50	84	16
F	3.50	89	20
(b) Fractions from further separation of active Fraction A:			
AA1	2.35	0	0
AA2	0.05	77	7
AA3	0.02	65	12
AA4	0.10	73	14
AA5	0.01	65.3	11

Untreated Controls: % seed germination:- 88-98%
Mean fresh weight (F.W.) of seedlings:- 15-18 mg

(c) **Bioactivity of Fraction AA1**

The highly active fraction AA1 which gave a needle-shaped, white, crystalline solid compound, was tested for further bioactivity in a dilution series and on a variety of test species. The same protocol that was used for the earlier bioassays was used. This fraction at concentrations as low as 0.47 mg/ml caused 100% inhibition of germination of tomato seeds (Fig. 4.2a, 4.3a). The I_{50} (concentration that caused 50% inhibition of seedling growth) for tomato seedling growth calculated from the two independent experiments was 0.352 mg/ml (Fig. 4.2b, 4.3b).

4.2.1.2 Chromatographic separation of ethyl acetate fraction

(a) Gel-filtration Chromatography

The active ethyl acetate fraction was subjected to gel-filtration chromatography on a Sephadex LH 20 column (150 x 4.5cm) in methanol-dichloromethane (1:1). The fourteen fractions collected (code named 1B to 14B) were concentrated and bioassayed (Table 4.3). The fraction 4B was found to be the most active in inhibiting the seed germination and seedling growth of tomato. Fractions 3B and 5B (Plate 4.1) were also clearly highly bioactive.

(b) Column-chromotography of Fraction 4B on silica gel

The active fraction 4B partially solidified on standing. The solid part was separated from the liquid part. Bioactivity was found to reside only in the liquid fraction. Hence the active liquid fraction was further separated on a silica gel coloumn in dichloromethane containing gradiently increasing concentrations of methanol from 0- 40%, into seven fractions (code named 4B1 to 4B7) which were bioassayed (Table 4.3). Only the Fraction 4B1 was found to be highly active.

When purified by recrystalization from aqueous methanol, this too was obtained as a white crystalline solid, identical to that of Fraction AA1 obtained from the dichlromethane fraction. The compound was shown to be identical with an authentic sample and the component in fraction AA1 by mixed melting points and TLC comparisons. Finally, the bioactive compound from Gliricidia maculata fresh leaves were identified as coumarin.

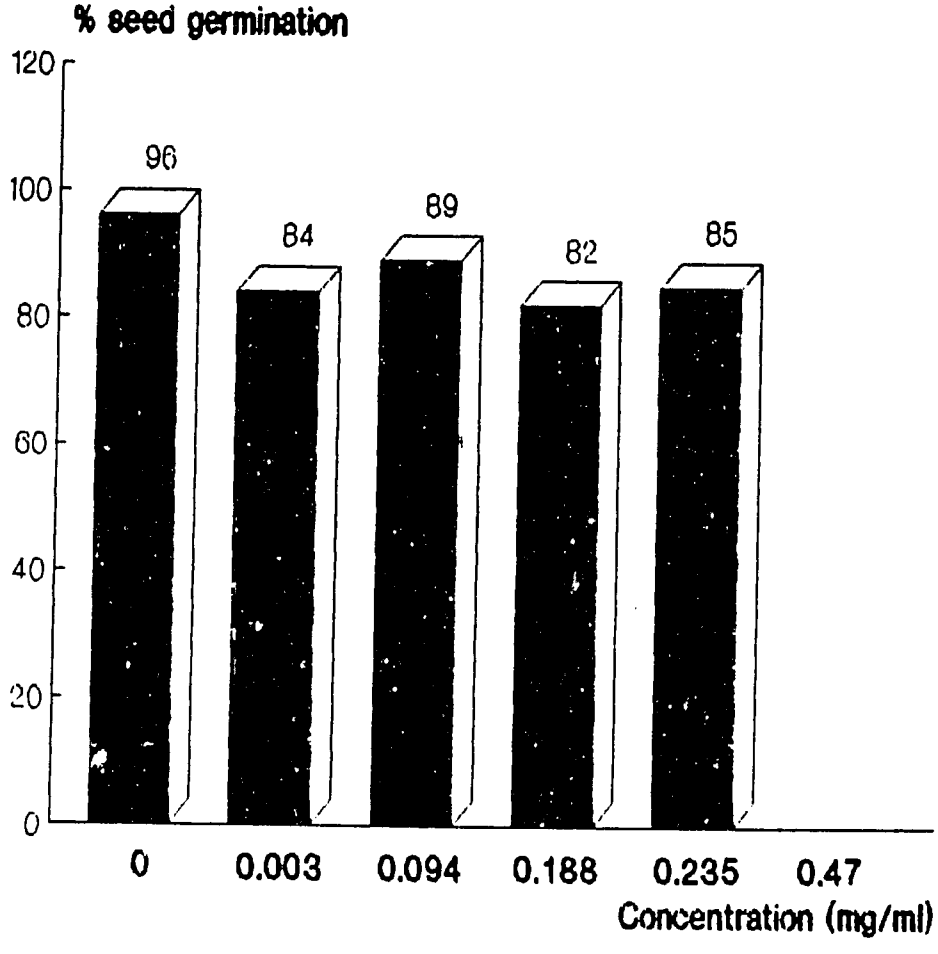

Figure 4.2a: The effect of a range of Coumarin concentrations on seed germination of Tomato 12 days after treatment (Trial I)

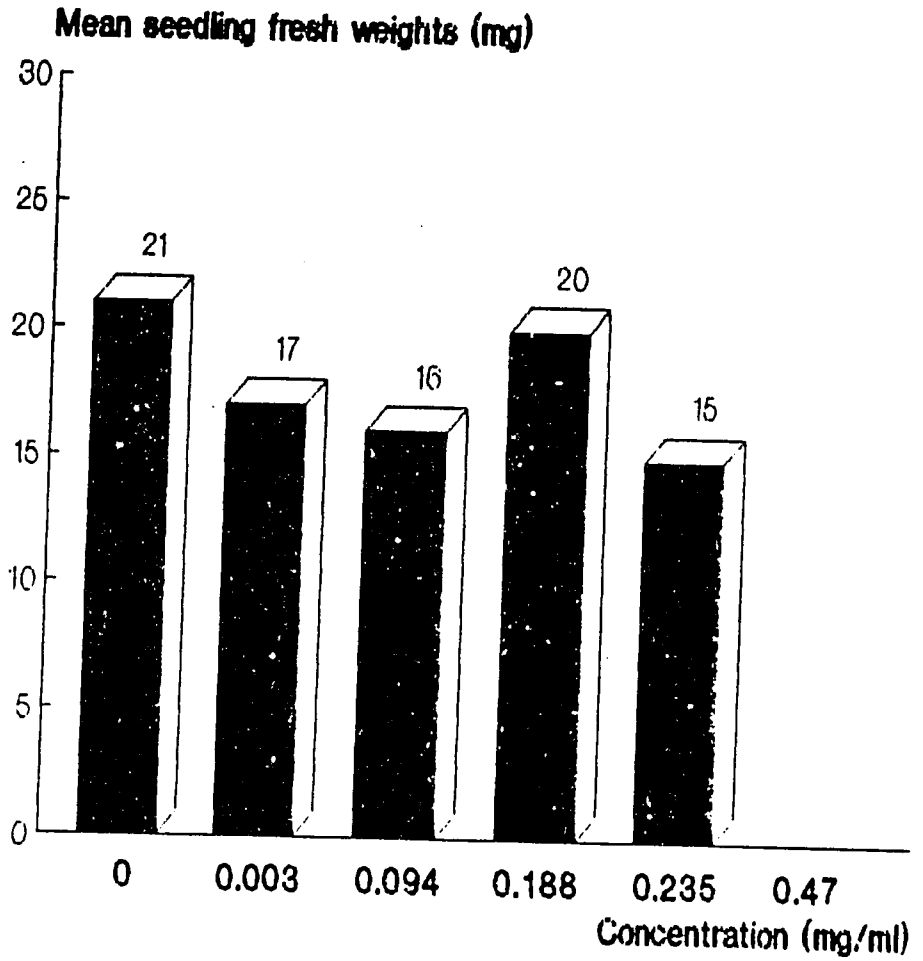

Figure 4.2b: The effect of a range of Coumarin concentrations on seedling growth of Tomato 12 days after treatment (Trial I)

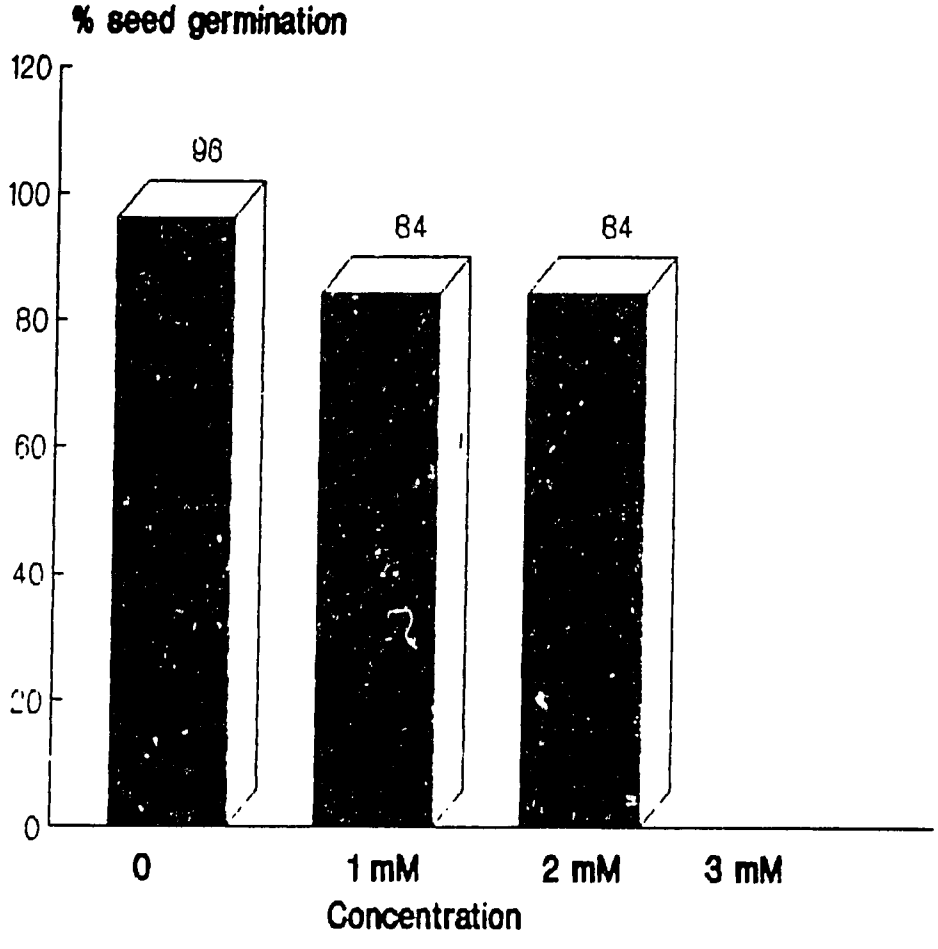

Figure 4.3a: The effect of a range of Coumarin concentrations on seed germination of Tomato 12 days after treatment (Trial II)

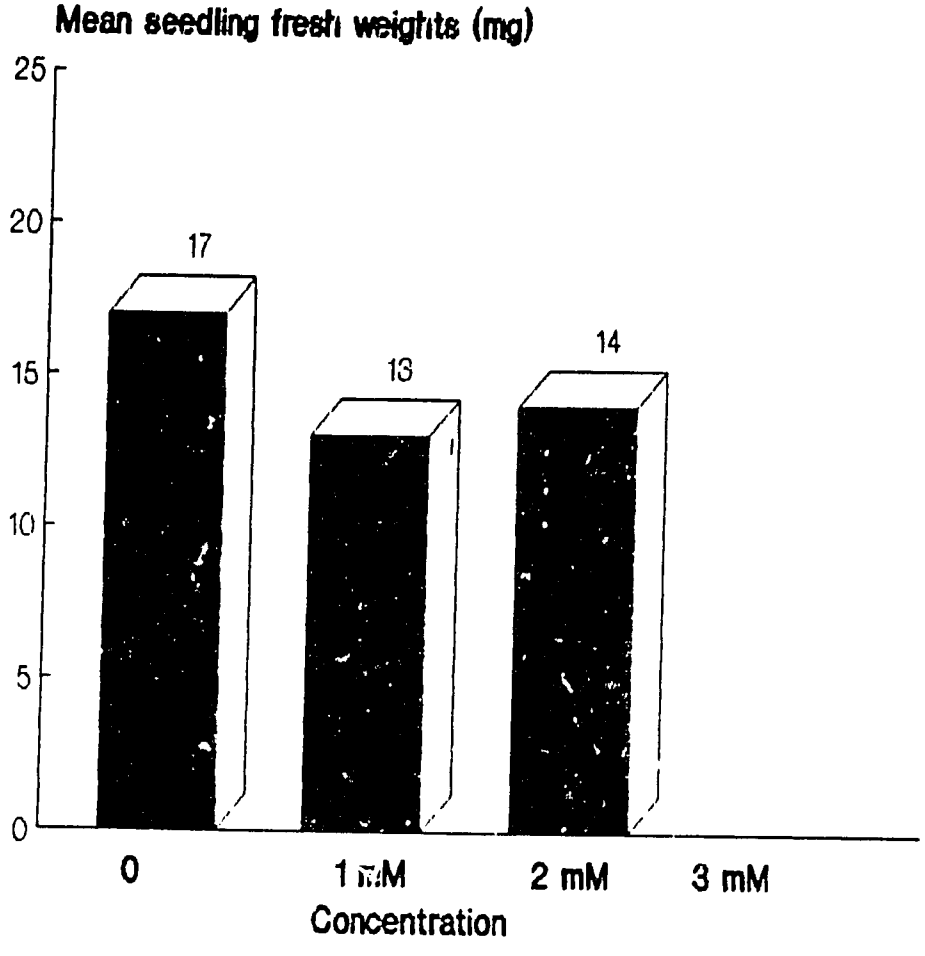

Figure 4.3b: The effect of a range of Coumarin concentrations on seedling growth of Tomato 12 days after treatment (Trial II)

Table 4.3: Effect of fractions obtained from ethyl acetate solubles of Gliricidia leaves on tomato seed germination and seedling fresh weight

Fraction	Concentration (mg/ml)	% Seed Germination	Mean fresh weight (mg)
(a) Fractions from ethyl acetate extract (Kupchen scheme):			
1B	1.00	86	21
2B	6.76	54	5
3B	3.56	0	0
4B	7.62	0	0
5B	22.40	0	0
6B	4.00	54	6
7B	0.10	80	14
8B	0.22	82	14
9B	0.25	76	16
10B	0.46	64	4
11B	0.16	88	16
12B	1.24	56	6
13B	1.32	72	13
14B	0.38	84	19
(b) Fractions from further separation of active Fraction 4B:			
4B1	0.34	0	0
4B2	0.25	75	23
4B3	0.42	85	26
4B4	0.44	68	19
4B5	2.22	95	23
4B6	0.55	80	21
4B7	0.82	70	18

Untreated Controls: % seed germination:- 88-96 %
Mean F.W.of seedlings:- 0.16-0.18 mg

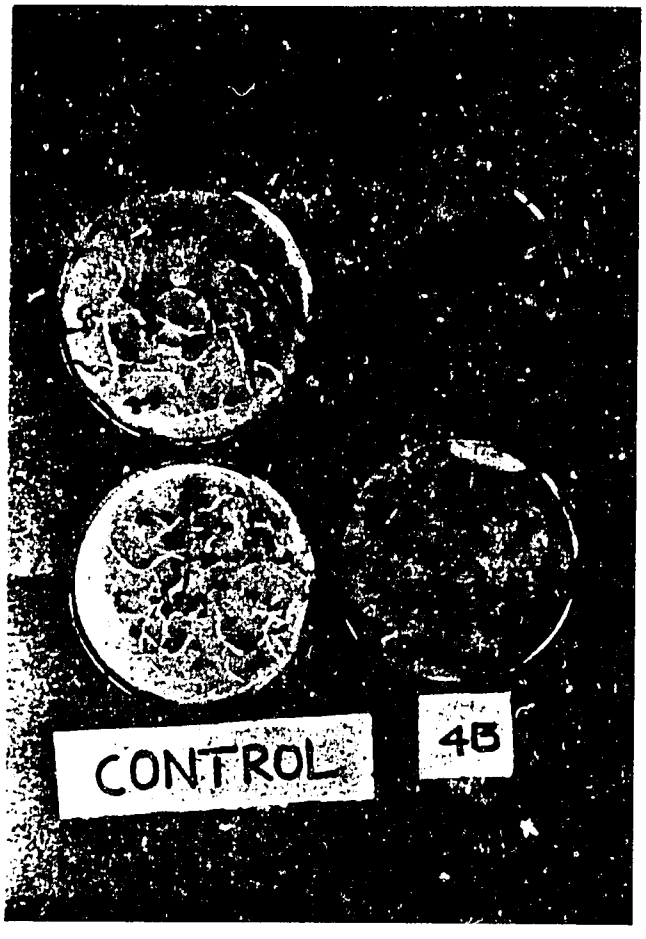

Plate 4.1: Activity of the highly bioactive faction 4B from the Ethyl Acetate fraction of Gliricidia fresh leaves

4.2.2 Isolation of soyasapogenol from Gliricidia dried leaves

Dried and powdered leaf materials of Gliricidia were dipped/soaked in distilled methanol (1.5 kg/litre). After one week, the extract was decanted off, concentrated and bioassayed (Table 4.4). The tomato seed germination bioassay was found to be quite sensitive and suitable to determine the inhibitory activity of the extract.

4.2.2.1 Solvent partitioning of the Methanol extract

The methanolic crude extract was redissolved in water-methanol (1:9), subjected to initial solvent partitioning by the modified Kupchen scheme and separated into hexane solubles, dichloromethane solubles, and ethyl acetate solubles. These fractions were concentrated and bioassayed (Table 4.5). Inhibitory bioactivity was found to reside mostly in the dichloromethane fraction and to a lesser extent in the ethyl acetate fraction. The hexane solubles were not inhibitory in the tomato seed germination bioassay.

4.2.2.2 Activity-directed fractionation of the active dichloromethane fraction

(a) Gel-filtration chromatography of the Dichloromethane Fraction

The dichloromethane fraction was subjected to gel-filtration chromatography on a Sephadex LH 20 column (150 x 4.5 cm) in methanol: dichloromethane (1:1) mixture. The resulting four fractions (code named GDM1, GDM2, GDM3, GDM4) were concentrated and bioassayed (Table 4.6). Fraction GDM3 was found to be extremely bioactive.

(b) Chromatograpic separation of fraction GDM3 on silica

The active fraction GDM3 obtained above was subjected to chromatograpic separation on silica-gel in hexane containing gradiently increasing concentrations of acetone from 0 to 100%. Seven different fractions (code named A, B, C, D, E, F, G) were collected, concentrated and bioassayd (Table 4.7).

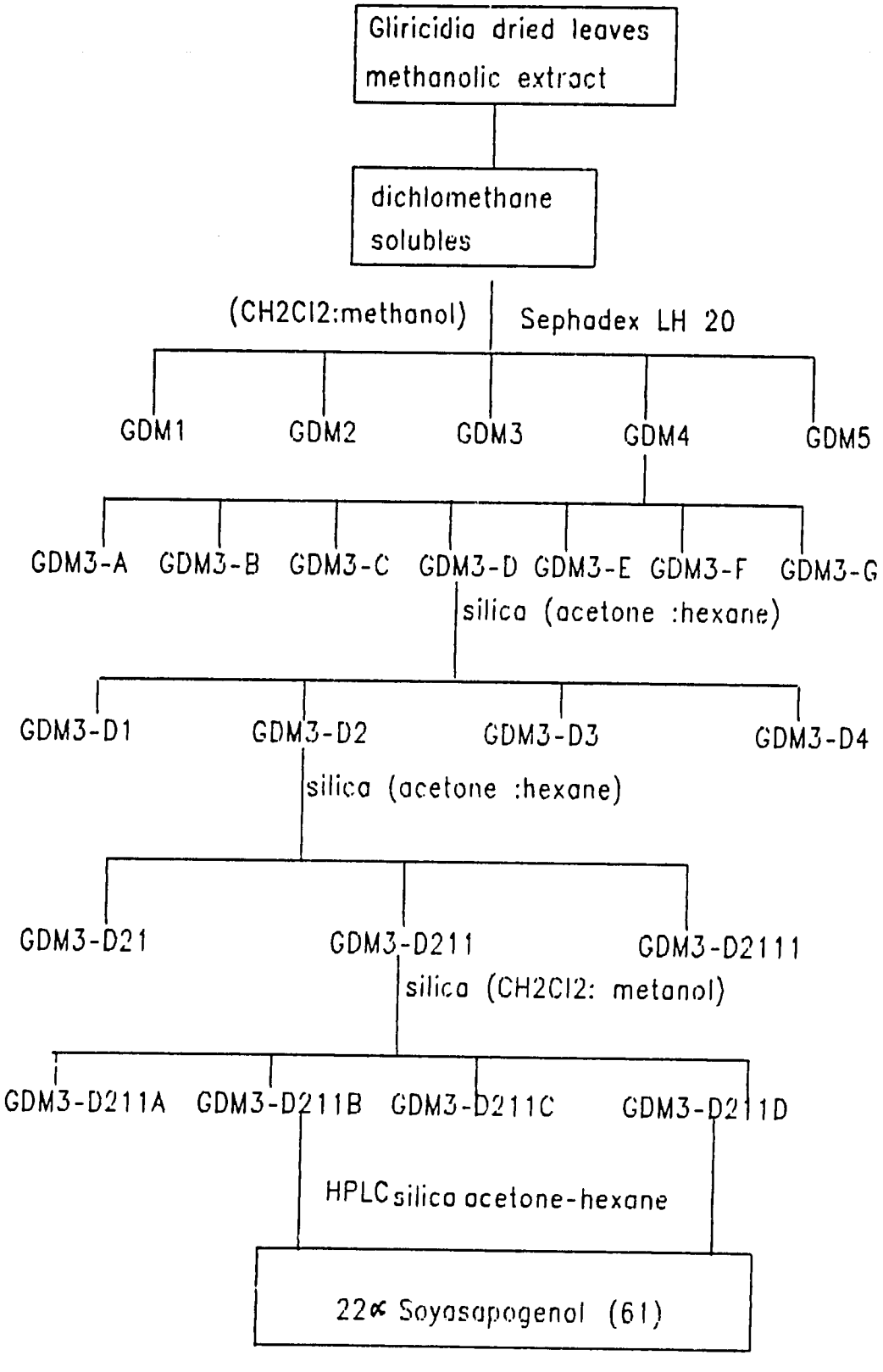

Figure 4.2: Extraction procedure for dried leaves of Gliricidia

Table 4.4 : The Effect of methanolic extract of Gliricidia maculata dried leaves on seed germination and seedling growth of Tomato and Achyranthes.

Assay spp.	Concentration (mg/ml)									
	0	0.25	2.5	12.5	25	0	0.25	2.5	12.5	25
	% Seed Germination					Mean Seedling Fresh Wt (mg)				
Tomato	84a	77a	77a	0b	0b	0.04a	0.02a	0.01b	0b	0b
Achyranthes	68a	5b	8b	0b	0b	0.01a	0b	0b	0b	0b

Table 4.5: The Effect of (a) hexane (b) ethyl acetate and (c) dichloromethane solubles of Gliricidia maculata dried leaves on seed germination and seedling growth of Tomato.

Fraction	Concentration (mg/ml)	% Seed Germination	Mean fresh weight (mg)
(a) Hexane solubles:			
	0	77a	0.02a
	2.5	76a	0.02a
	5.0	67a	0.013a
(b) Ethyl Acetate solubles:			
	0	77a	0.013a
	1.0	87a	0.02a
	2.0	0b	0b
(c) Dichloromethane solubles:			
	0	77a	0.013a
	1.6	0b	0b
	3.3	0b	0b

Table 4.6: The Effect of fractions obtained by gel-filtration chromatography of the dichloromethane Fraction on seed germination and seedling growth of Tomato.

	Fraction / Concentration (mg/ml)				
	Control 0	GDM1 (3)	GDM2 (1.96)	GDM3 (1.52)	GDM4 (0.22)
% Seed Germination	81a	76a	88a	0b	83a
Mean fresh wt (mg)	0.02a	0.01a	0.01a	0b	0.01a

Table 4.7: The Effect of fractions obtained by silica column chromatography of GDM3 on seed germination and seedling growth of Tomato

Fraction	Concentration (mg/ml)	% Seed Germination	Mean fresh weight (mg)
Control	0.00	71a	0.013a
GDM3-A	0.013	77a	0.006a
GDM3-B	0.065	1b	0b
GDM3-C	0.039	1b	0b
GDM3-D	0.36	0b	0b
GDM3-E	0.186	0b	0b
GDM3-F	0.27	0b	0b
GDM3-G	0.457	0b	0b

Fraction GDM3-A was not found to be active. Of the other active fractions GDM3-D (Plate 4.2) had the highest yield and was taken further for characterization. Based on the similarity of TLC characteristics, fractions GDM3-C, GDM3-E, GDM3-F and GDM3-G were considered to contain essentially the same type of bioactive compound(s).

(c) Column-chromatographic separation of fraction GDM3-D on silica gel

The active fraction GDM3-D was further chromatographed on silica in hexane containing gradiently increasing concentrations of acetone from 0 to 100%. The resulting fractions (code named D1, D2, D3, D4) were concentrated and bioassayed (Table 4.8). Inhibitory bioactivity was found to reside in the Fraction GDM3-D2 (Plate 4.3). All other fractions were inactive based on the results of the tomato seed germination bioassay.

(d) Column chromatography of fraction GDM3-D2 on silica gel

The active fraction GDM3-D2, obtained above was subjected to further chromatographic separation on silica in hexane containing gradiently increasing concentrations of acetone from 0 to 100%. The resulting fractions (code named D2i, D2ii, D2iii) were also concenrtated and bioassayed (Table 4.9).

The fraction GDM3-D2ii was found to be highly active (Plate 4.4), while the other fractions were not active.

(e) Column chromotography of fraction GDM3-D2ii on silica gel

The active fraction GDM3- D2ii was next chromatographed on silica gel in dichloromethane containing gradiently increasing concentrations of methanol from 0 to 80%. The resulting fractions (code named D2iiA, D2iiB, D2iiC, D2iiD) were concentrated and bioassayed (Table 4.9). The fractions GDM3-D2iiB and GDM3-D2iiD showed extremely potent inhibitory activity on tomato seed germination at very low concentrations (0.027 mg/ml and 0.06 mg/ml, respectively).

The fraction GDM3-D2iiB which gave a greater yield, and a single dark yellow spot at the range of Rf 0.45-0.5 in 0.5% methanol in dichloromethane. The purity was further confirmed by normal phase HPLC in 1:3 acetone: hexane. The compound was a amorphous solid (18-20 mg, 0.001%). IR λ_{max} ($CHCl_3$ cm^{-1} 3400 (OH), 2950, 1430, 1020, 830. EIMS m/z (rel. int.%) 458 (1.4) [M^+], 234 (100), 224 (10.6), 203 (9.4), 175 (39.3).

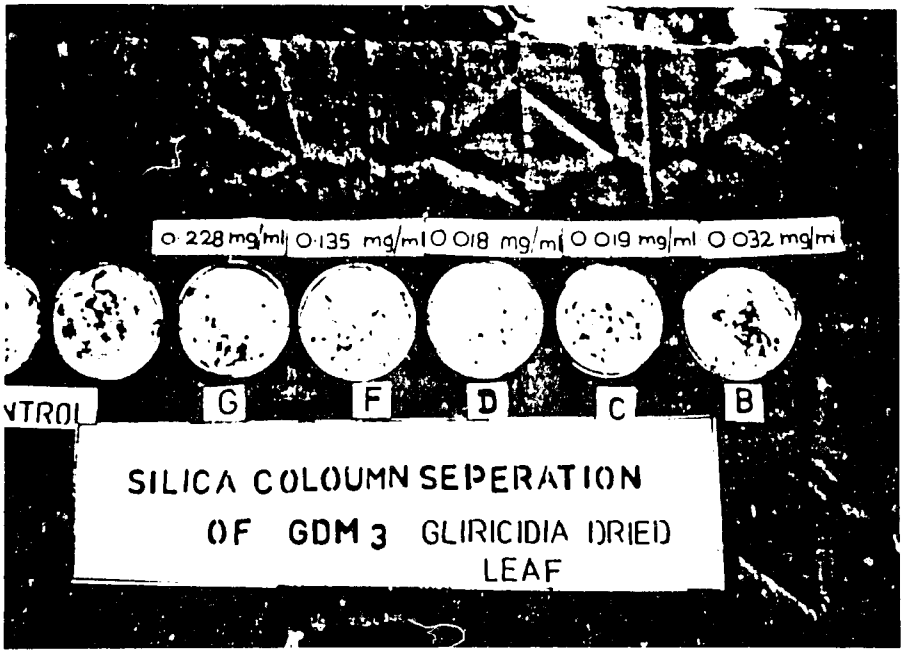

Plate 4.2: Activity of bioactive fraction GDM3-D obtained from Gliricidia dried leaves

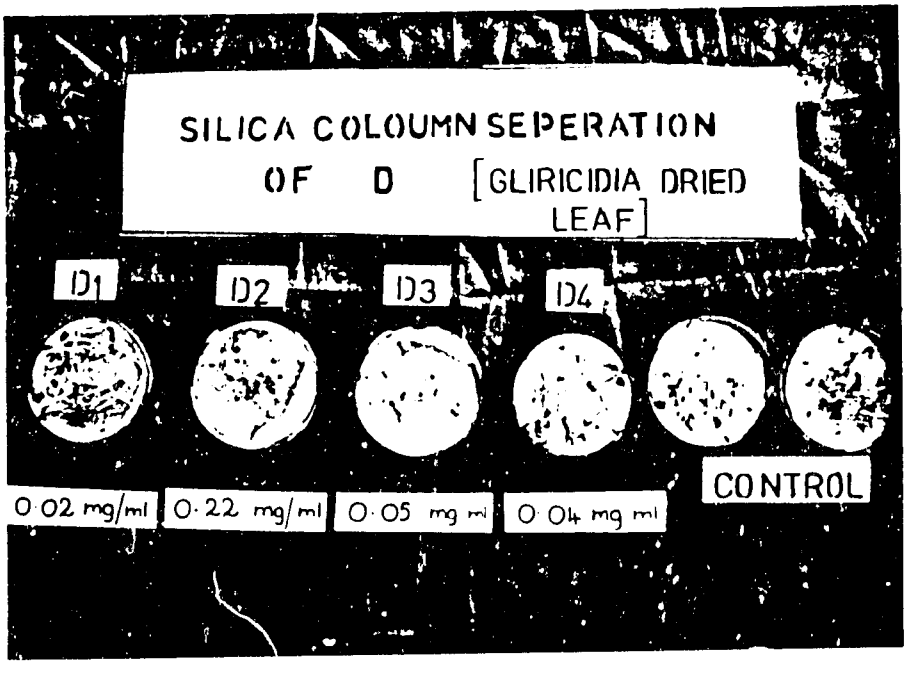

Plate 4.3: Activity of bioactive fraction GDM3-D2 obtained from Gliricidia dried leaves

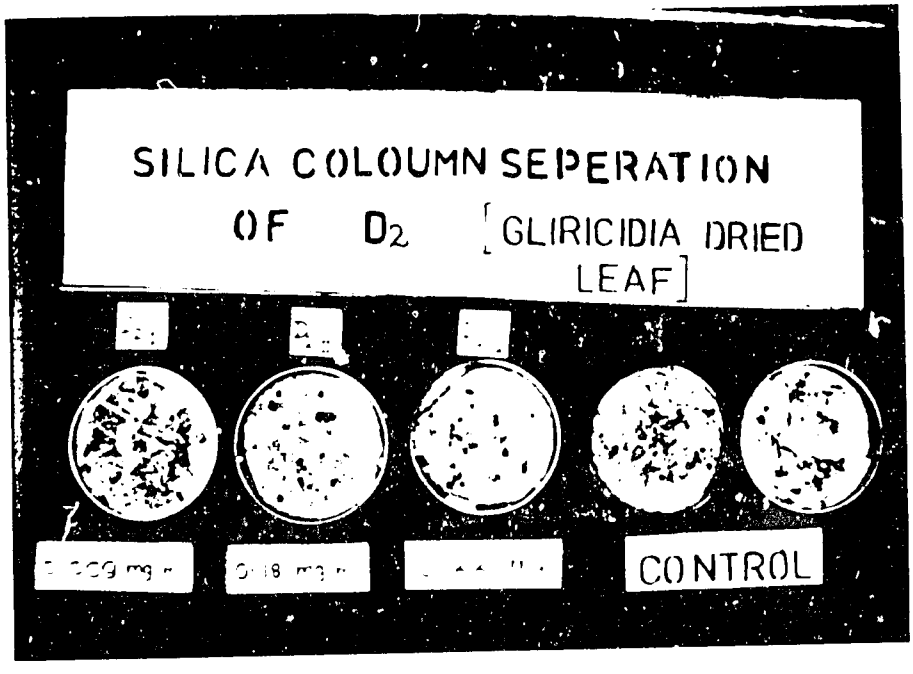

Plate 4.4: Activity of bioactive fraction GDM3-D211 obtained from Gliricidia dried leaves

Table 4.8 : The Effect of fractions obtained by silica column chromatography of Fraction GDM3-D on seed germination and seedling growth of Tomato.

Fraction	Concentration (mg/ml)	% Seed germination	Mean fresh weight (mg)
Control	0	73a	0.014a
GDM3-D1	0.026	81a	0.017a
GDM3-D2	0.229	0b	0c
GDM3-D3	0.0536	72a	0.006b
GDM3-D4	0.049	89a	0.015a

Table 4.9 : The Effect of fractions obtained by silica column chromatography of Fractions (a) GDM3-D2 and (b) GDM3-D2ii, on seed germination and seedling growth of Tomato.

Fraction	Concentration (mg/ml)	% Seed germination	Mean fresh weight (mg)
(a) GDM3-D2:			
Control	0	80a	0.018a
GDM3-D2i	0.009	83a	0.017a
GDM3-D2ii	0.018	0c	0b
GDM3-D2iii	0.038	68b	0.013b
(b) GDM3-D2ii:			
Control	0	63a	0.012a
GDM3-D2iiA	0.05	60a	0.001c
GDM3-D2iiB	0.027	24b	0.002c
GDM3-D2iiC	0.027	57a	0.006b
GDM3-D2iiD	0.06	24b	0.003bc

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.67 (m, H1a), 0.9 (m, H1b), 1.7 (m, H-2a), 1.1 (m, H-2b), 3.4 (dd, J=11.5 Hz, 4 Hz,H-3), 0.8 (m, H-5), 1.65 (m, H-6a), 1.35 (m, H-6b), 1.53 (m, H-7a), 1.35 (m, H-7b), 1.7 (m, H-9), 1.8 (m, H-11a, 11b), 5.3 (t, J= Hz, H-12), 1.7 (m, H-15a), 1.0 (m, H-15b, H-19b), 1.25 (m, H-16a, H16-b), 2.1 (dd, H-18), 1.72 (m, H-19a), 1.4 (m, H-21a, H-21b), 3.4 (dd, J=5, 3 Hz, H-22), 3.2 (dd, J=4.2, 11 Hz, H-23a, H-23b), 1.02 (s, H_3-24), 0.9 (s, H_3-25), 0.98 (s, H_3-26), 1.1 (s, H_3-27), 1.02 (s, H_3-28), 0.89 (s, H_3-29), 0.91 (m, H_3-30).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ 38.443 (C-1), 27.69 (C-2), 80.936 (C-3), 42.812 (C-4), 55.9 (C-5), 18.48 (C-6), 33.138 (C-7), 39.714 (C-8), 47.7 (C-9), 36.69 (C-10), 23.138 (C-11), 122.3 (C-12), 144.3 (C-13), 42.4 (C-14), 25.908 (C-15), 28.213 (C-16), 37.414 (C-17), 44.7 (C-18), 46.198 (C-19), 30.538 (C-20), 41.5 (C-21), 76.63 (C-22), 64.5 (C-23), 22.213 (C-24), 16.1 (C-25), 16.8 (C-26), 25.413 (C-27), 28.7 (C-28), 33.1 (C-29), 20.033 (C-30)

(f) Bioactivity of Fraction GDM3-D2iiB

The highly active fraction GDM3-D2iiB was further tested for bioactivity in a dilution series using tomato as the test species. The same protocol that was used for the earlier bioassays was used. This fraction at concentrations as low as 0.054 mg/ml caused 100% inhibition of germination of tomato seeds (Fig. 4.4a), and at 0.02 mg/ml caused 50% inhibition of seedling growth (Fig. 4.4b).

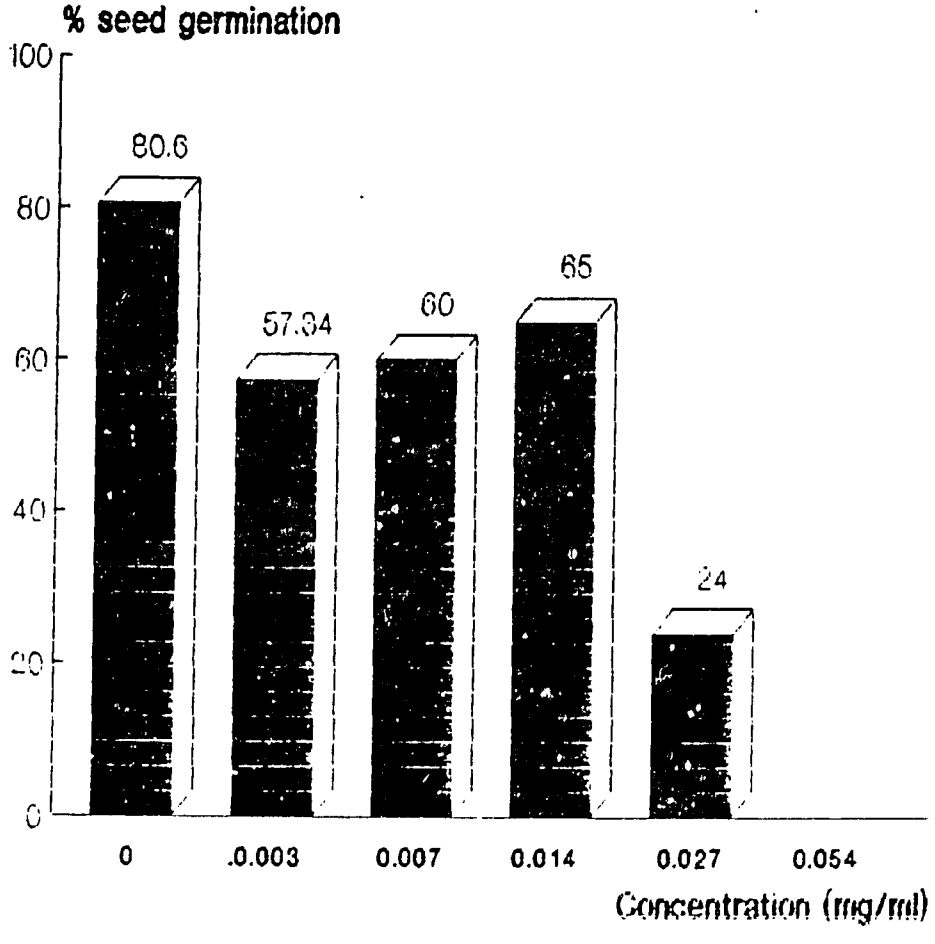

Figure 4.4a: The effect of a range of concentrations of Soyasapogenol on seed germination of Tomato 12 days after treatment

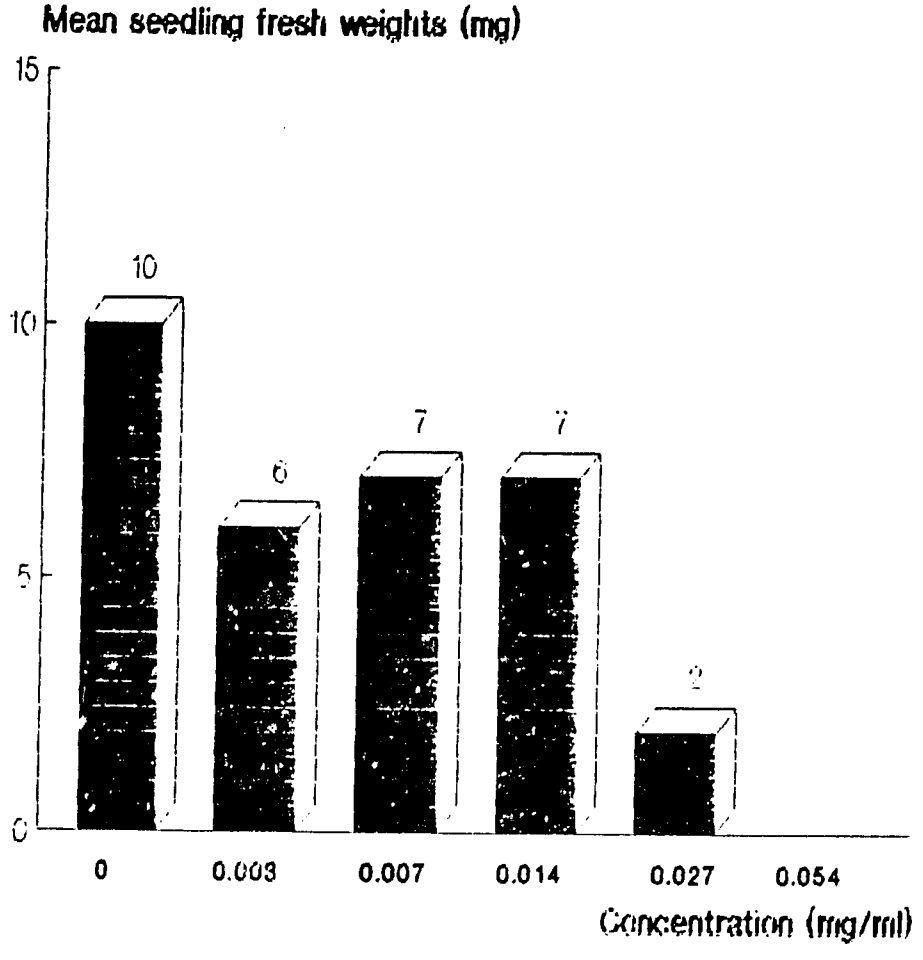

Figure 4.4b: The effect of a range of concentrations of Soyasapogenol on seedling growth of Tomato 12 days after treatment

S E C T I O N 4.3

ISOLATION OF ALLELOCHEMICALS FROM TITHONIA

4.3.1 Isolation of bioactive Allelochemicals from Tithonia dried leaves

The procedure followed is given in the flow-chart (Figure 4.). Dried Tithonia leaves (1.5 kg/litre) were soaked in distilled methanol for one week. The extract was collected by decanting and concentrated under vacuum at room temperature. The methanolic extract was then subjected to bioassay with several selected assay species. The results (Table 4.10) indicated that seeds of tomato and the two weed species were very sensitive to the extracts.

4.3.2 Solvent partitioning of the Methanol Extract

The crude methanol extract was redissolved in water- methanol (1:9) and subjected to the modified Kupchen scheme: the hexane, dichloromethane and ethyl acetate solubles were separated and bioassayed (Table 4.11). While the hexane fraction (non-polar) was relatively less inhibitory, the more polar dichloromethane and ethyl acetate fractions showed considerable inhibitory activity on both tomoto and chilli seed germination and seedling growth, in the bioassay. Hence, the above fractions were selected for further studies.

4.3.3 Chromatograpic separation of the Ethyl Acetate fraction

(a) Vacuum Liquid chromatography on silica gel

Separation of the ethyl acetate solubles was carried out by vacuum liquid chromatography on silica gel using dichloromethane containing increas-ing concentrations of methanol from 0% to 50%. Five fractions (code named TE-A to TE-E) were collected, concentrated, and bioassayed with tomato seeds (Table 4.12). The results indicated that the bioactivity was mostly in the fraction TE-A, which was then subjected to further fractionation.

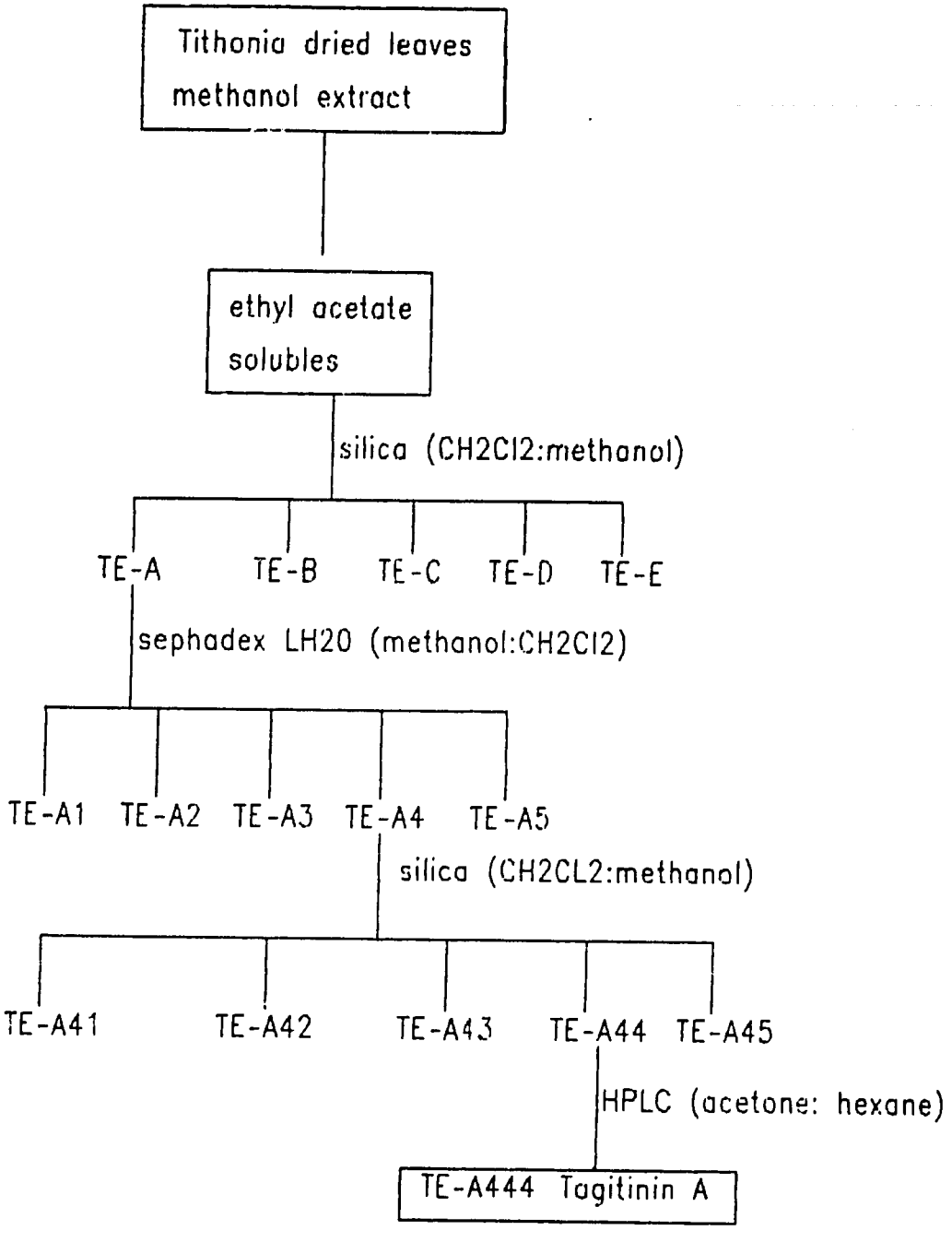

Figure 4.3: Separation procedure for Ethyl Acetate fraction of Methanolic extract of Tithonia dried leaves

Table 4.10: Effect of methanolic extract of *Lithonia diversifolia* dried leaves on the seed germination and seedling growth of selected crop and weed species

	Seed Germination %					Mean Seedling Fresh Weight (mg)				
	Extract concentration (mg/ml)					Extract concentration (mg/ml)				
Species	0	0.7	2.9	5.8	11.6	0	0.7	2.9	5.8	11.6
Tomato	92	90	71	28	5	20a	20a	7b	1c	0c
Chilli	79	80	75	67	36	20a	20a	10b	9b	1c
Tridax	85	77	84	48	8	6a	5a	4a	1b	0b
Achyranthes	55	52	37	27	7	5a	5a	3b	1c	0c

Germination and seedling fresh weight measured 10 days after treatment.
Values along rows followed by the same letter are not significantly different at $p<0.05$, according to the Duncan's Multiple range test

Table 4.11: Effect of (a) Hexane (b) Dichloromethane (c) Ethyl acetate solubles obtained from *Tithonia diversifolia* dried leaves on the seed germination and seedling growth of selected species

	Seed Germination (%)					Mean Seedling Fresh weight (mg)				
(a) Hexane solubles										
Concentration (mg/ml)	0	1.2	2.4	5.8	11.6	0	1.2	2.4	5.8	11.6
Tomato	81	76	14	12	15	20a	10b	2c	1c	1c
Chilli	84	80	74	74	66	20a	10b	10b	10b	10b
(b) Dichloromethane solubles										
Concentration (mg/ml)	0	0.7	1.7	2.8	5.6	0	0.7	1.7	2.8	5.6
Tomato	97	12	5	2	0	20a	3b	0b	0b	0b
Chilli	81	80	73	48	48	20a	10b	10b	3c	1c
(c) Ethyl acetate solubles										
Concentration (mg/ml)	0	0.1	0.2	0.4	0.8	0	0.1	0.2	0.4	0.8
Tomato	83	43	20	0	0	10a	5b	1c	0c	0c
Chilli	79	75	68	44	5	20a	10b	4c	1d	0d

Values along rows followed by the same letter are not significantly different at $p<0.05$, according to the Duncan's Multiple range test

Table 4.12: Effect of five fractions separated by Vaccum- liquid Chromatography from the ethyl acetate solubles obtained from *Tithonina diversifolia* dried leaves on the seed germination and seedling growth of Tomato

	Seed Germination (%)					Mean Seedling Fresh Weight (mg)				
(a) Fraction TE-A										
Concentration (mg/ml)	0	0.05	0.1	0.2	0.4	0	0.05	0.1	0.2	0.4
Tomato	83	80	84	82	46	20a	6b	8b	9b	9b
(b) Fraction TE-B										
Concentration (mg/ml)	0	0.003	0.007	0.015	0.03	0	0.003	0.007	0.015	0.03
Tomato	95	85	87	83	83	20	20	14	18	20
(c) Fraction TE-C										
Concentration (mg/ml)	0	0.01	0.027	0.05	0.1	0	0.01	0.027	0.05	0.1
Tomato	95	82	80	78	87	20	20	15	20	20
(d) Fraction TE-D										
Concentration (mg/ml)	0	0.01	0.02	0.04	0.09	0	0.01	0.02	0.04	0.09
Tomato	95	89	83	87	96	20	20	10	10	10
(e) Fraction TE-E										
Concentration (mg/ml)	0	0.1	0.2	0.4	0.8	0	0.1	0.2	0.4	0.8
Tomato	95	76	70	87	88	20	20	20	20	20

(b) Gel-filtration chromatography of Fraction TE-A

The bioactive fraction TE-A was chromatographed on a Sephadex LH 20 coloumn using methanol- dichloromethane (1;1). The resulting seven fractions (code named TEA1 -TEA5) were also bioassayed with tomato seeds (Table 4.13). Bioactivity was found in the fraction TE-A4, which caused severe growth inhibition and stunted growth of tomato seedlings (Plate 4.5).

(c) Chromatographic separation of Fraction TEA-4 on silica

The fraction TEA-4 was next subjected to column chromatography on silica in dichloromethane containing gradiently increasing concentrations of methanol from 0% to 50%. The resulting five fractions code named $TE\text{-}A4_1$ to $TE\text{-}A4_5$) were concentrated and bioassayed (Table 4.14). Whilst most fractions were inhibitory to seed germination, the most significant inhibition of seedling growth resulting in severe stunting was produced by fraction $TE\text{-}A4_4$. $TE\text{-}A4_4$ was obtained as a white, gummy material which gave a single spot on TLC with 20% acetone: hexane. This fraction was taken for further separation.

(d) Further separation of Fraction $TE\text{-}A4_4$ By HPLC

The final purification was carried out by normal phase HPLC separation (silica column, RI detector,) in 1:3 acetone : hexane mixture. The major component which gave a single peak was code named $TE\text{-}A4_{4\text{-}4}$. This and the materials corresponding to the other minor peaks (code named $TE\text{-}A4_{4\text{-}1}$, $TE\text{-}A4_{4\text{-}2}$, $TE\text{-}A4_{4\text{-}3}$ and $TE\text{-}A4_{4\text{-}5}$ were collected separately and bioassayed (Table 4.15). The fraction $TE\text{-}A4_{4\text{-}4}$ showed bioactivity. The compound was a white crystaline solid. m.p. 167°C, α_D^{25} 154 (EtOH): IR max ($CHCl_3$) cm^{-1} 3240 (OH), 1740, 1720.

^1H NMR ($CDCl_3$, 500 MHz): -δ 4.1 (dd, J=7 Hz, H-1), 2.2 (m, $H\text{-}2_a$), 1.9 (m, $H\text{-}2_b$), 2.12 (m, H-4), 1.9 (m, H-5a), 1.65 (m, H-5b),4.5 (m, H-6), 3.9 (m, H-7), 5.5 (m, H-8), 1.65 (m, H-9a), 1.95 (m, H-9b),6.2 (d, J=1.5 Hz, H-13a), 5.4 (d, J=1.5 Hz, H-13b), 1.5 (s, H_3-14), 1.0 (d, J=7 Hz, H_3-15), 2.3 (septlet, J=7 Hz, H-2), 1.03 (d, J=7 Hz, H-3), 1.01 (d, J=7 Hz, H-4).

^{13}C-NMR ($CDCl_3$), 100 MHz):-δ 78.5 (C-1), 46.75 (C-2), 105.6 (C-3), 44.4 (C-4), 37.69 (C-5), 81.9 (C-6), 47.73 (C-7), 69.9 (C-8), 34.5 (C-9), 81.58 (C-10), 137.2 (C-11), 169.3 (C-12), 121.8 (C-13), 24.79 (C-14), 19.2 (C-15), 176 (C-1), 34.05 (C-2), 18.7 (C-3), 18.6 (C-4)

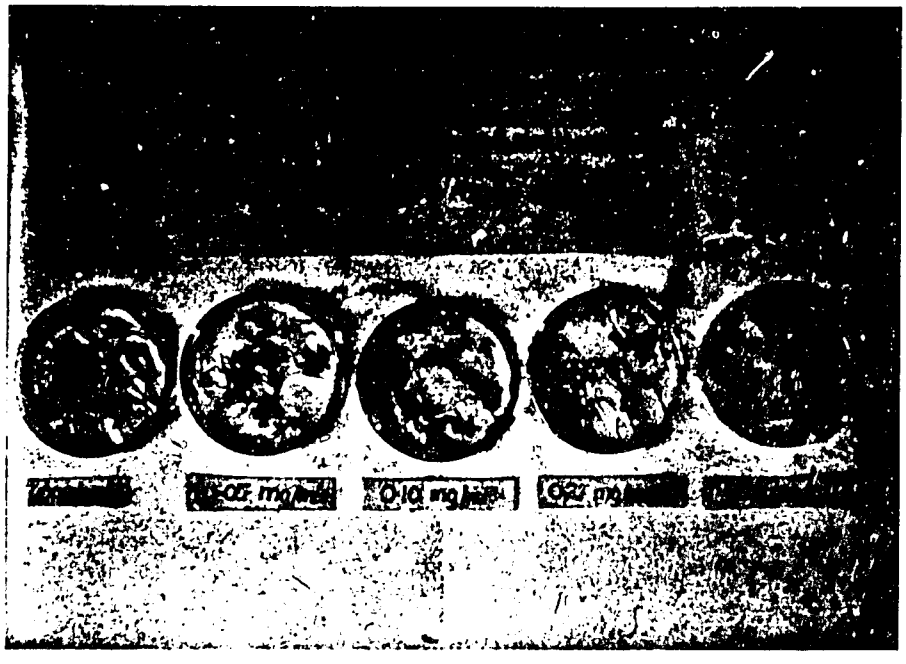

Plate 4.5: Activity of bioactive fraction TE-A4 obtained from *Tithonia* dried leaves

Table 4.13: Effect of five Fractions separeted by Gel- filtration Chromatography from the fraction TE-A on the seed germination and seedling growth Tomato

Fraction	Concentration (mg/ml)	% Seed Germination	Mean Fresh Weight (mg)
Control	0.00	89	20a
TE-A1	0.03	87	10b
TE-A2	0.12	79	10b
TE-A3	0.06	84	10b
TE-A4	0.21	76*	6c
TE-A5	0.04	84	10b

* Most seedlings showing severely stunted growth
Germination and seedling fresh weight measured 10 days after treatment.

Table 4.14: Effect of five fractions separated by Silica-column chromatography from the fraction TE-A4 (Ethyl Acetate solubles) on seed germination and seedling growth of tomato

Fraction	Concentration (mg/ml)	% Seed germination	Mean Fresh Weight (mg)
Control	0.00	89	10a
$TE\text{-}A4_1$	0.01	60	10a
$TE\text{-}A4_2$	0.04	60	10a
$TE\text{-}A4_3$	0.16	67	10a
$TE\text{-}A4_4$	0.05	65*	4b
$TE\text{-}A4_5$	0.03	64	10a

Table 4.15: Effect of the active Fraction $TE\text{-}A4_4$ (ethyl acetate solubles) on the seed germination and seedling growth of tomato

Fraction	Concentration (mg/ml)	% Seed germination	Mean Fresh Weight (mg)
Control	0.00	73	10a
$TE\text{-}A4_{4-1}$	0.17	53	8a
$TE\text{-}A4_{4-2}$	0.34	72	9a
$TE\text{-}A4_{4-3}$	0.69	65	7a
$TE\text{-}A4_{4-4}$	1.35	42*	0b

* Seedlings showing severely stunted radicle growth

Table 4.16: Effect of eleven fractions separated by silica- column chromatography of the dichloromethane solubles of Tithonia dried leaves on seed germination and seedling growth of tomato

Fraction	Concentration (mg/ml)	% Seed germination	Mean Fresh Weight (mg)
Control	0.00	89	10a
TM1	0.03	65	5bc
TM2	0.08	60	2c
TM3	0.04	65	4bc
TM4	0.03	66	4bc
TM5*	1.2	23	2c
TM6*	1.6	9	3c
TM7	0.37	48	2c
TM8	1.6	60	4bc
TM9	0.35	65	7b
TM10	0.41	70	6bc
TM11	0.53	72	7b

* Most seedlings showing severely stunted growth
Germination and seedling fresh weight measured 10 days after treatment

4.3.4 Bioactivity of pure compound TE-A4$_{4-4}$

The bioactivity of the pure compound TE-4$_{4-4}$ was tested again using the tomato seed germination and seedling growth bioassay. Surface-sterilized tomato seeds were exposed to a range of concentrations (0.17 to 1.38 mg/ml) in the same manner as for the bioassays, previously described. Seed germination was counted and the fresh weight of seedlings taken on day 12. The results (Fig. 4.5a, 4.5b) indicate that the I_{50} based on inhibition of seedling growth was 0·8 mg/ml.

4.3.5 Chromatographic separation of the Dichloromethane fraction

The flow-diagram (Fig. 4.3) describes the procedure followed.

(a) Column chromatographic separation on silica gel

The dichloromethane solubles were subjected to column chromatography on silica gel in dichloromethane, containing gradiently increasing concentrations of methanol from 0% to 50%. Eleven fractions were collected (code named TM1 to TM11) and bioassayed with tomato (Table 4.16). The most bioactive fractions were TM5 and TM6, which inhibited the seed germination and seedling growth of tomato very severely. Both TM5 and TM6 had similar TLC characteristics. Since the yield of TM5 (Plate 4.7) was considerably more than that of TM6, the former was considered for further separation. Other fractions were not inhibitory (Plates 4.6, 4.7, 4.8, 4.9, 4.10).

(b)Column chromatography of Fraction TM5 on silica gel

Further separation of fraction TM5 was carried out by column chromatography on silica gel in hexane with gradiently increasing concentrations of acetone (0% to 100%). The resulting four fractions (TM5-1 to TM5-4), were concentrated and bioassyed (Table 4.17). Fraction TM5-2 was found to be very potent in causing severe stunted growth of tomato seedlings.

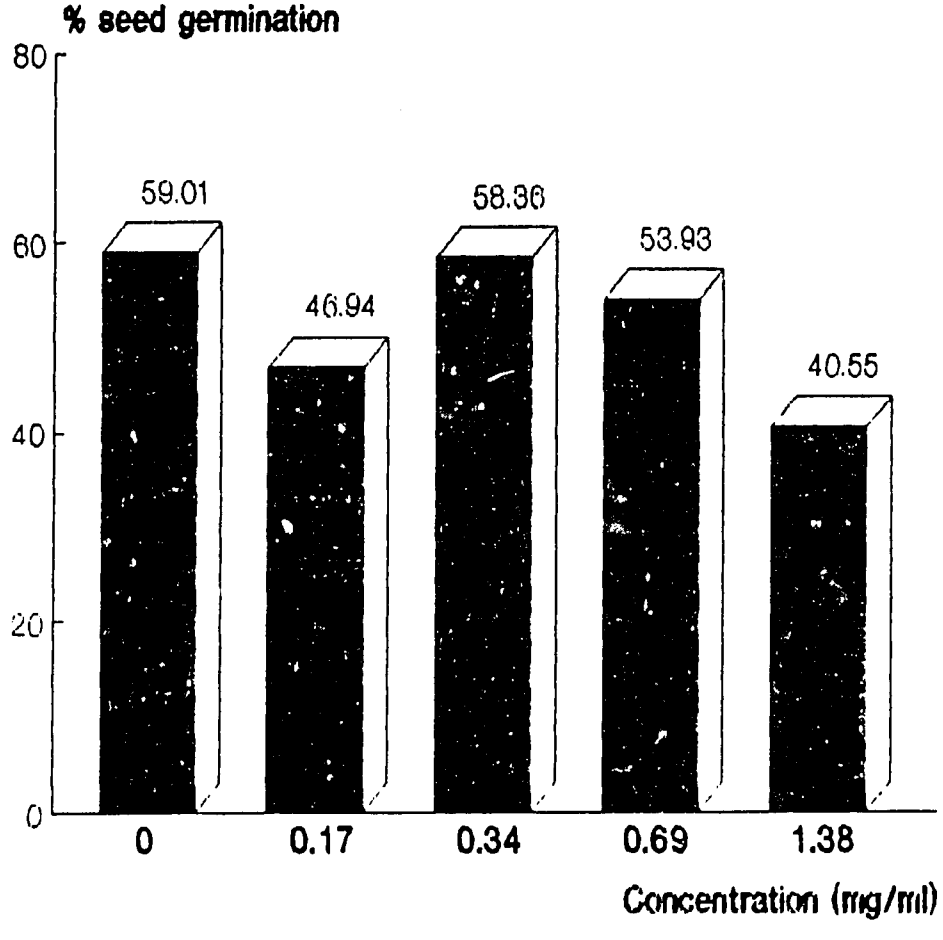

Figure 4.5a: The effect of a range of concentrations of TE-A_{44-4} (Tagitinin A) on seed germination of Tomato 12 days after treatment

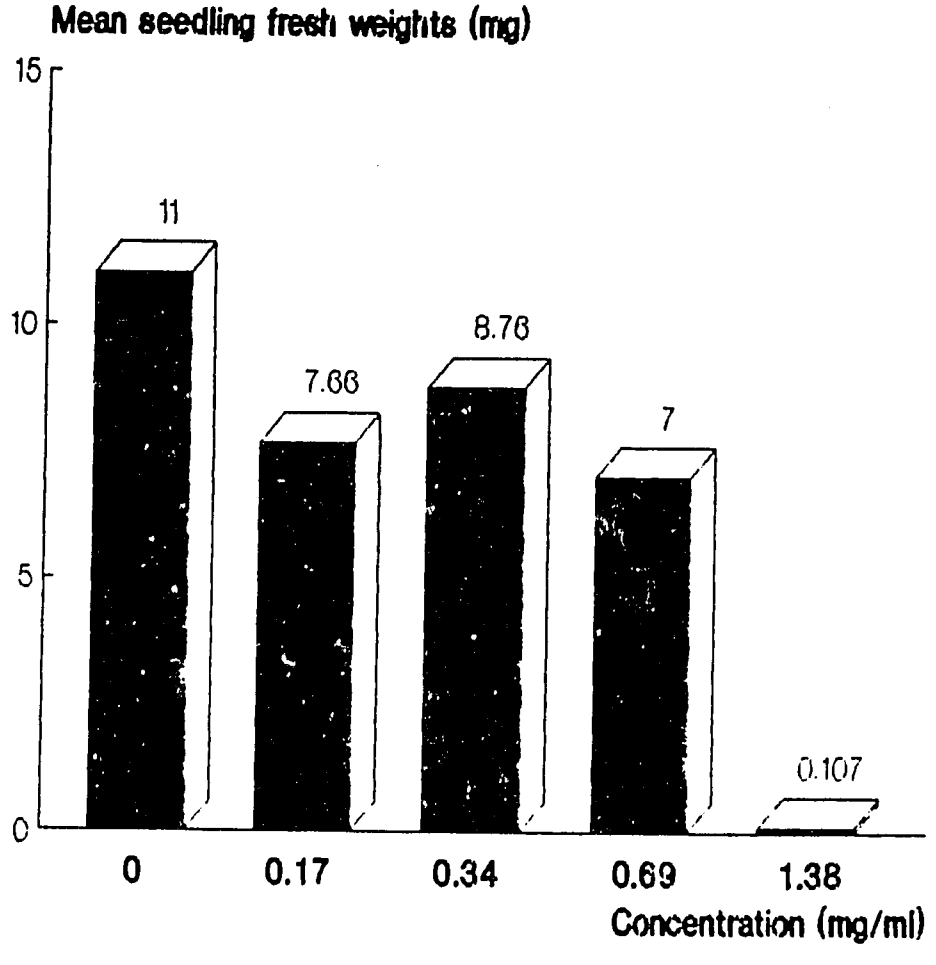

Figure 4.5b: The effect of a range of concentrations of TE- A_{44-4} (Tagitinin A) on seedling growth of Tomato 12 days after treatment

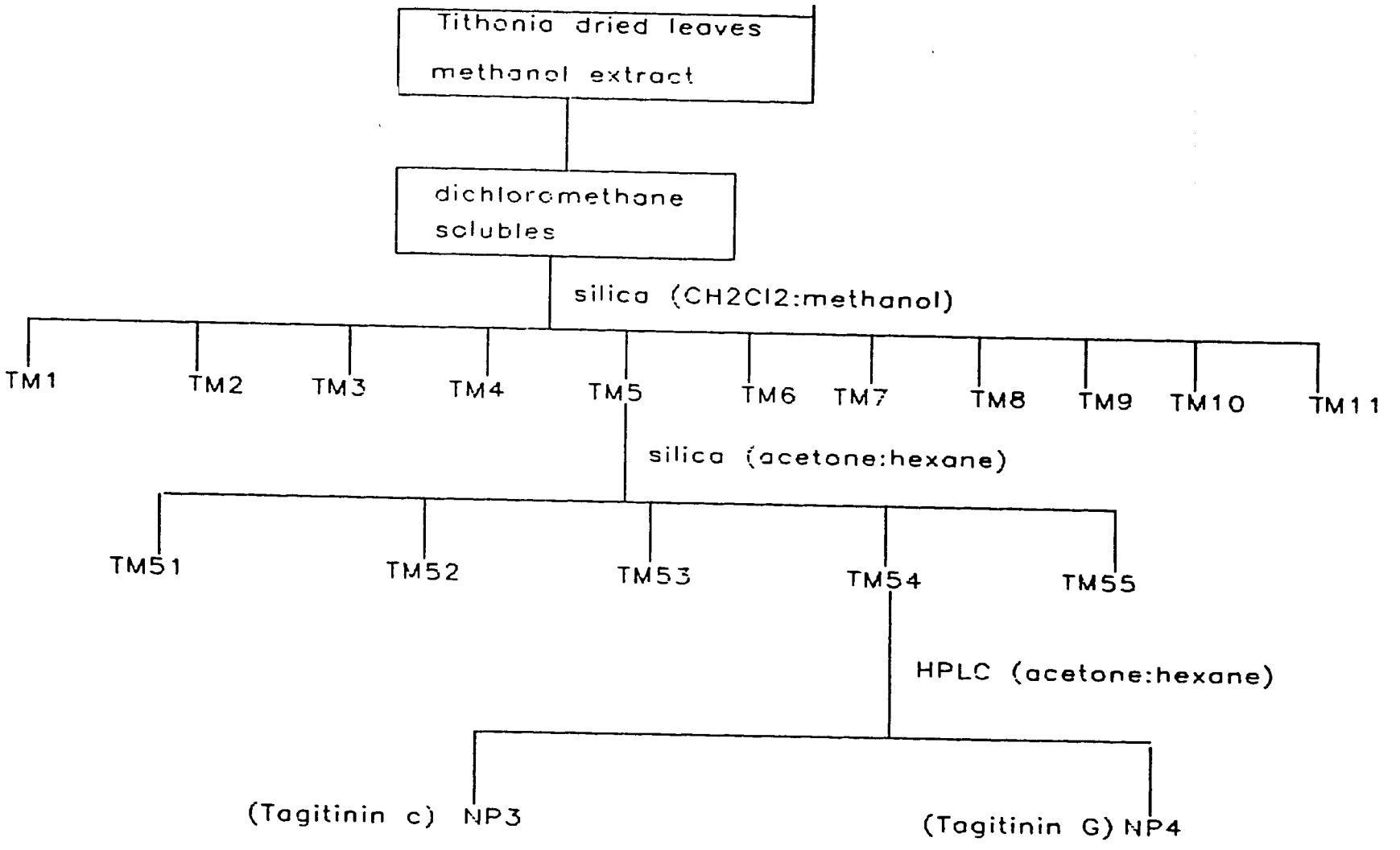

Figure 4.4: Separation procedure for Dichloromethane fraction of Methanolic extract of Tithonia dried leaves

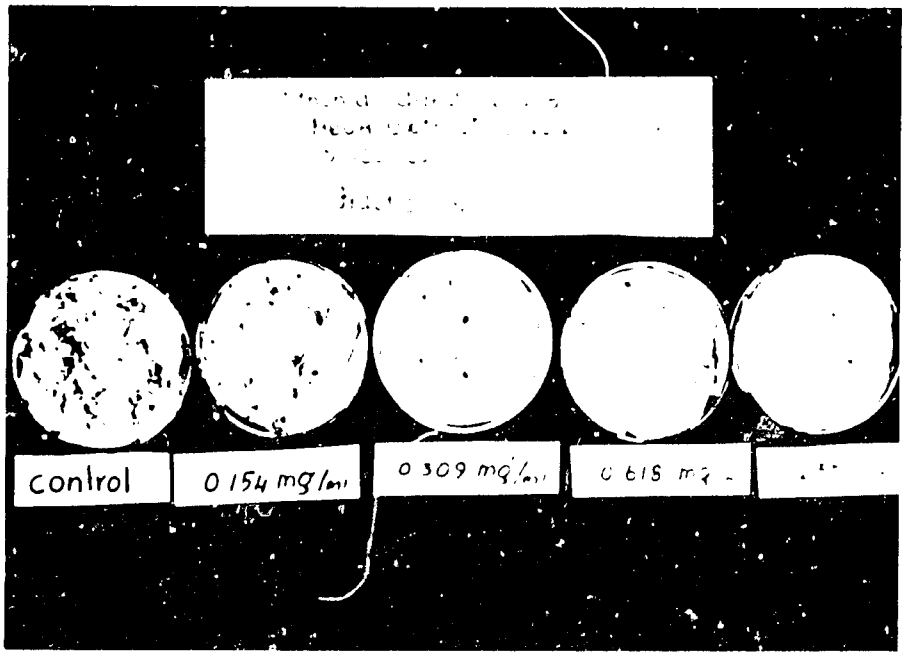

Plate 4.6: Activity of bioactive fraction IM5 obtained from Tithonia dried leaves

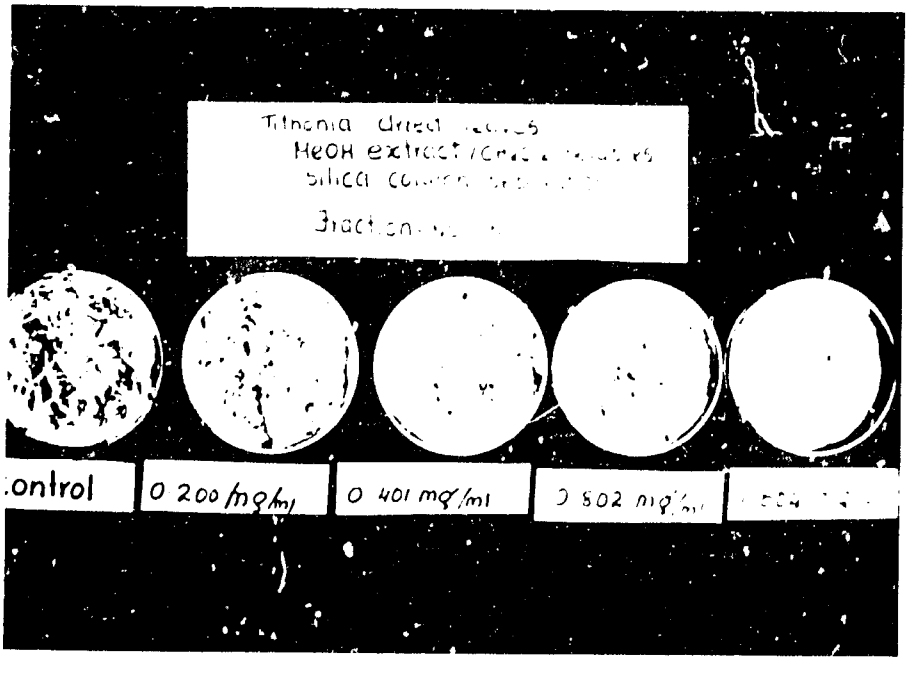

Plate 4.7: Activity of bioactive fraction IM6 obtained from Tithonia dried leaves

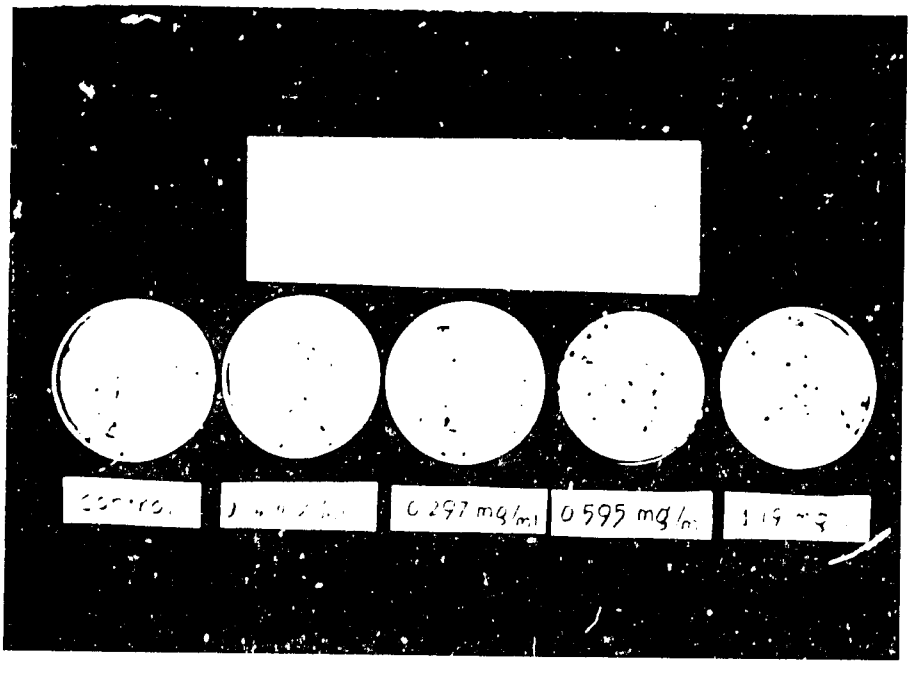

Plate 4.8: Relatively inactive fraction TM1 obtained from Tithonia dried leaves

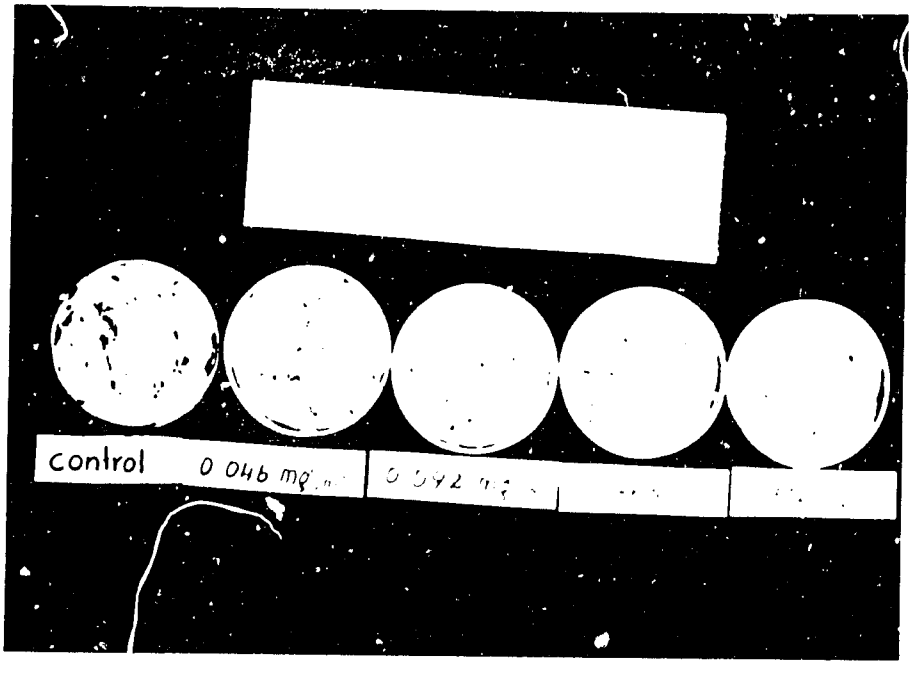

Plate 4.9: Relatively inactive fraction TM7 obtained from Tithonia dried leaves

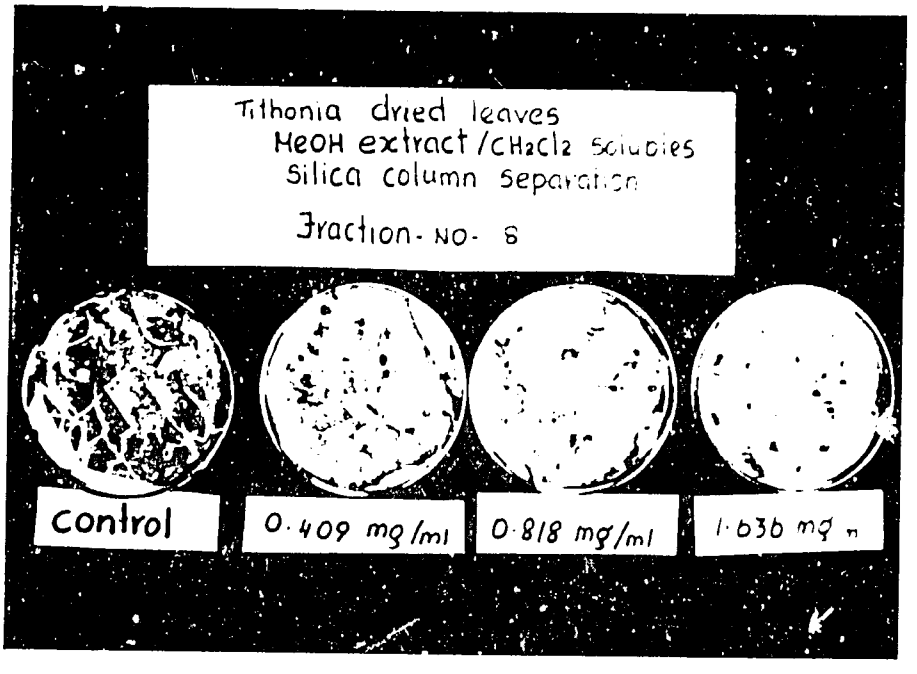

Plate 4.10: Relatively inactive fraction TM8 obtained from Tithonia dried leaves

(c) Purification of the Fraction TM5-2 by HPLC

Purification of the fraction TM5-2 was carried out by normal phase HPLC (silica, RI detector, 1:3 acetone:hexane). Three major fractions were collected and code named NP1, NP3 and NP4. Since the yield of NP1 was much less than NP3 and NP4, only the latter two major fractions were bioassayed (Table 4.18). These showed very strong inhibition of tomato seed germination and seedling growth. As shown on Plate 4.11, both NP3 and NP4 caused a considerable delay in the germination of tomato seeds, compared to controls, which achieved maximum germination (80-100%) within the first 4-6 days. Even after 12 days, seeds treated with the bioactive fractions made very little or severely stunted growth (Plate 4.12).

The compound NP3 was purified by repeated column chromatography and finally by HPLC. It was a gummy material. IR ν max $CHCl_3$, cm^{-1} 3240, 1740, 1720. α_D^{25}=204, EtOH]. EIMS m/z (rel. int. %) 348 (15.81) M^+, 330 (5.04), 305 (6.25), 277 (43.06), 260 (10.78), 242 (50.47) 71 (100).

^1H NMR ($CDCl_3$, 500 MHz): δ 6.89 (d, J=17 Hz, H-1), 6.21 (d, J=17 Hz, H-2), 5.87 (dd, J=11.5 Hz, 1.5 Hz, H-5), 5.36 (dd, J=11.5 Hz, 1.5 Hz, H-6), 3.51 (ddd, J=6 Hz,4.2 Hz, 2.3Hz, H-7), 5.31 (m, H-8), 2.4 (m, H-9a), 2.0 (m, H-9b), 5.78 (d, J=1.8, H-13a), 6.3 (d, J=1.8, H-13b), 1.5 (s, H_3-14), 1.9 (s, H_3-15), 2.4 (septiet, J=7 Hz, H-2'), 1.03 (d, J=7 Hz, H_3-3'), 1.03 (d, J=7 Hz, H-4').

^{13}C-NMR ($CDCl_3$, 100 MHz): δ160 (C-1), 127.9 (C-2), 190.85 (C-3), 138.9 (C-4), 137.1 (C-5), 76.05 (C-6),47.05 (C-7), 73.9 (C-8), 48.4 (C-9), 72.05 (C-10), 136.11 (C-11), 169.0 (C-12), 124.5 (C-13), 29.04 (C-14), 19.65 (C-15), 176.18 (C-1'), 24.05 (C-2'), 18.62 (C-3'), 18.7 (C-4').

The compound NP4 was also a white gummy material, which gave a rf value of 0.45 in TLC (silica, 1:3 acetone: hexane) and appeared as a yellow spot when sprayed with Vanillin- H_2SO_4. α_D^{25}= 25 (in EtOH).
IR ν max $CHCl_3$, cm^{-1} 1750, 1650, 3450 (OH). EIMS m/z (rel. int.) 364 (0.53), 293 (0.64), 248 (1.64), 71 (100).

^1H NMR ($CDCl_3$, 500 MHz): δ 3.6 (d, J= 2.35 Hz, H-1), 3.2 (d, J= 2.35 Hz, H-2), 6.34 (d, J=1.5 Hz, H-5), 5.6 (br.s, H-6), 3.25 (br.s, H-7), 5.3 (m, H-8), 1.94 (m, H-9a), 2.07 (dd, J= 4.2 Hz, 4.2 Hz, H-9b), 6.32 (d, J= 1.5 Hz, H-13a), 5.82 (d, J= 1.5 Hz, H-13b), 1.92 (m, H-14), 1.25 (s, H-15), 2.45 (d, J= 7, H-2'), 1.08 (d, J= 7 Hz, H_3-3'), 1.08 (d, J= 7 Hz, H_3- H4').

Table 4.17: Effects of four fractions separated by silica- column chromatography of fraction TM5 on seed germination and seedling growth of tomato

Fraction	Concentration (mg/ml)	% Seed germination	Mean Fresh Weight (mg)
Control	0.00	80	13a
TM5-1	0.73	80	6b
TM5-2*	0.20	42	0c
TM5-3	0.11	87	8b
TM5-4	0.14	87	10ab

* Most seedlings showing severely stunted growth
germination and seedling fresh weight measured 10 days after treatment.

Table 4.18: Effects of fractions obtained by HPLC of TM5-2 on seed germination and seedling growth of tomato

Fraction	Concentration (mg/ml)	% Seed germination	Mean Fresh Weight (mg)
Control	0.00	80	10a
NP3	0.33	0	0b
NP4	0.25	0	0b

^{13}C-NMR ($CDCl_3$, 100 MHz): δ 58.5 (C-1), 65.0 (C-2), 193.0 (C-3), 137.1 (C-4), 141.0 (C-5), 73.6 (C-6), 49.7 (C-7), 75.1 (C-8), 42.0 (C-9), 70.0 (C-10), 135.6 (C-11), 168.9 (C-12) 125.0 (C-13), 25.9 (C-14), 20.24 (C-15), 176.2 (C-1'), 34.1 (C-2'), 18.6 (C-3'), 18.8 (C-4').

4.3.6 Bioactivity of pure compounds NP3 and NP4

The bioactivity of the pure compounds NP3 and NP4 was also tested using the tomato seed germination and seedling growth bioassay in the same way as for the previous compounds. Tomato seeds were exposed to a range of concentrations (0.046 to 0.37 mg/ml for NP3 and 0.001 to 0.012 mg/ml for NP4) in the same manner as for the bioassays, previously described. Seed germination was counted and the fresh weight of seedlings taken on day 12. The results (Fig. 4.6a, 4.6b) indicate that the I_{50} for NP3, based on inhibition of seedling growth was 0.135 mg/ml. It was evident that the compound caused a severe inhibition of seed germination and also a severe stunting of growth after the radicle had emerged.

The results of NP4 (Fig. 4.7a, 4.7b) indicated that the I_{50}, based on inhibition of seedling growth was 0.007 mg/ml. As with the bioactive compound NP3, the major effect of NP4 was also severe stunting of seedling growth after emergence of the radicle.

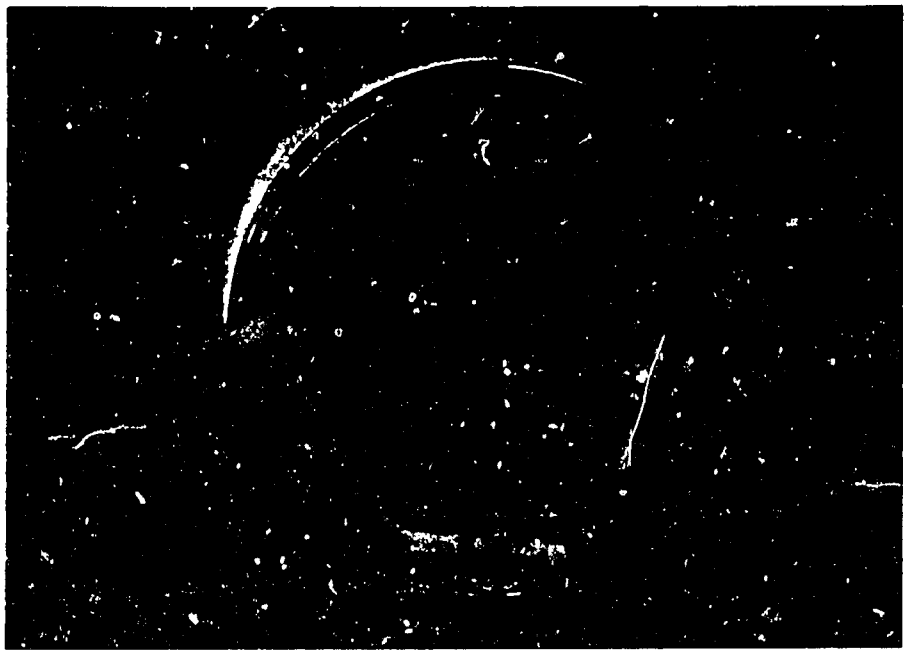

Plate 4.11: Activity typical of NP3-tomato seeds showing delayed germination (5 days after treatment)

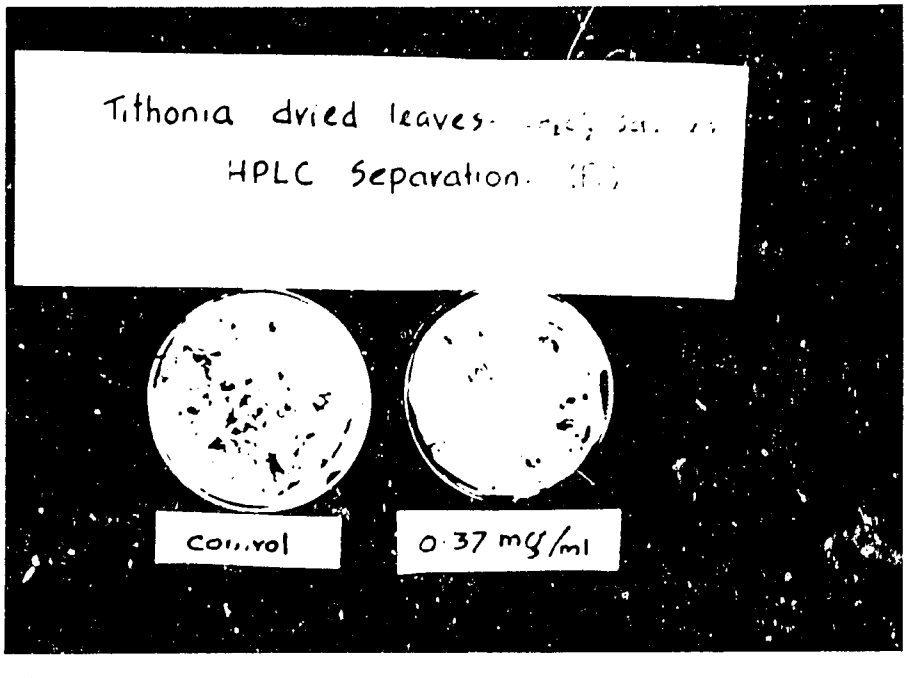

Plate 4.12: Activity typical of NP3 or NP4- tomato seeds 12 days after treatment showing severely stunted growth

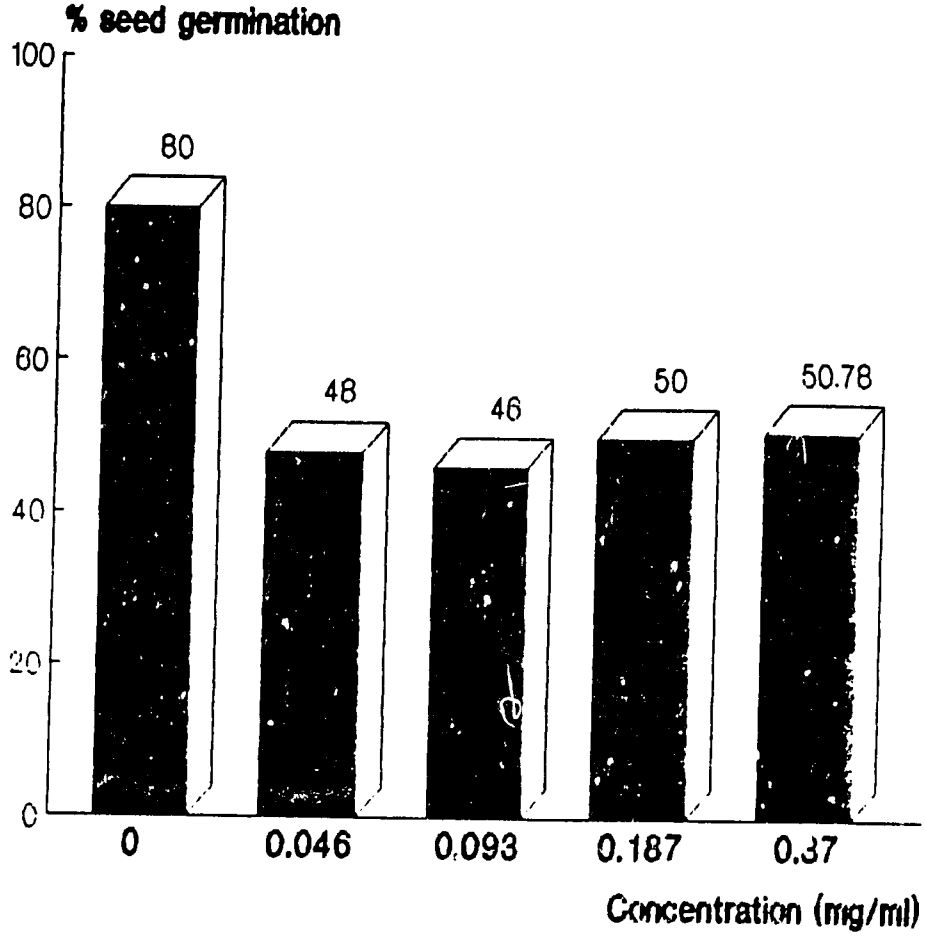

Figure 4.6a: The effect of a range of concentrations of NP_3 (Tagitinin C) on seed germination of Tomato days after treatment

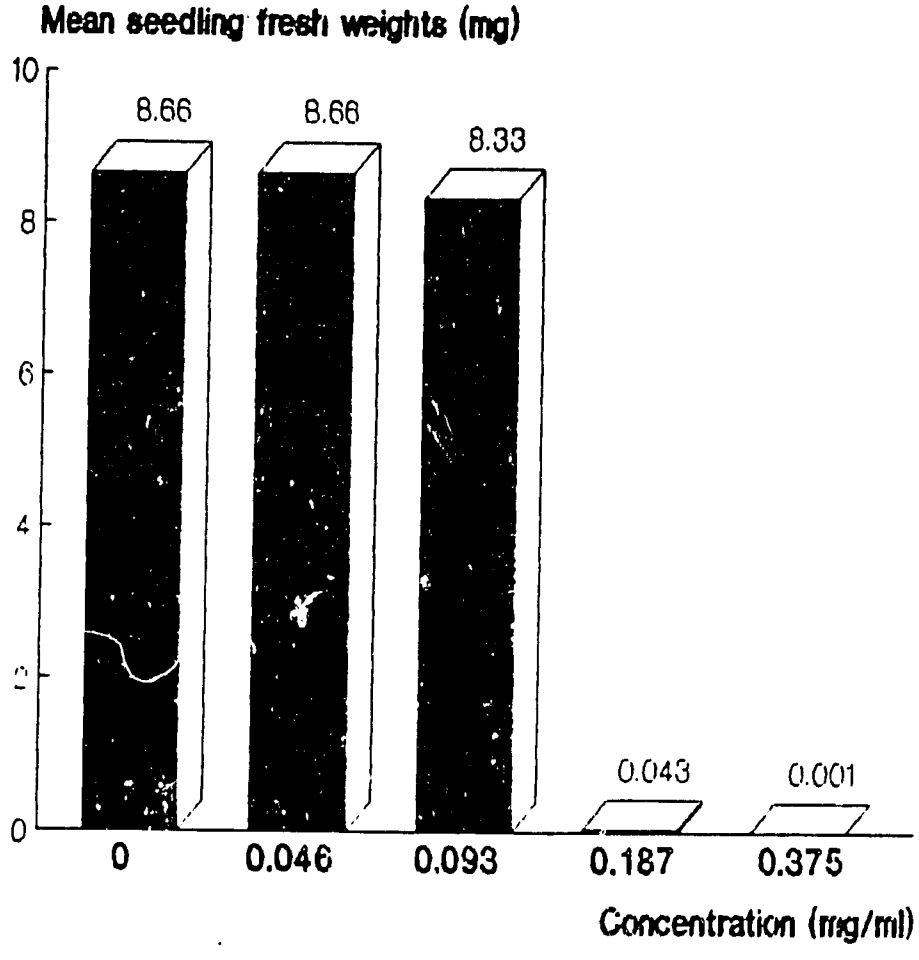

Figure 4.6b: The effect of a range of concentrations of NP_3 (Tagitinin C) on seedling growth of Tomato days after treatment

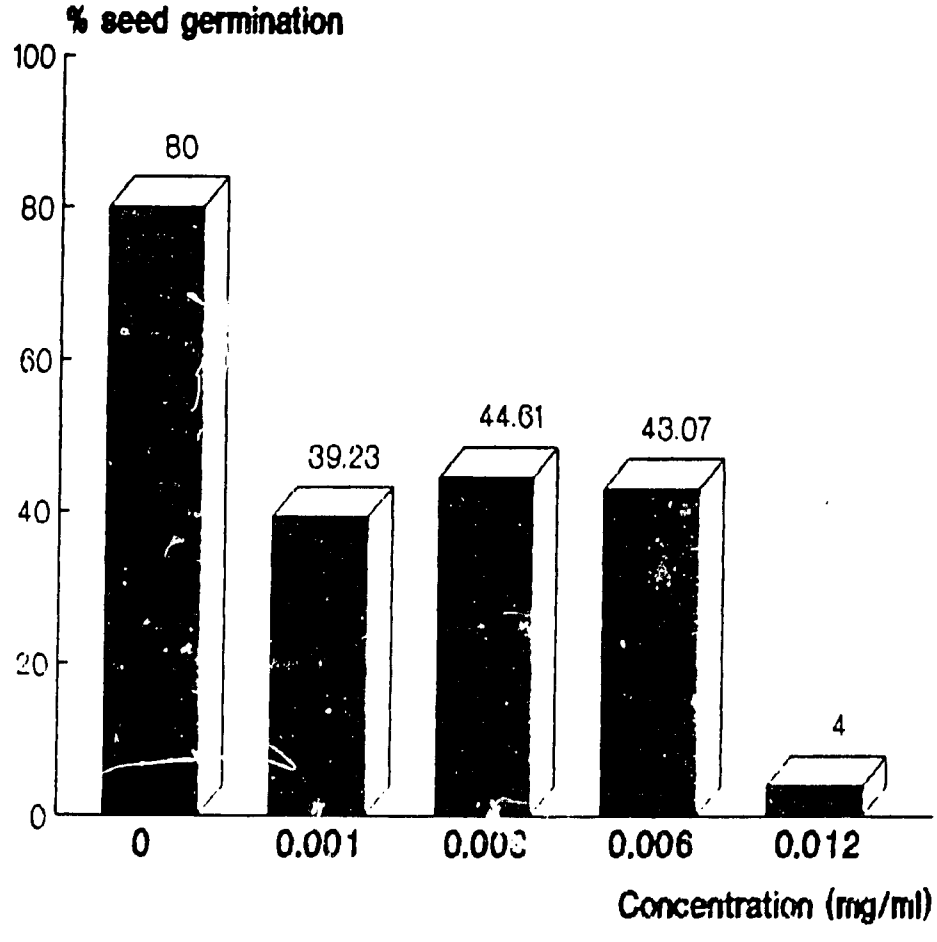

Figure 4.7a: The effect of a range of concentrations of NP_4 (Tagitinin G) on seed germination of Tomato 12 days after treatment

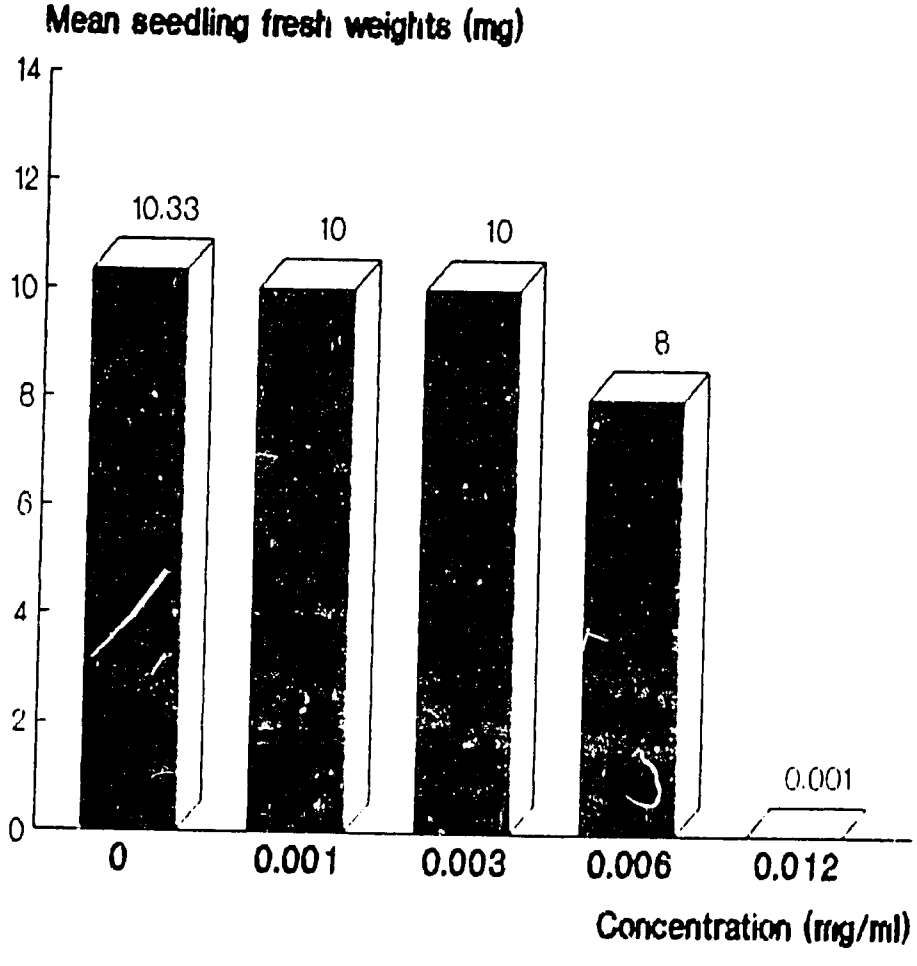

Figure 4.7b: The effect of a range of concentrations of NP_4 (Tagitinin G) on seedling growth of Tomato 12 days after treatment

CHAPTER 5

STRUCTURE ELUCIDATION OF ALLELOCHEMICALS FROM GLIRICIDIA AND TITHONIA

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5.2.3 Structure elucidation of NP_4 as Tagitinin G

5.1 DETERMINATION OF THE STRUCTURE OF THE ACTIVE COMPOUNDS IN GLIRICIDIA

5.1.1 Coumarin from Gliricidia fresh leaves

The active fractions (Fractions AA1 and 4B1) obtained as described previously in Chapter 4 (Section 4.2.1), were purified by recrystallisation from water-methanol; The compound melted at 68 ^{0}C. The ^{1}H- NMR, 500 MHz ($CDCl_3$) spectrum (Fig. 5.1) of the compound showed the presence of two mutually coupled doublets corresponding to 1 H each centered at 6.01 ppm (J=9.5) and 7.46 ppm (J=9.5) and two multiplets, corresponding to 2 H each centered at 7.2 ppm and 6.95 ppm. These values correspond well with those reported for coumarin (Murray et al, 1982). The EI (Electron Impact) Mass Spectrum of the coumpound gave the molecular ion peak (Fig. 5.2) as 146 m/z as the base peak. The molecular ion peak suggested the compound to be simple coumarin (Fig. 5.2) It was further confirmed by melting point determination and co-TLC studies.

5.1.2 Structure elucidation of the active compound in fraction GDM3-D2iiB from Gliricidia dried leaves, as 3-β ,22-α , 23-trihydroxyolean-12-ene (22α-soyasapogenol)

TLC analysis of the compound (in 1:3 Acetone:hexane solvent system) GDM3-D2iiB obtained from the dichloromethane fraction of the Gliricidia dried leaf extract (Section 4.2.2), gave a dark yellow spot (Rf 0.32) with vanillin- H_2SO_4.

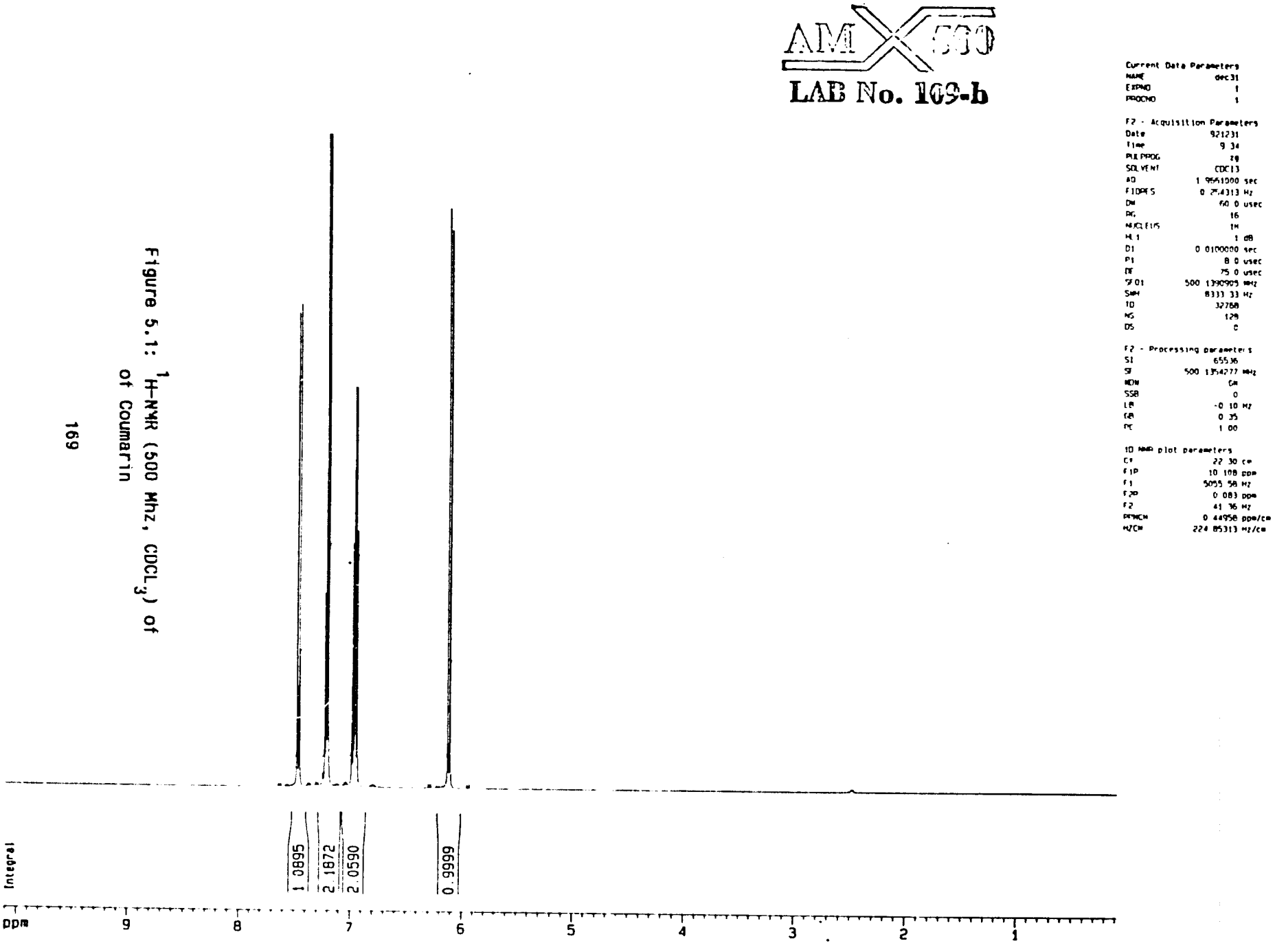


Figure 5.1: ^{1}H-NMR (500 Mhz, $CDCL_3$) of of Coumarin

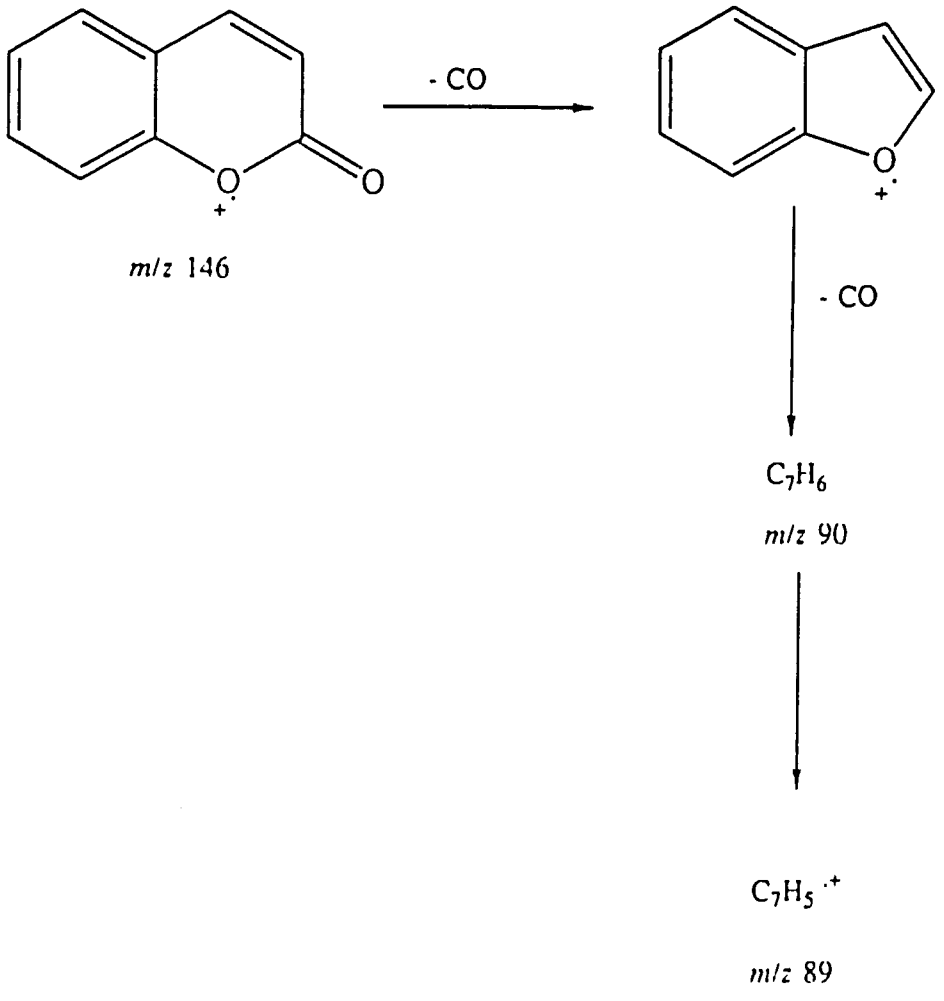


Figure 5.2: Mass fragmentation of Coumarin

The information collected from EI, High Resolution and FD mass spectra suggested the molecular formula of the compound to be $C_{30}H_{50}O_3$, indicating six sites of unsaturation. The key evidence for the structure of the compound was derived from its mass spectrum, which showed major fragment ions at m/z 234 and 224, suggesting a retro-Deils- Alder fragmentation (Fig. 5.3), of an olean-12-ene triterpene bearing two oxygen atoms on the A-B ring fragment (Budzikiewicz, et. al, 1964).

The Broad Band decoupled spectrum of the compound (Fig. 5.4) showed 30 distinct resonances attributable to seven methyl carbons, ten methylene carbons, six methine carbons and seven non protonated carbon atoms. The IR (CHCl3) spectrum of the compound showed the presence of hydroxyl groups with absorption bands at 3400 cm^{-1} and 3500 cm^{-1}. The carbon atoms which resonated at 122.3 ppm and 144.4 ppm were strongly indicative of a compound in the oleanane series (Tori et al, 1974). The basic skeleton of the compound, as an oleanane was drived on the basis of its mass spectrum and ^{13}C- data. The ^{13}C- NMR spectrum of the compound showed three oxygenated carbon atoms (Fig. 5.4). Two were methine carbons (80.1 ppm, 76.4 ppm), and one was methylene (64.5 ppm).

The position of the primary hydroxyl group was confirmed by the mass spectra on the basis of the loss of CH_2OH (31) unit from the 223 fragment. Further, the mass spectral data suggested that two hydroxyl groups should be linked to the A-B ring system. One hydroxyl group was assumed to be linked to C-3 carbon on biogenetic consideration. ^1H- NMR (500 MHz) spectra in C5D5N and CDCl3 (Fig. 5.5a, 5.5b) showed at least 20 different chemical environments in the molecule. In the spectrum recorded in $CDCl_3$ an olefinic proton triplet at 5.3 ppm. (J= 3.5 Hz) was very characteristic of H-12 of an oleanane type structure. An AB type double doublet at 4.2 ppm and 3.2 ppm indicated the geminal protons of the methylene group of the primary hydroxyl group. Two overlapping doublets of doublets corresponding to H-3 and H-22 appeared at 3.4 ppm. However, they appeared well separated at δ 3.63 (J= 10 Hz, 4 Hz) and δ 3.72 (J= 5.3 Hz) in the spectrum recorded in C_5H_5N. Seven 3 proton singlets in the upfield region of the H-NMR spectrum indicated of the presence of seven tertiary methyl groups in the molecule.

The hete onuclear shift correlation (Hetero-COSY) studies of the compound (Rahman et al, 1991; Rahman, 1986; 1989), gave direct carbon-hydrogen connectivities. The position of the protons which resonated at 3.63 ppm and 3.72 ppm (5 Hz, 3 Hz) were fixed to carbon atom number three (C-3) and twenty two (C-22) according to the HMQC spectrum recorded in in C_5D_5N. It was further confirmed by the coupling constant of the doublet which appeared at 1.22 ppm (J= 5 Hz). The hydroxyl group which belongs to the DE-ring system was fixed to C-22 on the basis of the chemical shift

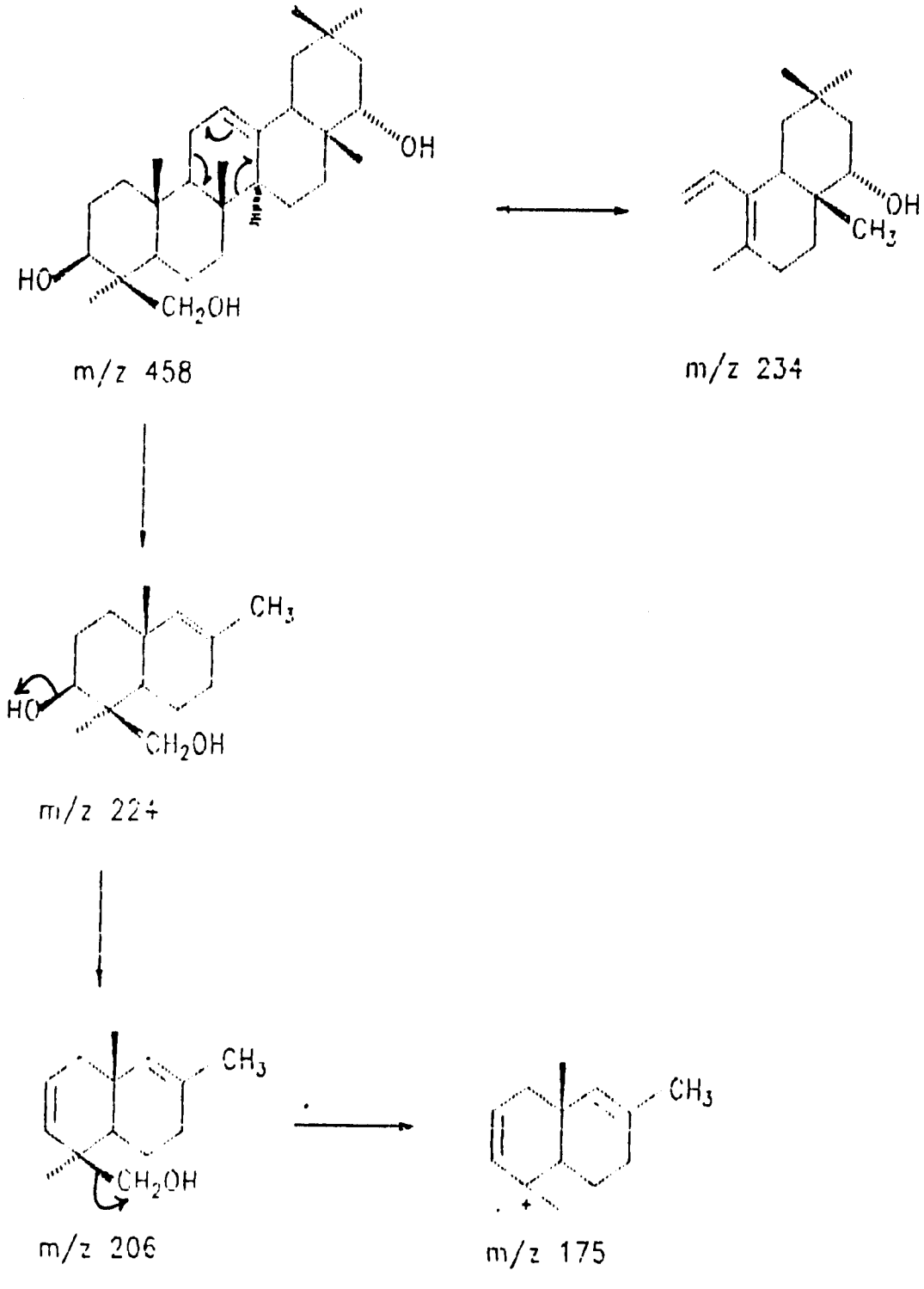


Figure 5.3: Mass fragmentation of Soyasapogenol

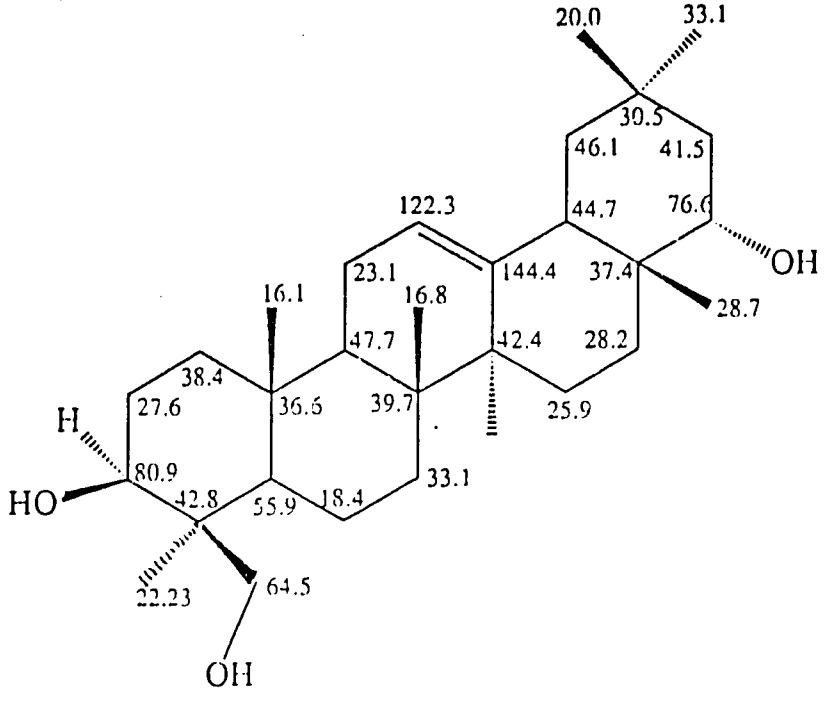


Figure 5.4: ^{13}C-NMR Chemical Shift assignments of 3β, 22α, 23-trihydroxyoleane-12-ene (22α-Soyasapogenol)

values of C-22 and H-22 (80.1 ppm, 3.72 ppm) (Fig. 5.6). The protons of the methylene group of the primary hydroxyl group (3.4 ppm, 4.3 ppm) interacted with C-3 which resonated at 80.1 ppm. Hence, the group $-CH_2OH$ was fixed to C-4 in the ring A.

The stereochemistry of the hydroxyl group at C-3 was β due to the axial-axial and axial-equatorial coupling of H-3 with H-2a and H-2b, respectively (J = 11.5 Hz, 3 Hz). The stereochemistry of the hydroxyl group at C-22 was α because of the low coupling constants of H-22 with H-21a and H-22b, corresponding to axial-equatorial and equatorial-equatorial couplings (J = 5 Hz, 3 Hz). The NOE experiment of the compound gave clear picture of the steriochemistry of the compound. The β- configuration of the CH_2OH was confirmed on the basis of NOE interactions. The ^{13}C- values of the compound are given in Table 5.1.

Table 5.1: ^{13}C- values of active compound GDM3-D2iiB from <u>Gliricidia maculata</u> dried leaves

CH_3	CH_2	CH	C
16.16	18.4	44.7	36.9
16.88	23.77	47.7	37.4
18.4	25.9	55.9	30.53
20.03	28.21	76.65	39.7
22.43	33.18	80.93	42.5
25.41	41.51	122.37	39.7
32.8	46.19	144.9	---
	64.5		

The vicinal and geminal couplings were established by Homo-COSY 45^0 spectrum of the compound (Fig. 5.7). By comparison of spectral data with those reported in literature (Tori, 1964; Kumar & Seshadri, 1976; Xue et al, 1988; David, 1992; Kinoshita, 1992; Papanov et al, 1992; Sashida et al, 1992; Reher & Budesinsky, 1992; Aliotta et al, 1992; Napoli et al, 1992) and the structure was confirmed as 3-β, 22-α, 23- trihydroxy olean-12-ene, which is a new compound of the soyasapogenol series. The 22 epimer of this compound 3-β, 22-α, 23-trihydroxyolean- 12 -ene (soyasapogenol B) is the closest compound to this reported in the literature (Lynn, 1985).

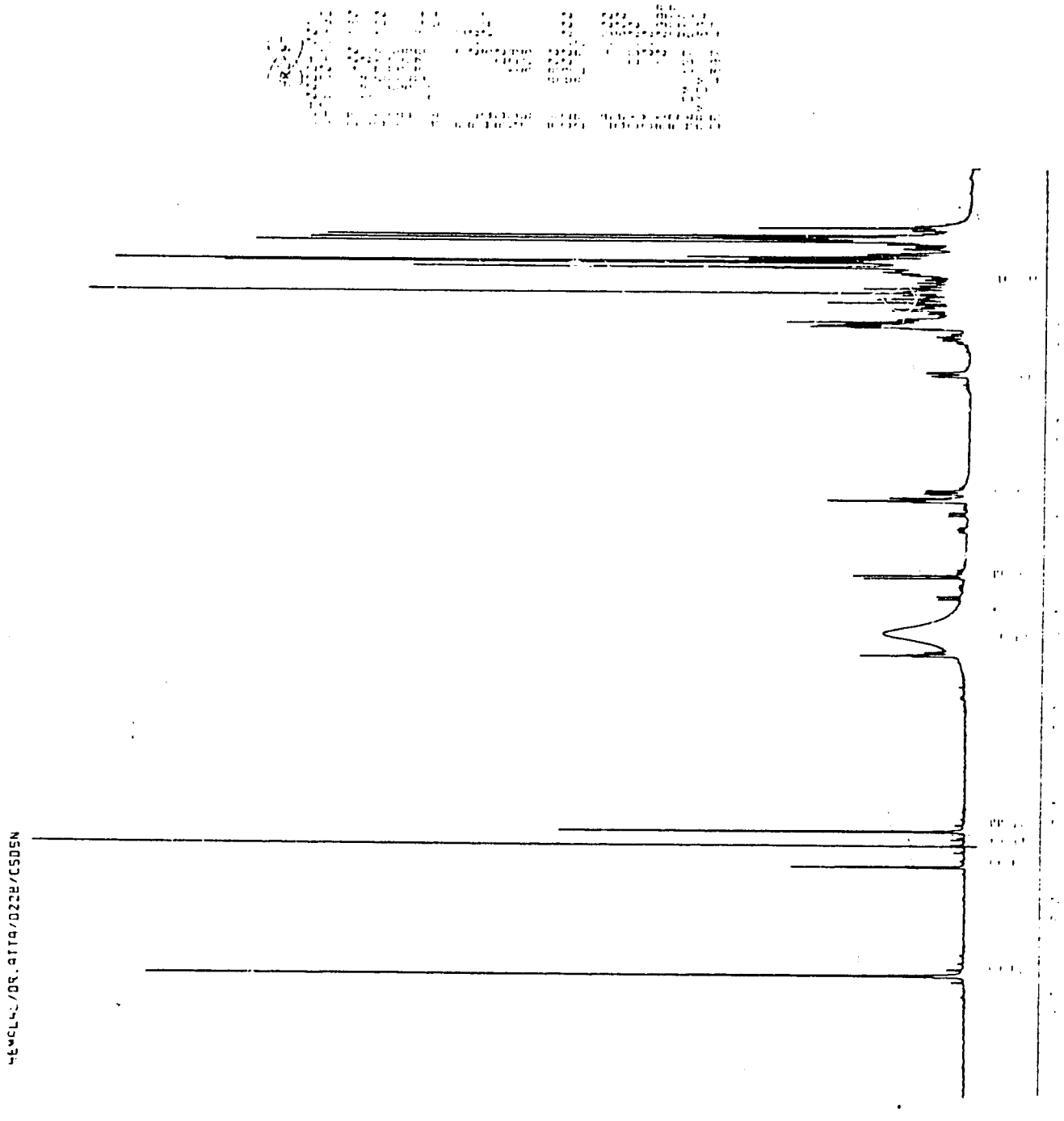
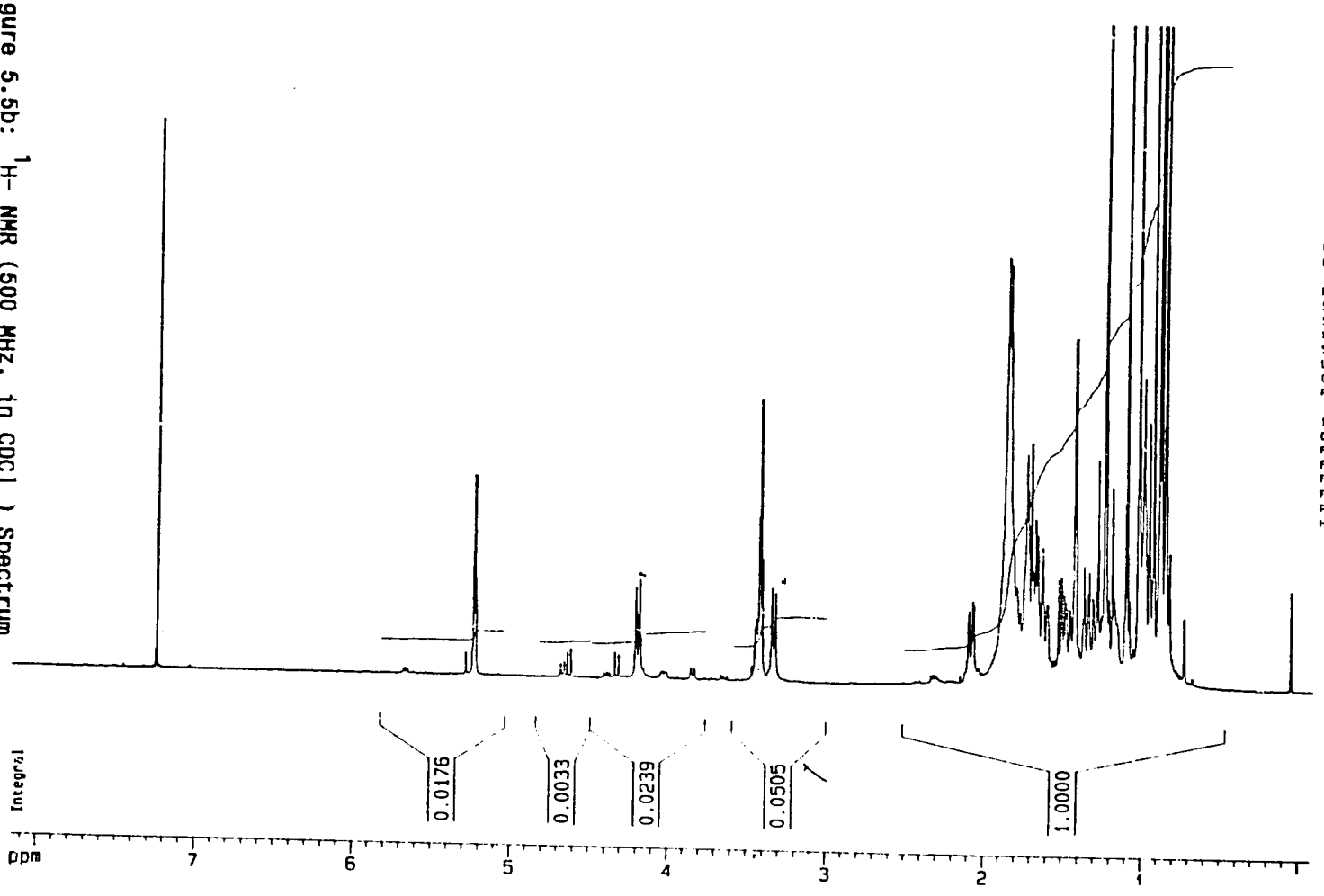


Figure 5.5a: ^{1}H- NMR (500 MHz, in C_5D_5N) Spectrum of GDM3-D2iiB (= 22 -Soyasapogenol)

AMX 500

LAB No. 109-b

HEMALAL/DR.A.REHMAN/D22B/CDCL3



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NL1 1 dB
D1 0 0100000 sec
P1 8.0 usec
DE 75.0 usec
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SWH 8333 33 Hz
TD 32768
NS 128
DS 0

F2 - Processing parameters
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SSB 0
LB -0 10 Hz
GB 0 35
PC 1 00

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F1P 8.165 ppm
F1 4083 56 Hz
F2P -0.083 ppm
F2 -41.62 Hz
PPMCM 0 41448 ppm/cm
HZCM 207 29536 Hz/cm

Figure 5.5b: ^{1}H- NMR (500 MHz, in $CDCl_3$) Spectrum of Soyasapogenol

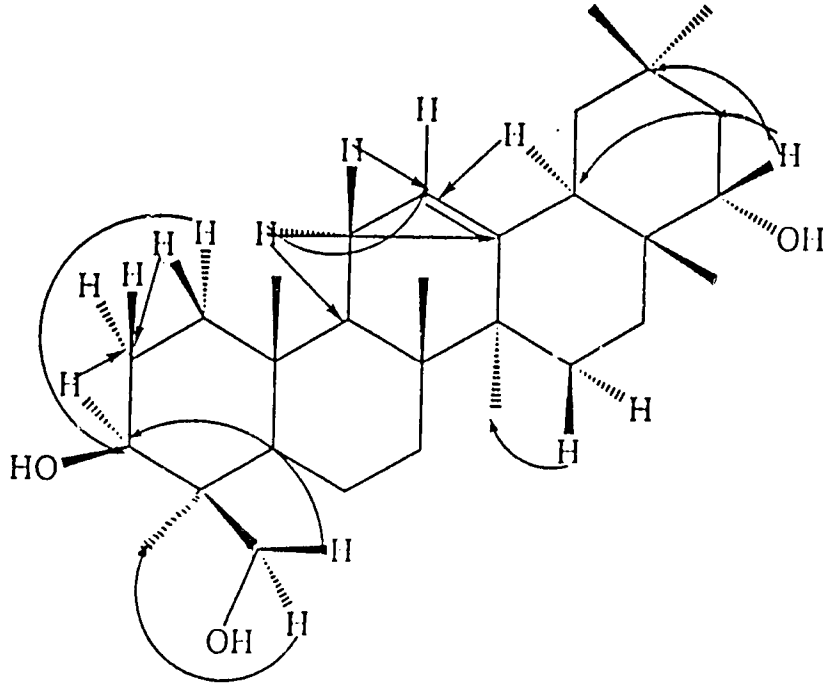


Figure 5.6: HMBC Interactions of 3β, 22α, 23-trihydroxyoleane-12-ene

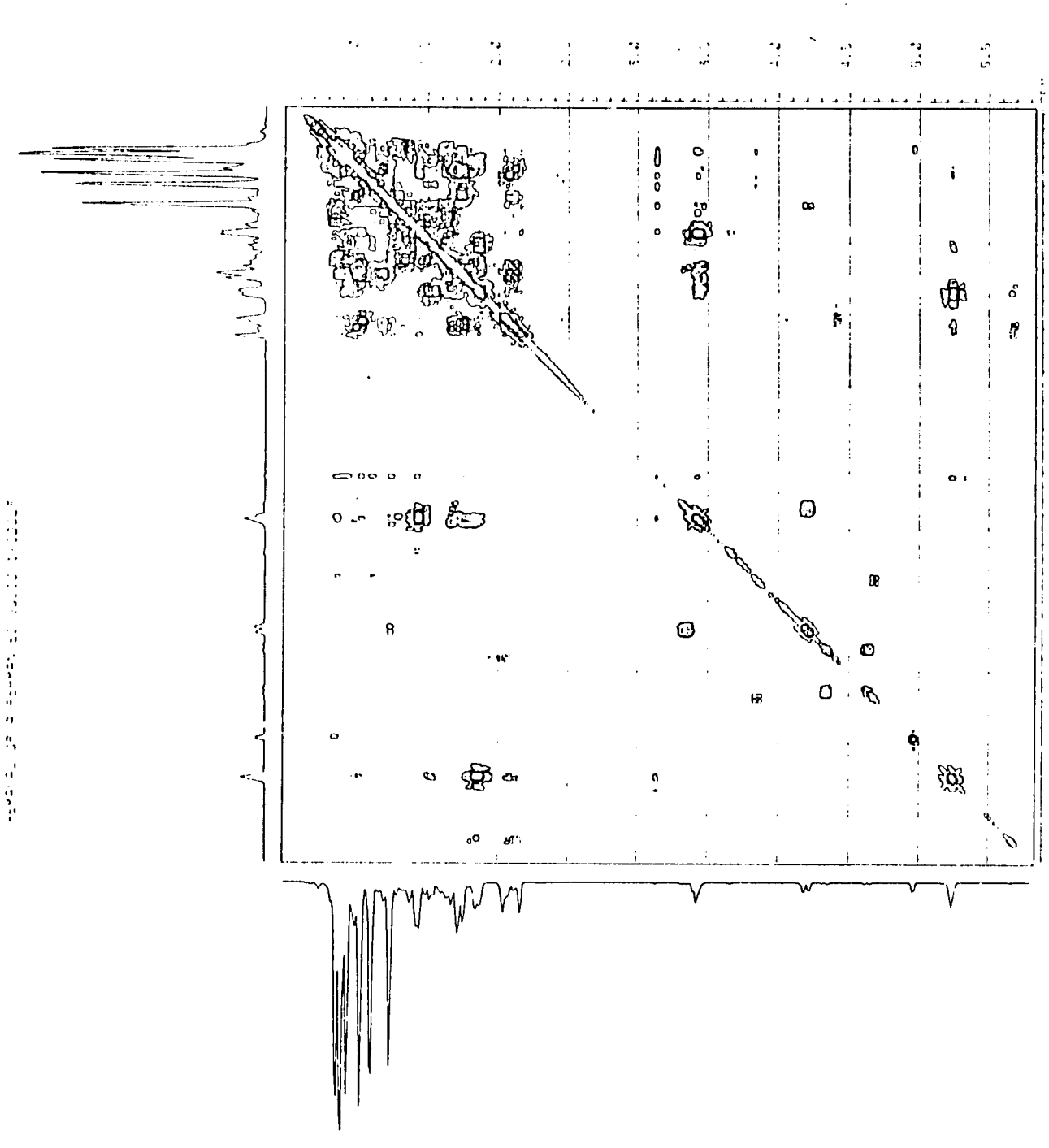


Figure 5.7: Homo-COSY 45^{0} Spectrum of GDM3-D211B
(= 22 -Soyasapogenol)

5.2 DETERMINATION OF THE STRUCTURE OF THE ACTIVE COMPOUNDS IN TITHONIA

5.2.1 Structure elucidation of TE-A4$_{4-4}$ as 12 carboxy-1,3,6- trihydroxy-3, 10- epoxy- 8 -isobutyryloxy germacra- 11 (13) ene γ -lactone (Tagitinin A)

The Fraction TE-A4$_{4-4}$, which was isolated from the ethyl acetate fraction of Tithonia, (Section 4.3.1) gave large diamond shaped cystals from benzene:ethyl acetate (1:4). On TLC in acetone: hexane (1:3), TE-A$_{44-4}$ produced a dark yellow spot (Rf 0.5) when sprayed with Vanillin- H_2SO_4.

The coumpound melted at 167^{0}C and showed a specific rotation of 154^0 in ethanol. High Resolution Mass Spectrometry gave the molecular formula C_{19} H_{28} O_7, indicating six double bond equivalents, in the molecule. The Molecular ion peak and fragmantation pattern were confirmed by Field Desorbtion (FD) and link scanning mass spectroscopy methods (Budzikiewicz et al, 1964).

The presence of hydroxyl groups, and an ester carbonyl group, was evident from the IR absoption bands at 3429 cm^{-1} and 1720 cm^{-1} respectively. Bands at 1740 cm^{-1} and 920 cm^{-1} indicated the presence of an unsaturated lactone moiety with an exomethylene group. The postulation of the compound TE-A$_{44-4}$ as a sesquiterpene was based on the presence of three isoprene units and the positive answer to the Vanillin -H_2SO_4 test.

The 500 MHz ^{1}H- NMR Spectrum (Fig.5.8) of the compound showed 16 different proton chemical environments in the molecule, as shown in Table 5.2 and Fig. 5.9a. A three proton singlet reasonating at 1.3 ppm was ascribed to methyl group attached to a quatenary carbon atom. The three protons doublet at 1.02 ppm (J = 7 Hz) represented a methyl group attached to a secondary carbon atom. The six proton triplet which appeared at 1.03 ppm (J = 7 Hz) represented the two methyl groups of an isopropyl group. The triplet was formed due to the overlapping of two doublets. The methylene proton of the isoprophyl group appeared as a septet at 2.3 ppm (J= 7Hz).

The ^{13}C Broad Band decoupled spectrum of TE-A4$_{4-4}$ revealed the presence of nineteen carbon atoms in the molecule (Table 5.3; Fig. 5.9b). The multiplicity of each carbon atom was determined by Distortionless Enhancement by Polarization Transfer (DEPT) experiment, which showed the presence of four methyl carbons, five methylene carbons, five methine carbons and hence by difference from the broad band spectrum, five tertiary carbons.

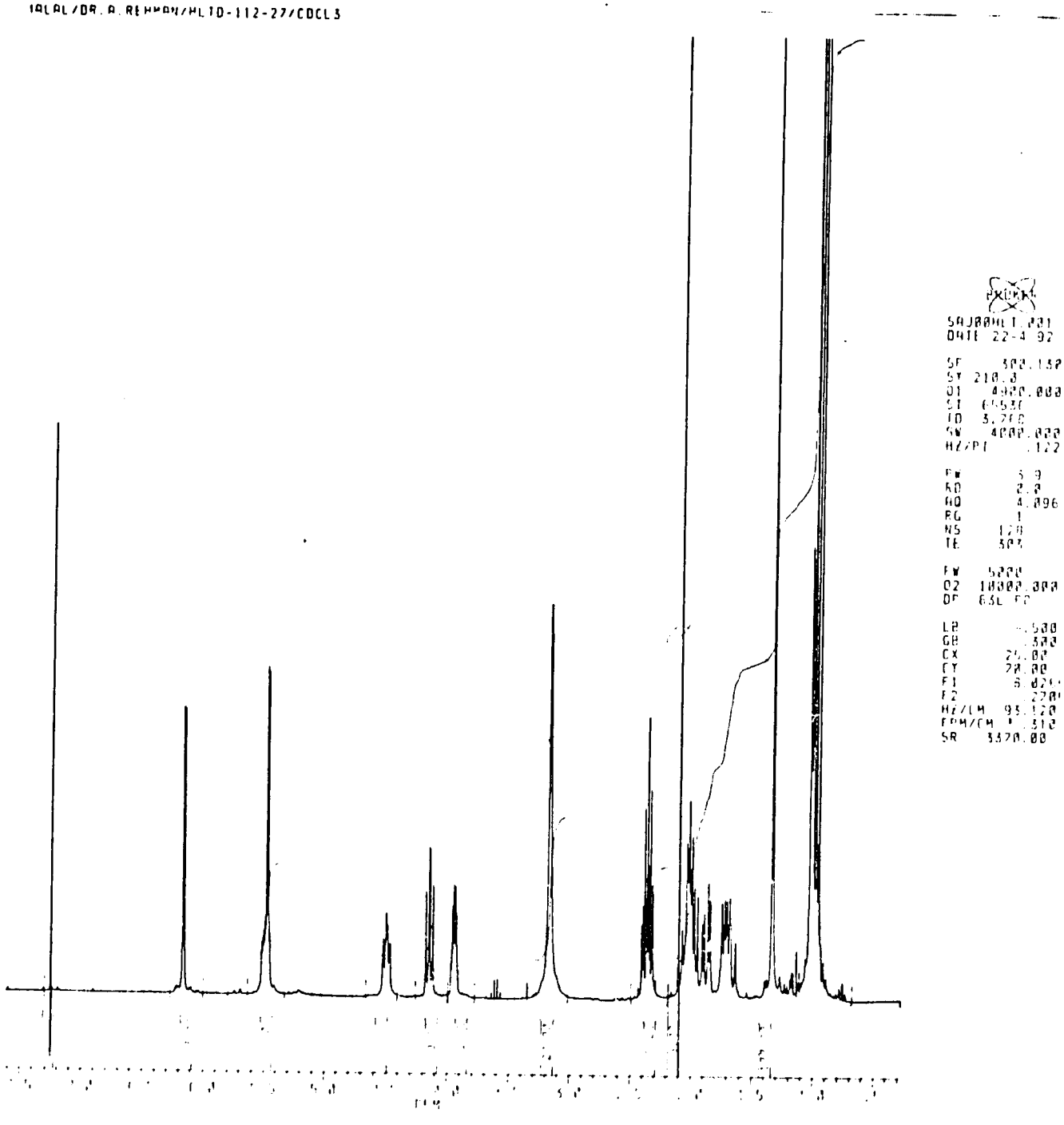


Figure 5.8: ^{1}H- NMR (500 MHz, $CDCl_3$) Spectrum of $TE\text{-}A_{44\text{-}4}$ (= Tagitinin A)

Table 5.2: A comparison of ^{1}H - NMR chemical shifts of compound $TE\text{-}A_{44\text{-}4}$ from Tithonia diversifolia with those previously reported for Tagitinin A (Baruah et al, 1979)

	$TEA4_{4\text{-}4}$	TAGITININ A
H-1	4.1, m	4.23, m
H-2a	2.2, m	2.44, m
H-2b	1.9, m	2.1, m
H-4	2.12, m	2.1, m
H-5a	1.9, m	2.1, m
H-5b	1.65, m	2.1, m
H-6	4.5, ddd	4.55,ddd
H-7	3.9, m	3.99, m
H-8	5.5, ddd	5.59, ddd
H-9a	1.65, dd	1.81, dd (13,8)
H-9b	1.95, dd	1.95, dd (13,5)
H-13a	6.2, d	6.25, d (3,5)
H-13b	5.4, d	5.53, d (3)
H-14b	1.5, br.	1.43, br.
H-15b	1.0	1.11, d (6.51)
H-2'	2.3, m	2.44, m
H-3'	1.03	1.07, d (7)
H-4'	1.01	1.04, d (7)

Table 5.3. A comparision of ^{13}C chemical shift (ppm) of compound $TE\text{-}A_{44\text{-}4}$ from Tithonia diversifolia with those previously reported for Tagitinin A (Baruah et al, 1979)

	TE-A44-4	Tagitinin A
C_1	78.5	78.46
C_2	46.75	46.94
C_3	105.6	105.69
C_4	44.4	44.38
C_5	37.69	37.81
C_6	81.9	81.86
C_7	47.73	47.84
C_8	69.9	69.90
C_9	34.5	34.65
C_{10}	81.58	81.69
C_{11}	137.2	137.01
C_{12}	169.3	169.75
C_{13}	121.8	121.73
C_{14}	24.79	24.96
C_{15}	19.2	19.18
C^1	176.0	176.45
C^2	34.05	34.11
C^3	18.7	18.76
C^4	18.6	18.42

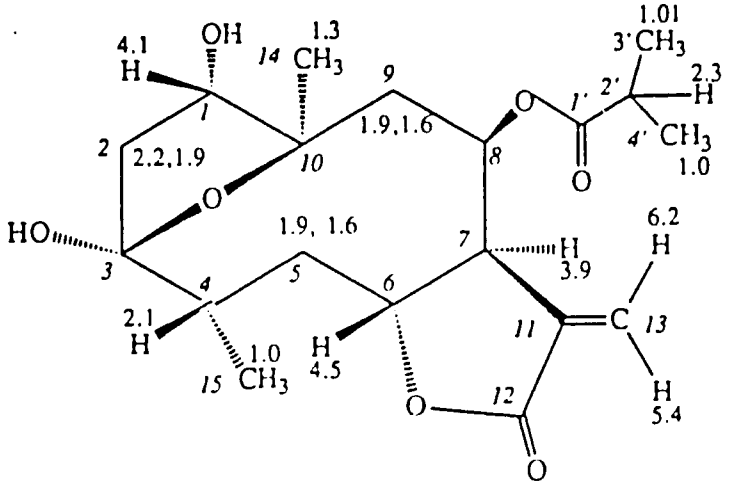


Figure 5.9a: ^{1}H-NMR Chemical shift assignments of TE-A4$_{4-4}$

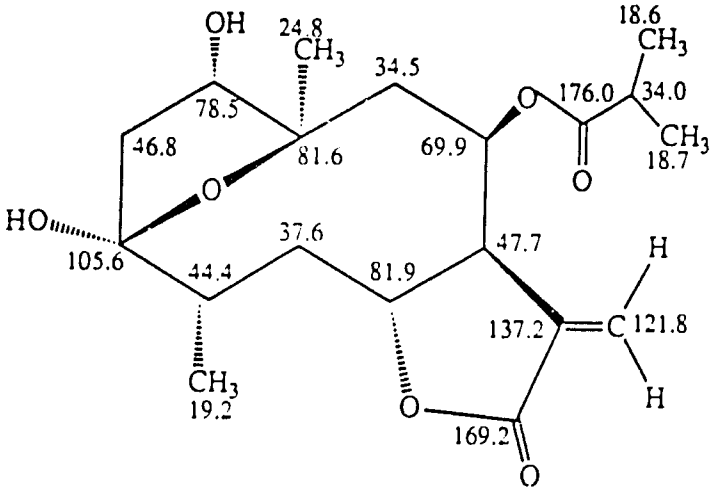
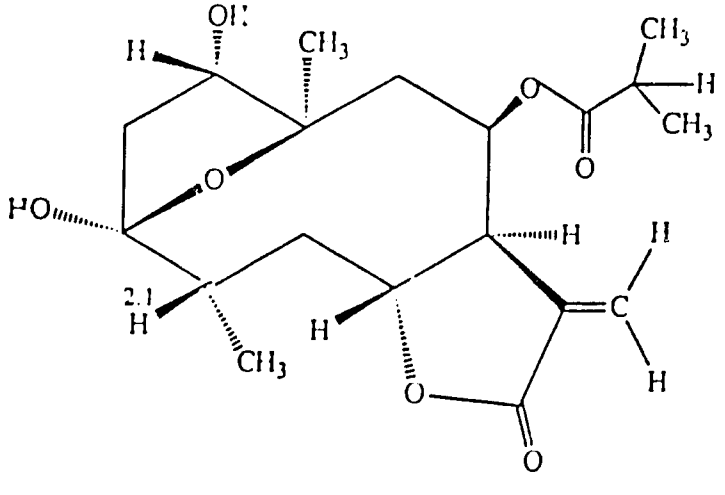


Figure 5.9b: ^{13}C-NMR Chemical shift assignments of TE-A4$_{4-4}$

The peak at m/z 71 in the EI mass spectrum showed that the isopropyl group is incorporated in an isobutyryloxy group. The IR absorption at 1680 cm^{-1} on the IR spectra was due to the presence of the carbonyl group in the isobutyryloxy group.

The carbon hydrogen direct connectivities were obtained by the Heteronuclear Multiple Quantum Shift Correlation (HMQC) experiment. The ^{13}C-NMR, IR, ^{1}H-NMR spectral data and inverse heterocosy experiments suggested a ten membered ring system, one lactone ring and an isobutyryloxy ester group. Biogenetically the lactone ring is transfused to carbon atoms number six and seven (Fischer, et al,1979; Yashiyoka, et al, 1973). This is further confirmed by HMBC Heteronuclear multiple bond correlation experiments. The presence of an epoxy bridge between carbon atoms number ten and three was also identified on the basis of HMBC connectivities (Fig 5.10). The Homo COSY 45^{0} spectrum of TE-$A4_{4-4}$ is given in Figure 5.11 with the major H- H couplings.

The ^{13}C chemical shift values of TAGITININ A, a previosly documented well-known germacronolide (Baruah et al, 1979) known to occur widely in the genera Tithonia and Helianthus of the Family Asteraceae (Rahman & Ahmad, 1992), were exactly the same as those of TE-$A4_{4-4}$. Therefore, the structure of TE-$A4_{4-4}$ was established as Tagitinin A. The melting point of the compound (i.e. 167^{0}) was the same as given for Tagitinin A in literature (Fischer et al, 1979).



TAGITININ A (= TE-$A4_{4-4}$)

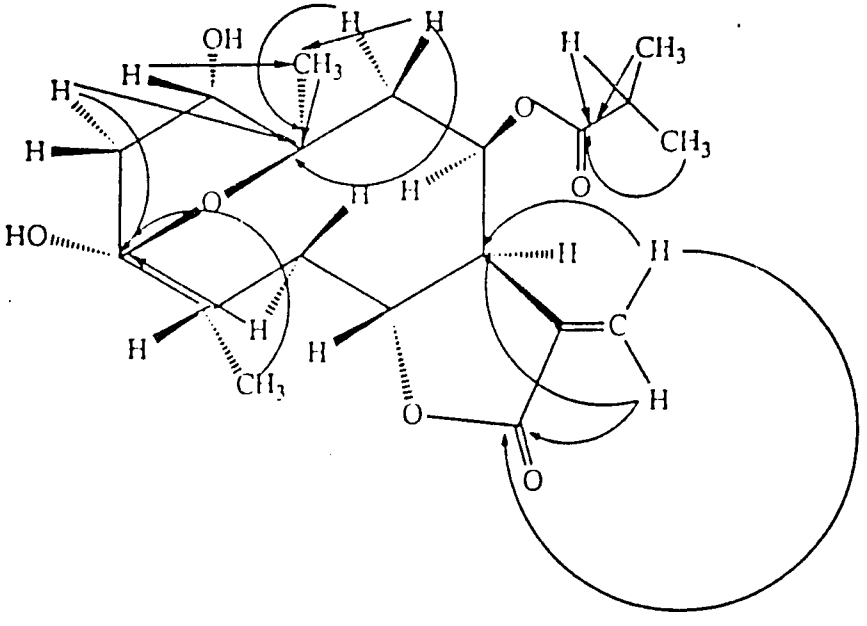


Figure 5.10: HMBC interactions of TE-A4$_{4-4}$

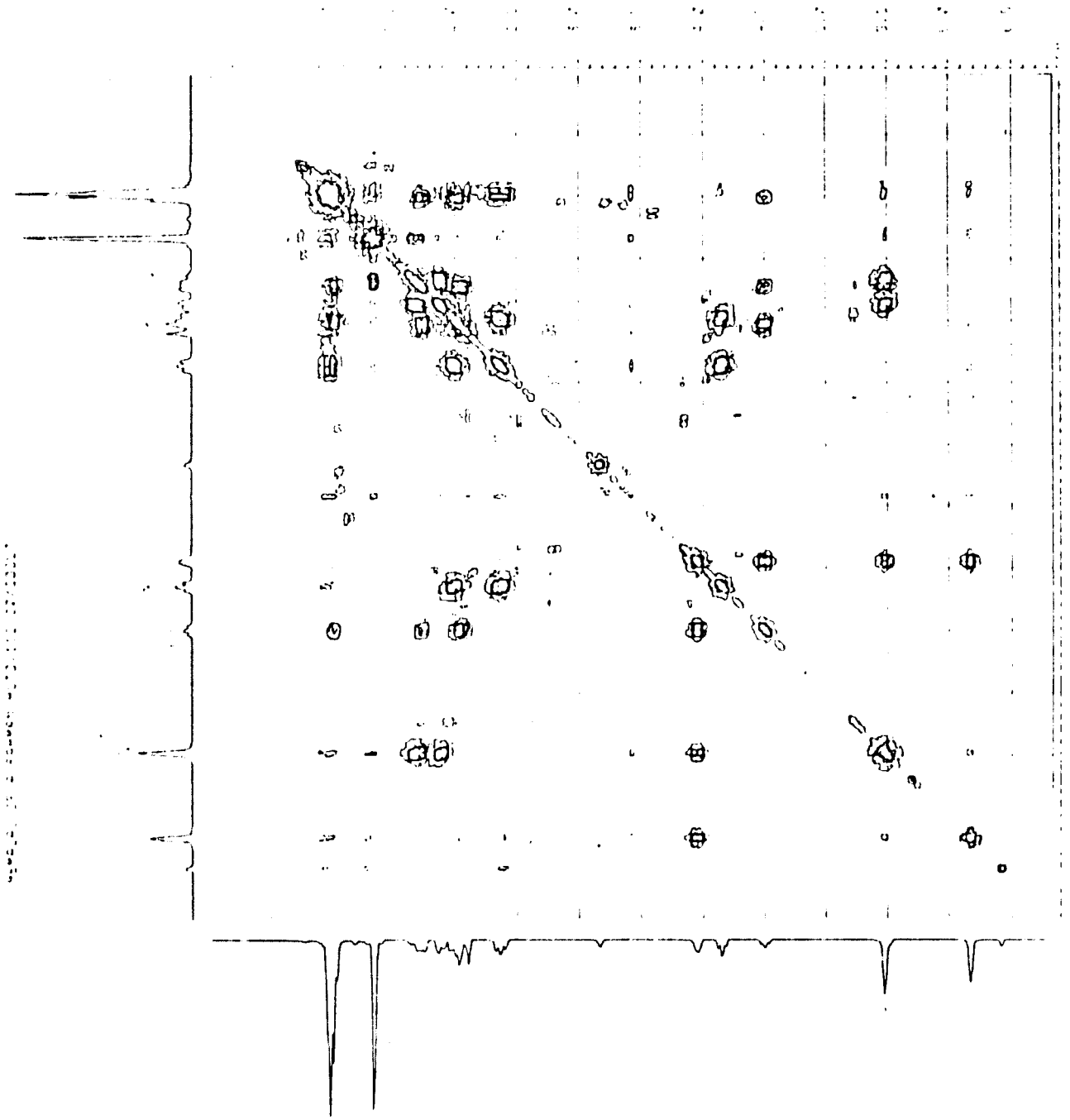


Figure 5.11: Homo-COSY Spectrum of TE-A4$_{4-4}$
(= Tagitinin A)

5.2.2 Structure elucidation of compound NP_3 as 12-carboxy -6,10-dihydroxy-8 - isobutyryloxy -3- oxogermacra- 1,4, 11(13) triene γ -lactone (Tagitinin C)

The active compound code named NP_3 from the dichloromethane fraction of Tithonia (Section 4.3.4) showed a dark yellow spot in 1:3 acetone : hexane (Rf 0.45),on TLC when sprayed with vanillin-H_2SO_4.

The presence of an -CH group, and a carbonyl group was confirmed by the IR ($CHCl_3$) absoption bands at 3240 cm^{-1} and 1740 cm^{-1}, respectively. The molecular formula of NP_3 was established by High Resolution Mass Spectrometry as $C_{19}H_{24}O_6$ (M^+ 348 m/z). On the basis of the molecular formula (three isoprene units), NP_3 was also considered to be a sesquiterpene γ - lactone. The degree of unsaturation of the compound was calculated as 8.

The ^{1}H-NMR 500 MHz (CDCl3) spectrum of the compound (Fig. 5.13) indicated the presence of 13 different proton chemical environments in the molecule (Fig 5.13, 5.14). The two doublets in the olefinic region at 6.9 ppm (J=12) and 6.2 ppm, suggested the presence of trans vicinal protons attached to a double bond.

The ^{13}C -Broad Band decoupled spectum showed the presence of 19 carbon atoms in the molecule (Fig.5.15). The multiplicity of each cabon atom was determined on the basis of DEPT spectra and were found to be, four methyl carbons, two methylene carbons (one of which is olefinic), seven methine carbons (3 of them olefinic) and by difference from the broad band spectrum, 6 quaternary carbons. The hetero nuclear H-C quantum shift correlation (inverse Hetero-cosy) expreriments showed the direct connectivities of carbon atoms and their respective protons.

The Mass Spectroscopic data of the compound indicated the exsistence of an isobutyryloxy ester group, corresponding to the mass 71. It was further confirmed by the ^{1}H NMR doublets at 1.03 ppm and 1.01 ppm (J=4.5) for the two methyl groups and the isolated septet at 2.34 ppm for the methine protons of the iso-propyl group.

The spectral data (IR, Mass, NMR) of the compound, suggested the presence of a ten membered ring system, an isobutyryloxy ester group and an exomethylene lactone ring. The positions of the functional groups were determined by the coupling constants of the respective protons. Biogenetically the lactone ring is fused to the ten membered ring at carbon atoms six and seven in the trans manner (Fischer et al, 1979), as

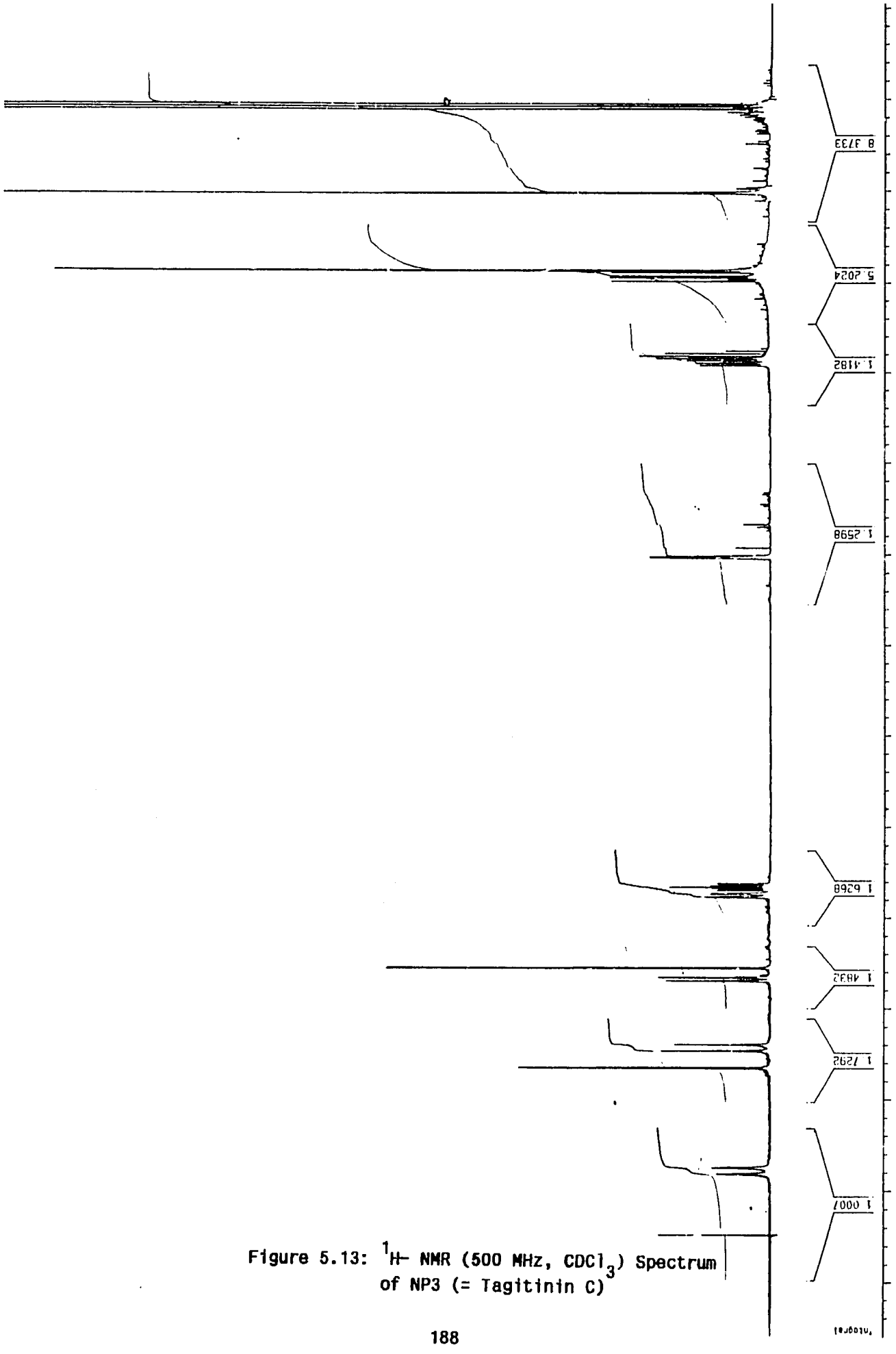


Figure 5.13: ^{1}H- NMR (500 MHz, $CDCl_3$) Spectrum of NP3 (= Tagitinin C)

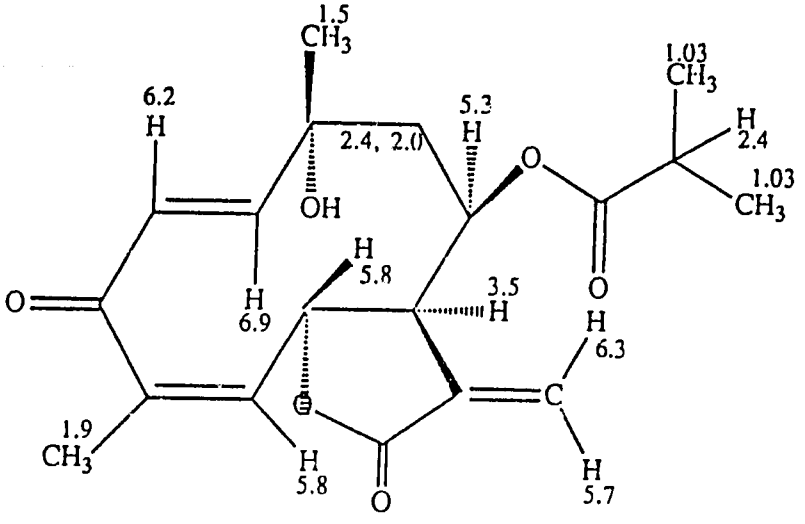


Figure 5.14: ^{1}H-NMR Chemical shift assignments of NP3

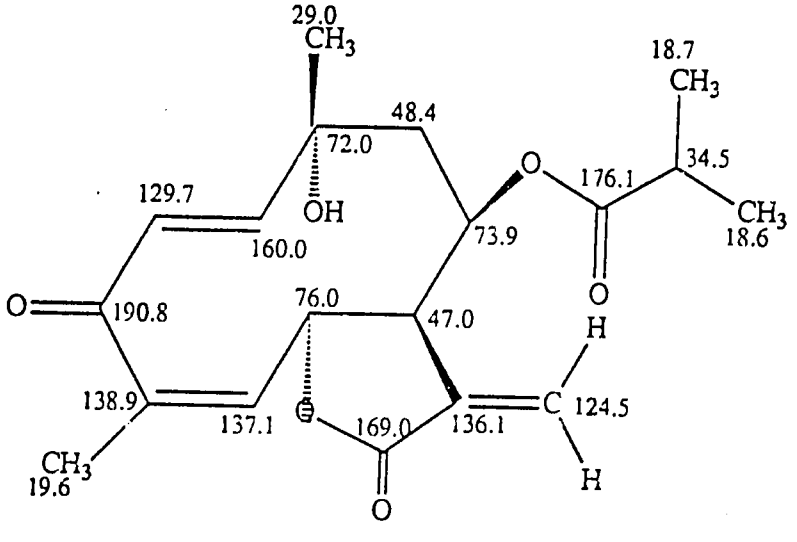


Figure 5.15: ^{13}C-NMR Chemical shift assignments of NP3

described previously with Tagitinin A. It was further confirmed on the basis of coupling constants of H-7 which resonated at 5.83 ppm (J=11 Hz) and H-7, which resonated at 5.78 ppm (J=11 Hz) (Fig. 5.16). The position of the isobutyryloxy group was confirmed on the basis of hetero COSY, i.e. HMQC and HMBC spectral data (Fig 5.17, Table 5.4). The stereochemisty of the isobutyryloxy group was β due to equatorial-axial and equatorial-equatorial coupling between H8 -H9a and H8- H9b protons.

Table 5.4. ^{13}C Chemical shift values and HMQC connectivities of NP_3

CH_3	CH_2	CH	C
18.62-1.03	48.44-2.4, 2	34.05-2.4	72.05
18.79-1.03	124.50-6.3,5.75	47.05-3.5	136.11
19.65-1.9		73.90-5.3	138.9
29.04-1.5		76.05-5.35	169.0
		129.7-6.2	176.0
		137.1-5.8	196.7
		160.6-6.9	

The spectral data of NP_3 totally superimposed with those reported for Tagitinin C (Baruah et al, 1979) which had been isolated previously from Tithonia species. The Table 5.5, below, summarize and compares the ^{13}C and 'H- NMR chemical shift values of NP_3 and Tagitinin C. ^{13}C chemical shift values of NP_3 were consistent with those reported with Tagitinin C (Table 5.5). Hence, the structure of NP_3 was established as 12- carboxy -6, 10- dihydroxy -8- isobutyryloxy -3- oxogermacra- 1,4, 11(13) triene γ - lactone, as shown below in Fig. 5.18

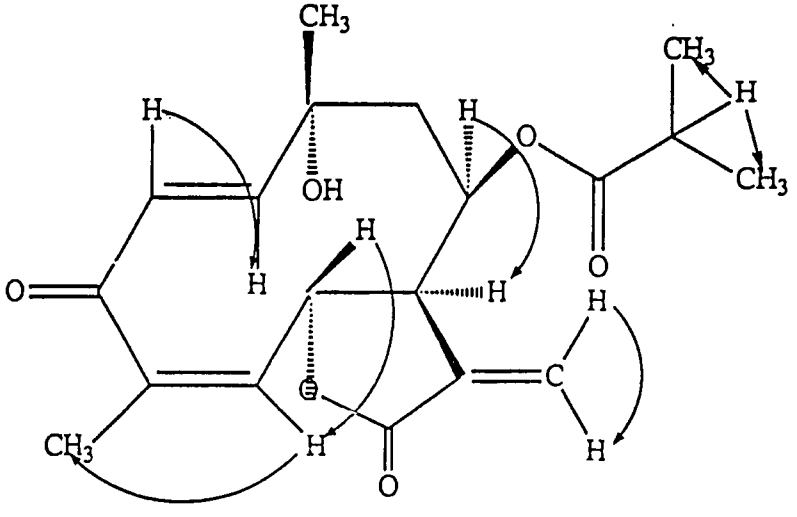


Figure 5.16: Homo-COSY Spectrum of NP3

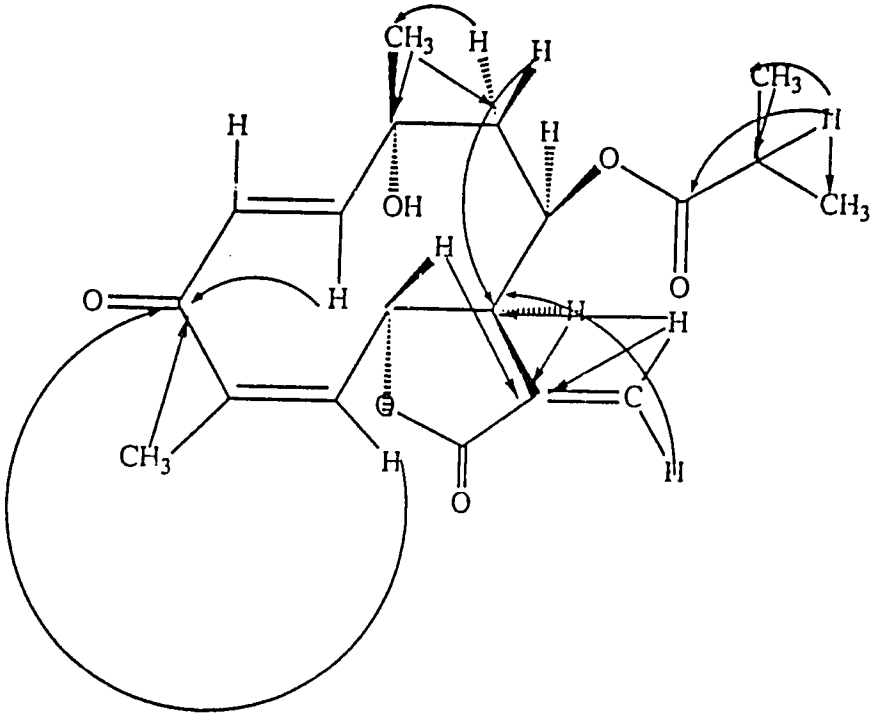


Figure 5.17: HMBC interactions of NP3

Table 5.5: A comparison of ^{13}C- NMR Chemical shift Values of compound NP_3 from Tithonia with those reported for Tagitinin C. (Baruah et al, 1979)

Tagitinin C 13C	NP3 13C
18.64	18.62
18.8	18.80
19.65	19.67
28.88	29.04
34.06	34.05
47.05	47.06
48.38	48.44
71.69	72.05
74.01	73.9
76.05	76.05
124.43	124.5
129.57	129.7
136.11	136.1
137.14	137.1
138.84	138.9
160.49	160.0
169.5	169.7
176.18	176.2
196.85	196.7

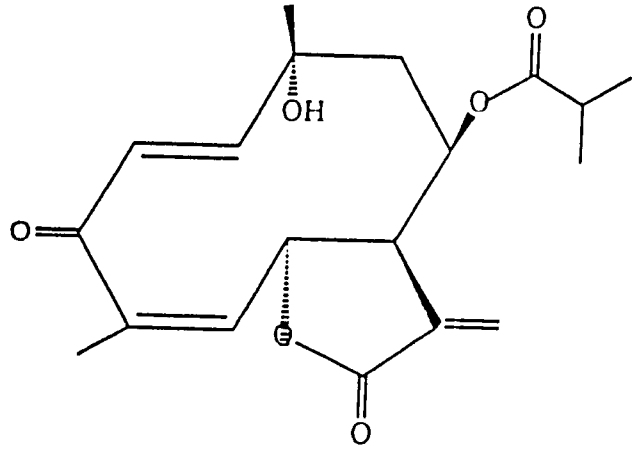


Figure 5.18: TAGITININ C (=NP3)

5.2.3 Structure elucidation of NP_4 as 12 carboxy -6,10- dihydroxy - 1,2 - epoxy - 8 - isobutyloxy germacra - 11(13) ene γ -lactone (Tagitinin-G)

The compound code named NP_4 isolated from the dichloromethane fraction of the Tithonia extract (section 4.3.4) was a white gummy material which gave a yellow coloured spot (Rf 0.5) on TLC in acetone : hexane (1:3), when sprayed with vanillin H_2SO_4.

The compound NP_4 contained an -OH group (at 3400 cm^{-1}) and an unsaturated lactone moiety with an exomethylene group (1750 cm^{-1}, 1650 cm^{-1}), as indicated by its IR (CHCl3) spectrum. The presence of an ester group in the compound was also confirmed by IR bands at 1727 cm^{-1} and 1120 cm^{-1}.

The EI Mass spectrum of NP_4 had the molecular ion peak at 364 (m/e) It was further confirmed by FD technique. The High Resolution Mass Spectrum of NP_3 suggested the molecular formula $C_{19}H_{24}O_7$. The calculated degree of unsaturation was 7.

The DEPT and Broad Band decoupled ^{13}C spectra of NP_4 (Fig.5.19, Table 5.6) led to the identity of nineteen carbon atoms in the molecule. The multiplicity of each carbon atom in the molecule was determined on the basis of DEPT spectrum and found to be four methyl carbons, two methylene cabons, seven methene carbons and by difference from the broad band spectrum, six quaternary carbon atoms. The Hetero-COSY inverse experiment HMQC, indicated the connectivities of Carbons and their respective protons (Table 5.6).

The 500 MHz ^1H- NMR spectrum of NP_4 (Fig. 5.20 and 5.21) showed the presence of an isopropyl group $(CH_3)_2$- CH-) with its (2*3H) doublets centered at 1.07 ppm and 1.05 ppm (J=7 Hz). The isopropyl group was attributed to the presence of an isobutyric ester grouping in the molecule on the basis of the peak at m/e 71, in the EI spectrum. The heptet, located at 2.45 ppm (J=7 Hz) region, was due to the methine proton of the isopropyl group.

The presence of a 10-membered ring, an exomethylene lactone ring, and an isobutyryloxy ester group was evident form ^{13}C, ^1H-NMR, IR, and mass spectral data, on the basis of similarities to the characteristic features of sesquiterpene lactones discribed earlier.

Table 5.6 : ^{13}C Chemical shift values and HMQC carbon - hydrogen connectivities of NP_4

CH3	CH2	CH	C
18.65- 1.08	125.0- 6.32,5.82	141.55- 6.34	70.12
18.81- 1.08	42.4- 2.07,1.94	75.14- 5.6	135.6
20.23- 1.25		73.6 - 5.3	137.1
25.9 - 1.92		58.5 - 3.6	168.9
		49.7 - 3.25	176.2
		65.5 - 3.2	193.9
		34.1 - 2.45	

The functional groups attached to the 10-membered ring were assigned. Biogenetically, the lactone ring was determined to be fused to carbon atoms number six and seven in the trans manner (Fischer et al,1979). The fusion of the lactone ring in a trans manner was confirmed by the coupling constants of H- 6 and H- 5 protons (J=11 Hz) (Fig. 5.22, Fig. 5.23).

The isobutyryloxy group was located on carbon atom number eight due to the down field ^{13}C chemical shift value of 75.1 ppm. HMBC interactions (Fig. 5.24). The β orientation of the isobutyryloxy group was assigned on the basis of coupling constants and by comparison with reported data (Fischer et al, 1979, Baruah et al, 1979).

The identities of the protons were established by analysing the ^{1}H- NMR (Fig.5.20) and the COSY 45° spectra (Fig. 5.22). The doublet at 3.2 ppm is due to the H- 2 proton. Hence, It shows coupling with the signal at 3.6 ppm (J= 2.3 Hz). The doublet at 3.6 ppm originated due to the H-1 proton. The multiplet at 6.34 ppm, is due to the H-5 which shows coupling with H-6 at 6.34 ppm and long range coupling with metnyl protons at 1.92 ppm.

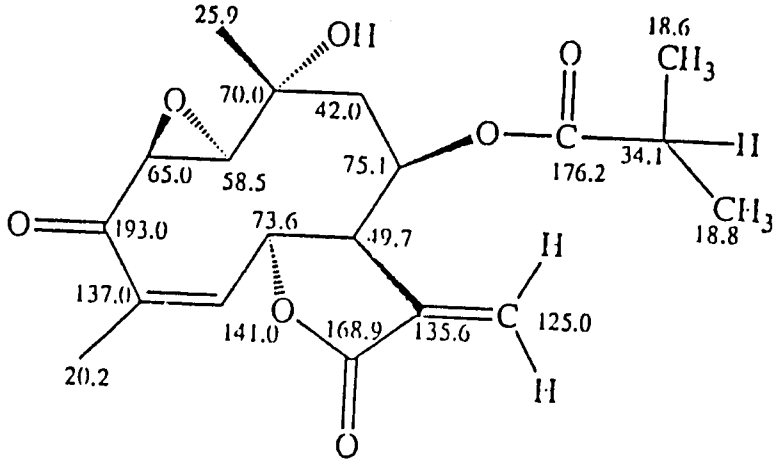


Figure 5.19: ^{13}C-NMR Chemical shift assignments of NP4

HEMALAL/DR.A.REHMAN/NP4/CDCL3

AMX 500

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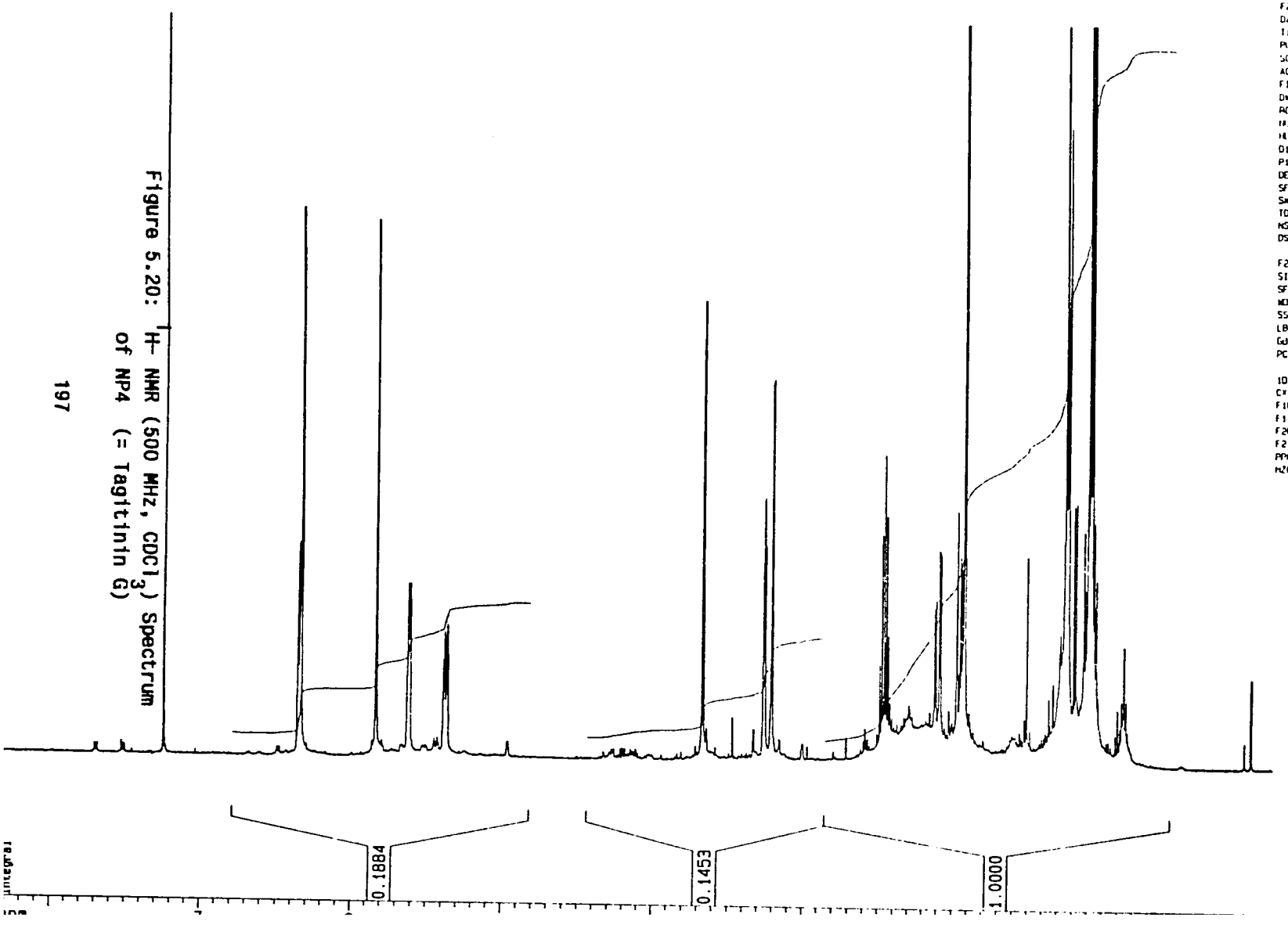

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F1 4166 54 Hz
F2P -0 131 ppm
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HZCM 188 92249 Hz/cm

Figure 5.20: ^{1}H- NMR (500 MHz, $CDCl_3$) Spectrum of NP4 (= Tagitinin G)

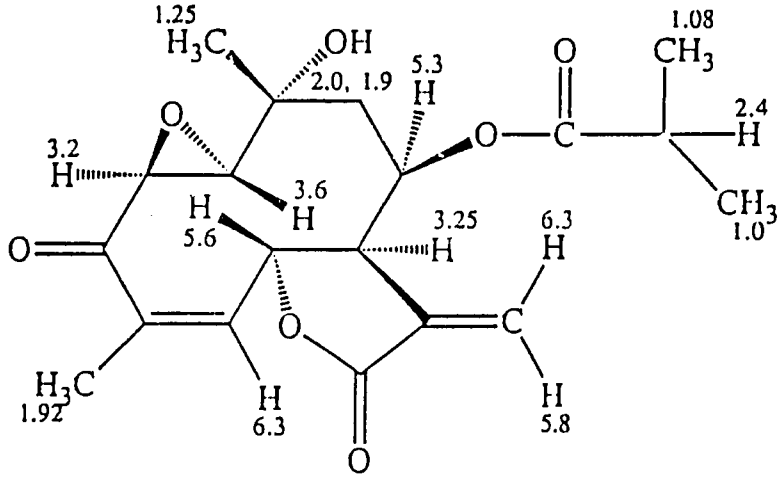


Figure 5.21: ^{1}H-NMR Chemical shift assignments of NP4

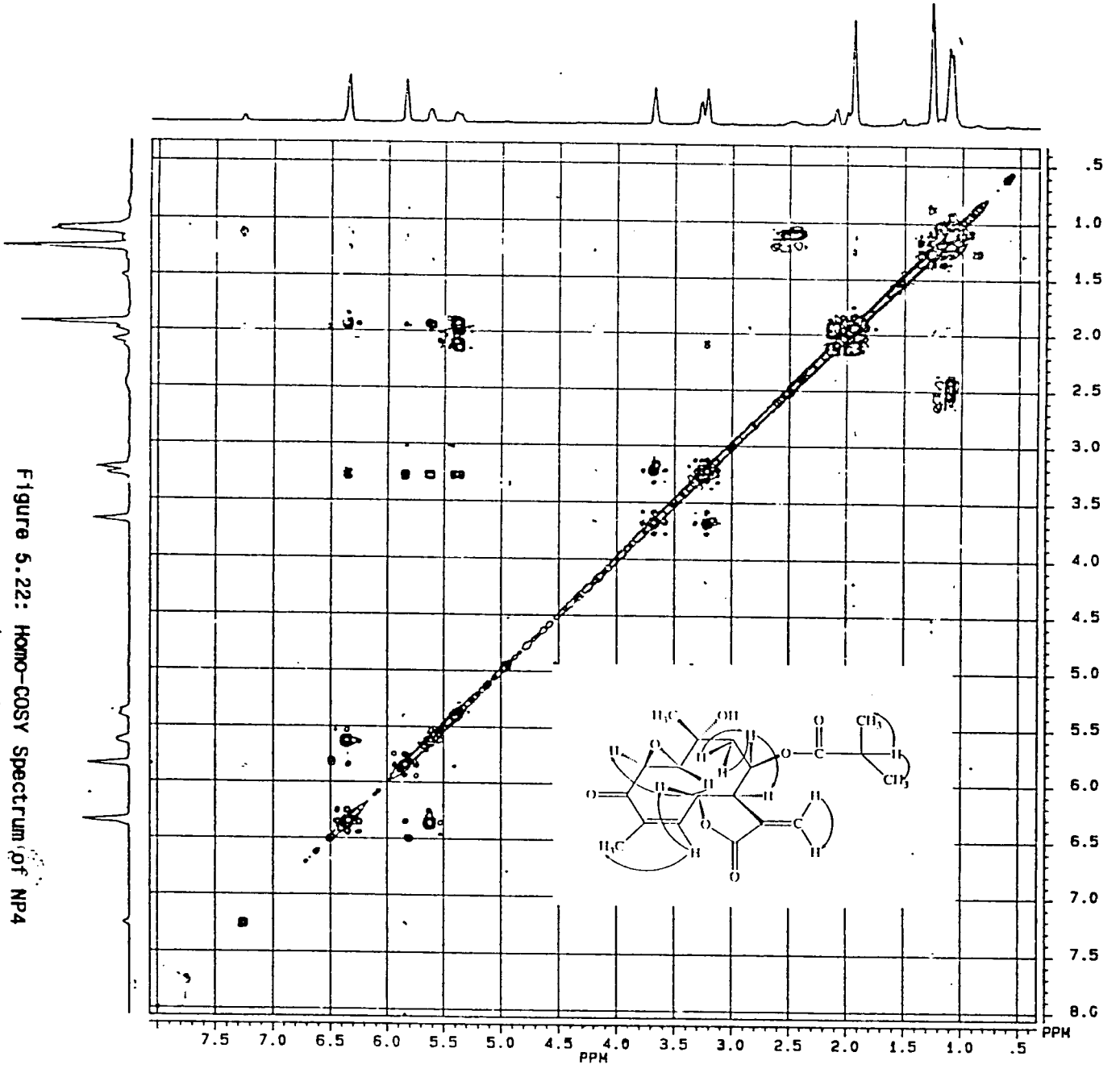


Figure 5.22: Homo-COSY Spectrum of NP4 (= Tagitinin G)

The stereochemistry of the compound ascertained by NOE difference measurements (Rahman, 1986; Rahman & Pervin, 1991) at certain points in the molecule, was found to be in accordance with the structure given in Figure 5.23. The strong NOE interactions of H-6 (5.6 ppm) and H-1 (3.6 ppm), H-2 (3.2 ppm) and H-8 (5.3 ppm) (Table 5.7) indicated the β configuration of H-1 (3.6 ppm) and H-6 (5.6 ppm) and the α configuration of the H-2 (3.2 ppm), H-8 (5.3 ppm) and H-7 (3.25 ppm). On biogenetic considerations the lactone ring is transfused to carbon atoms number six and seven (Fischer, 1979). Therefore, the H-6 has a β -configuration and the H-7, a α -configuration.

Table 5.7: Results of NOE Experiment with NP4

Signal irradiated		Signal enhanced	
Assignment	Chemical shift (ppm)	Assignment	Chemical shift (ppm)
H-6	5.60	H-1	3.60
H-8	5.30	H-7	3.25
H-2	3.20	H-8	5.30

Finally, the stucture for the compound was suggested and confirmed with related data collected from literature (Fischer et al, 1979, Baruah at al, 1979, Choudry et al, 1980, Hertz and Sharma, 1975, Raghwendra Pal et al, 1976; 1977, Rahman and Ahmad, 1992). The known compound Tagitinin-E is closely related to the present compound which may be named Tagitinin-G, (Fig. 5.25), because the location of the epoxide bridge is different. The comparison of the chemical shift values of related tagitinins is the same (Fischer et al, 1979; Baruah et al, 1979; Chouwdry et al, 1980; Herz & Sharma, 1975; Raghwendra Pal et al, 1976, 1977; Rahman & Ahmad, 1992) However, the location of the epoxide bridge was not previously reported to

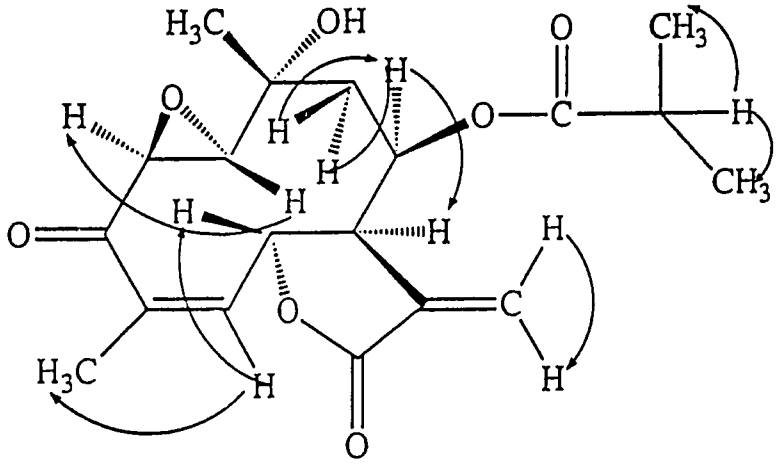


Figure 5.23: Homo-COSY 45^0 interactions of NP4

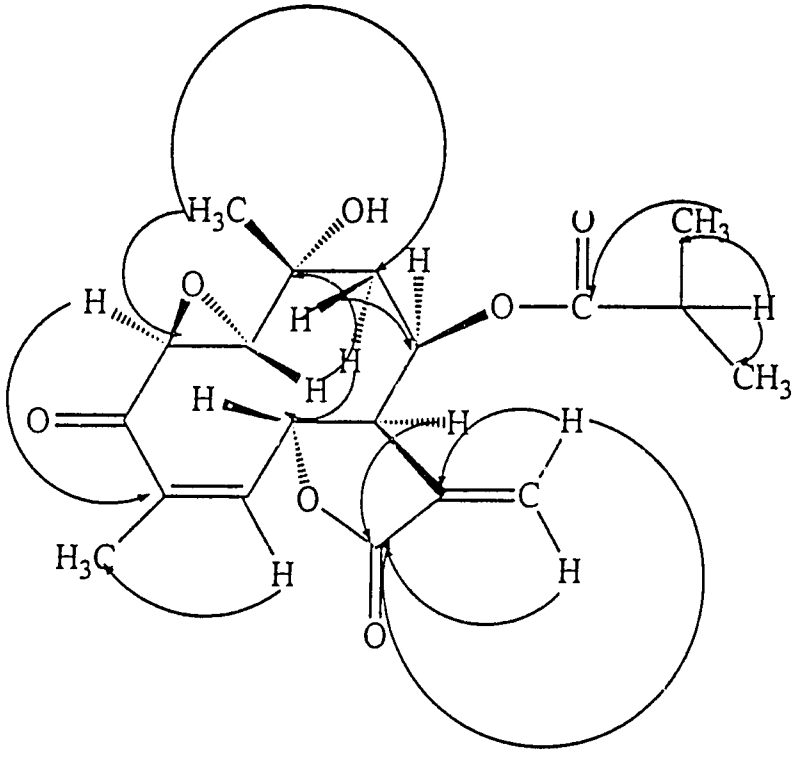


Figure 5.24: HMBC interactions of NP4

be on C-1 and C-2. This is the first instance of the natural occurrence of a tagitinin derivative carrying an epoxide bridge at C-1 in α- and C-2 in β- configurations.

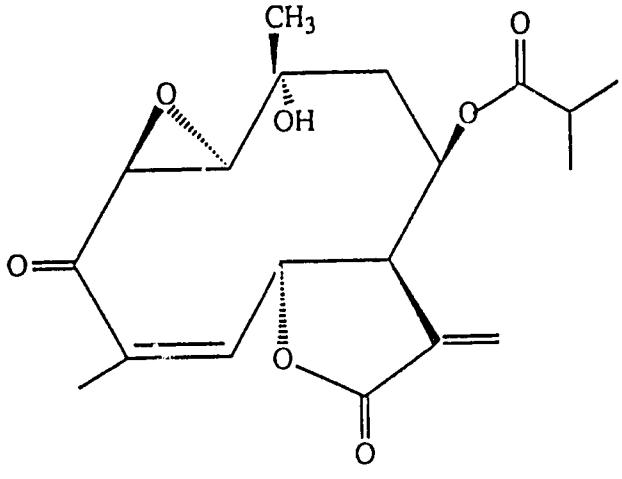


Figure 5.25: TAGITININ G (= NP4)

SECTION 5.3

DISCUSSION

Contents:

5.3.1 Allelochemicals from Gliricidia maculata
5.3.2 Allelochemicals from Tithonia diversifolia

5.3.1 Allelochemicals from Gliricidia maculata

The bioactive compound determined to be active in the fresh leaf extracts and leachates of Gliricidia in our studies, was coumarin, a well-known allelochemical. As stated previously coumains are especially common in grasses, legumes and citrus fruits (Robinson, 1980). The isolation of coumarin was first reported by Vogel in 1820 (loc.cit. Murray et al, 1982) from legume plants including clover (Meliotis officinalis). Coumarins, which are phenolic compounds, contain very powerful toxins. Coumarin itself is known to be a strong anti-coagulant of blood and a common ingredient of rat poison. (Whitlon et al. 1978).

The bioactivity of naturally occurring coumarin has been reported in a number of publications. Niccolier and Thompson (1982) found coumarin to be a more potent growth inhibitor than most aromatic acids and all flavonoids tested. Exposure to only 30 uM coumarin inhibited wheat root growth by as much as 50% within 4 days. Coumarin is about two orders of magnitude more potent as a germination inhibitor than ferulic or caffeic acid (Duke et al, 1984). The authors also suggested that the kinetics of coumarin interactions with water indicated a different mechanism of action than that of the weaker inhibitors. Coumarin inhibits both oxidative phosphorylation and photophosphorylation (Duke, 1986) and has effects much like an uncoupler of electron transport (Svensson, 1972). It also inhibits microfibril formation (Burgess & Linstead, 1977). Moreland et al (1966) reported that coumarin is more potent as a growth inhibitor than other coumarins such as umbelliferone, scopoletin, daphnetin, aesculin, limettin and aesculin. Van Sumere et al (1972) found that lettuce seed germination was inhibited 100, 53, 28, 11 and 0% by 100 nmoles per seed coumarin, herniarin, umbelliferone, esculetin, and scopoletin, respectively, after a 120 h exposure.

The presence of coumarin as an inhibitor has been reported from certain other legumes as well. Nicollier et al (1985) found that a legume, white clover (Meliotis alba Desr.) extract was allelopathic towards tomato and radish, inhibiting seed germination as well as seedling growth. They

found that coumarin, o-coumaric acid and melilotic acid accounted for the major portion of the activity of the crude extract. It was also reported (Winter, 1961) that white clover leaves contain appreciable amounts of coumarin, and mulching soil with leaves of this species resulted in the presence of coumarin in the soil and of a coumarin derivative in wheat plants growing in the soil. Schreiner and Reed (1908) had reported that coumarin and esculin are very potent inhibitors of the growth of wheat seedlings. Evenari (1949) also listed coumarin as a very powerful inhibitor of seed germination.

Although it is well known that phenolic compounds such as simple phenols, coumarins play important roles in allelopathy by either inhibiting germination or reducing growth of seedlings (Rice, 1984; Bewley & Black, 1985; Putnam & Tang, 1986), a clear insight into the precise physiological perturbation caused by these substances has not been obtained because of the difficulty in determining the specific site of action (Rice, 1984; Einhellig, 1986). According to Avers and Goodwin (1956) and Jankay and Muller (1976), coumarin appears to inhibit the elongation of cells of the differentiating zone of the root. Aliota et al (1993) confirmed these findings, and also reported that the elongation zone of coumarin-treated radish roots is much shorter than control roots, and that root hair differentiation of coumarin-treated root had shifted apically to form a tuft, not observed in the controls. Furthermore, radish root cells in the meristematic zone treated with coumarin appeared highly vacuolated compared with the control (Aliota et al, 1993).

Cornman (1946) had earlier reported that coumarin blocks all mitoses in onion and lily roots within 2-3 hours, an effect similar to that resulting from colchicine treatment, destruction of the spindle with the resultant interruption of anaphases and accumulation of metaphases. The interrupted mitoses apparently form tetraploid nuclei or binucleate cells. Coumarin also prevented the entry of cells into mitosis. Avers and Goodwin (1956) demonstrated that scopoletin and coumarin decreased mitoses in Phleum pratense roots and that scopoletin was more effective. Jankay and Muller (1976) reported that the coumarin-relative, umbelliferone decreased the cell elongation rate in cucumber roots, but increased radial expansion. Previously, Hogetsu et al (1974) demonstrated a retardation of cellulose synthesis in bean epicotyls by coumarin.

The above review shows the potential of coumarin and its relatives to be extremely effective allelochemicals by being potent growth inhibitors. Our studies have established that coumarin is released by Gliricidia leaves by leaching or during decay. It is highly probable that coumarin, so released will make an impact on the seeds that it comes into direct contact with. The present studies did not extend to determining the soil persistence and interactions of coumarin with microorganisms. In

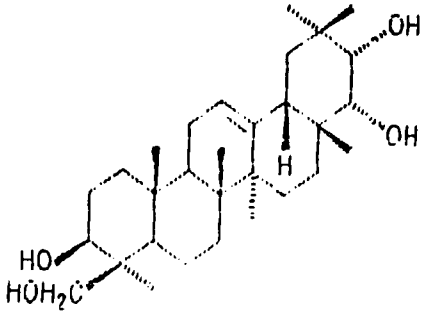
general, it is known that coumarin does not persist in soil for long. In some of our exploratory studies also, coumarin could not be detected from treated soil 48-72 hours later. Studies of coumarin dynamics, in particular, how bioactive coumarin could be in the presence of soil and its microbes, are extremely important areas of study for the immediate future.

The extremely potent bioactivity that was detected in the present studies with Gliricidia dried leaves was ascribed to a triterpene Soyasapogenol. In general, available literature lists several Soyasapogenols that have been characterized as Soya-bean saponins. The Dictionary of Organic Compounds (5th Edition, 1982) refers to Soyasapogenol A (67), Soyasapogenol B (68), Soyasapogenol C (69), Soyasapogenol D (70), and Soyasapogenol E (71). The compound that was isolated in the present studies from the dried leaves of Gliricidia, as a potent growth regulatory allelochemical appears to be a new compound, being a triol and the 22 α epimer of Soyasapogenol. It is different from Soyasapogenol A, a tetrol, with two -OH groups attached to the C-21 and C-22, and Soyasapogenol B, a triol, which is the 22 β epimer. This compound is quite different from Soyasapogenol C, Soyasapogenol D and Soyasapogenol E, which are all diols.

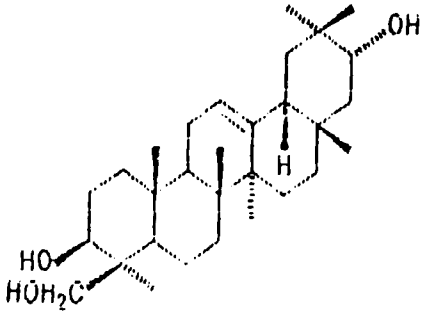
The best record of bioactivity of Soyasapogenol comes from its involvement as a haustorial inducer. The haustoria of parasitic angiosperms do not form when plants are grown axenically, but are rapidly induced in the presence of host roots and host-root exudates (Lynn, 1985). Several haustorial inducing compounds have been characterized, one of which is Soyasapogenol-B.identified from the roots of a legume Lespedeza sericea. In the absence of published literature, it has to be therefore concluded that the 22 α -Soyasapogenol is a highly potent inhibitor and may have a role to play in allelopathy interactions of Gliricidia residues. Further studies are needed on the bioactivity and significance of this new Soyasapogenol from Gliricidia.

5.3.2 Allelochemicals from Tithonia diversifolia

From the present studies based on the seed germination and seedling growth bioassay of tomato, the phytoactive (inhibitory or stimulatory) compounds found in Tithonia diversifolia extracts were identified as sesquiterpene lactones belonging to the group 'germacranolides'. As stated previously, there was relatively little bioactivity in the fresh leaf extracts in our test system; in contrast, the dried Tithonia leaves demonstrated very high activity. The germacranolides- Tagitinin A, Tagitinin C and possibly a new Tagitinin, Tagitinin G, were the main compounds isolated from the chemical extraction of the dried Tithonia leaves. As stated previously (Chapter 1), sesquiterpene lactones are known to occur commonly in the family Asteraceae, and in addition, several



soyasapogenol A (67)



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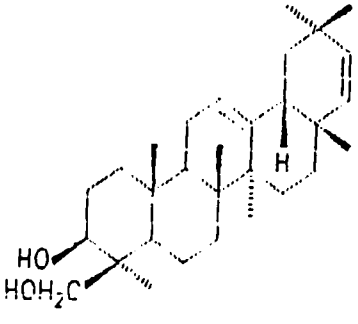

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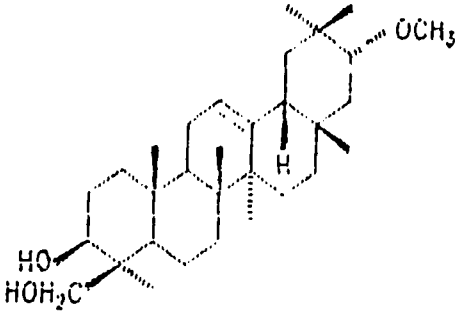

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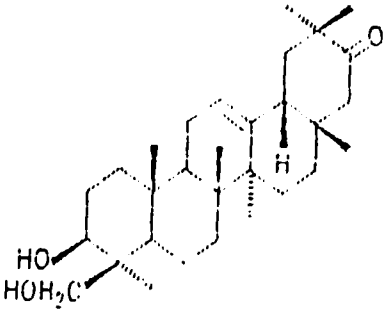


soyasapogenol E (71)

Tagitinins (Tagitinin A, B, D, E, F) have already been reported to occur in Tithonia species (Baruah et al, 1979; Herz, 1975). The unsaturated γ- lactone sesquiterpenes have been extensively studied for their bioactivity, which has been ascribed mainly to the presence of the exomethylene - γ - lactone group.

In spite of the vast number and the wide variety of naturally occurring sesquiterpenes (over 3500), that have been chemically characterized, little work has been done on their ecological significance which includes antibacterial, antifungal, cytotoxic, allergenic, allelopathic and deterrent activity, and toxicity and antifeedant activity (Rodriguez et al, 1976; Fischer et al, 1979; Marcias et al, 1992). Several studies have suggested that sesquiterpene lactones may act as plant growth regulators. Some sesquiterpene lactones have been reported to be responsible for the allelopathic properties of certain plants, by affecting the germination and growth of other species (Marcias et al, 1992). Certain sesquiterpene lactones are known to selectively promote and inhibit seed germination at concentrations as low as 10^{-6} M (Fischer et al, 1990).

Sesquiterpene lactones are thought to be involved in the allelopathic interactions of Ambrosia cumanensis, an important species in the ruderal vegetation in Mexico, which grows vigourously and in almost pure stands. As described by Anaya et al (1987), root and leaf aqueous leachates of Ambrosia cumanensis produced a strong inhibition of growth of weed species. Aqueous extracts of soil collected under Ambrosia during its flowering in July, were strongly allelopathic towards weed growth. Decomposition of leaves/roots in pots caused inhibition of some weeds also. Based on the differences of results from sterile and non-sterile soil, microorganisms were thought to be involved in this process. Bioassays with several sesquiterpene lactones from A. cumanensis showed that these compounds produce different effects (stimulatory or inhibitory) on the germination and growth of several species of secondary vegetation, which interact with Ambrosia. It has also been suggested that the allelopathic potential of Ambrosia cumanensis may contribute to autotoxicity (autocontrol) of its own population as well, by preventing growth of seedlings of its own kind.

Stevens and Merill (1983) identified numerous sesquiterpene lactones isolated from two major weeds Russian knapweed (Centaurea repens) and yellow starthistle (Centaurea solstitialis). Of these compounds, solstitiolide (32), repin (33), acroptilin (34) and centaurepensin (35) stimulated root elongation of lettuce at 20 ppm and inhibited growth at high concentrations.

Fischer et al (1989) recently reported on the ability of four naturally occurring sesquiterpenes, which are structurally very similar to

the germination stimulant strigol, the germination stimulant of the obligate root parasite witchweed (Striga asiatica), to stimulate Striga seed germination. The compounds tested were, confertiflorin and desacetyl-confertiflorin (both from Ambrosia confertiflora DC.), 11, 13-dihydro-parthenolide (from Ambrosia artimisiifolia) and parthenin from Parthenium hysterophorus). Parthenin and Confertiflorin significantly promoted Striga germination at 10^{-4} M' parthenin and desacetylconfertiflorin did the same at 10^{-3} M. Dihydropathenolide induced 70% germination of witchweed across a concentration range of 10^{-7} to 10^{-9}, showing very high bioactivity. The aim of these studies was to find potential germination stimulants of witchweed to promote seed germination in the absence of a suitable host, which results in death of the witchweed seedling (Fischer et al, 1990).

Continuing this work further, Fischer et al (1990) tested twenty-four natural and synthetic sesquiterpene lactones as germination stimulants of Striga, in order to determine the essential structural requirements for the stimulation of germination cased by dihydroparthe-nolide. Highest activity observed in these studies was with a mixture of two eudesmanolides, santamarin and reynosin. Also all of the 10 germacranolides induced 40-65% germination of witchweed across a broad concentration range of 10^{-5} to 10^{-9} M. These researchers concluded that it is the spatial arrangement of the germacrane-eudesmane skeleton in conjunction with the five-membered lactone ring, which is important in the promotion of witchweed germination, rather than the presence of any specific functional group in the molecule.

The function of sesquiterpene lactones as growth regulators has been reviewed by Fischer and Quijano (1985). The lactones heliangine, helianginol, pyrethrosin and cyclopyrethrosin acetate promote adventitious root formation on hypocotyls of cuttings taken from Phaseolus mungo seedlings and inhibit elongation of Avena coleoptile sections. Compounds with the methylene-γ-lactone group reduced to the saturated lactone showed no effect on root formation. The sesquiterpene dilactone vernolepin from Vernonia hymenolepis A. Rich. inhibits extension growth of wheat coleoptile sections at 1.8×10^{-5} M, an effect that is reduced by simulataneous addition of auxin.

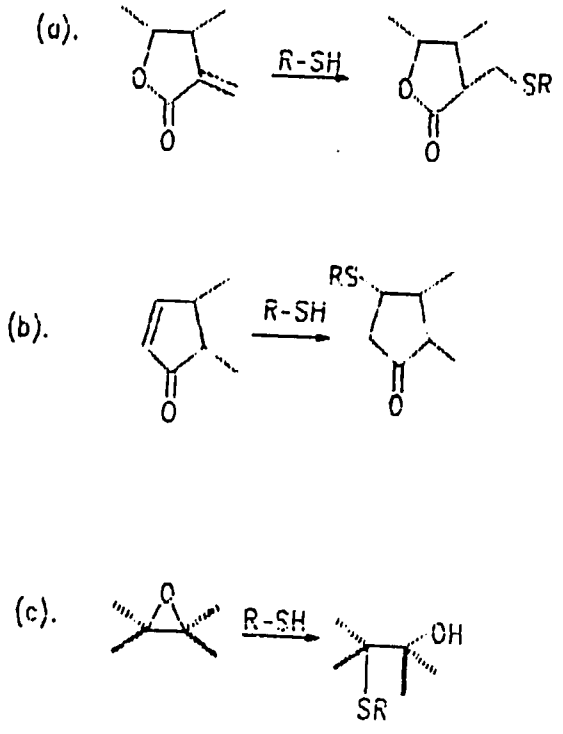
Parthenin at 50 ppm had no effect on germination of bean Phaseolus vulgaris but inhibited the development of radicles and hypocotyls. In other studies Fischer and Quijano (1985) reported about structure-activity relationships of sesquiterpene lactones with emphasis on growth regulator type activity. A 0.1 mM solution of the simplest germacranolide, costunolide, significantly promoted germination of sorghum (111%), carrot (121%), and cucumber (114%), but inhibited ryegrass (75%), wheat (91%) and Palmer amaranth (82%). In contrast, 11,13-dihydroparthenolide from Ambrosia artimisiifolia which lacks the methylene moiety in the γ-lactone

group, inhibits sorghum (92%) and Palmer amaranth (93%), but significantly promotes germination of wheat (116%), clover (111%), carrot (113%) and cucumber (111%). This demonstrated that a compound without a nucleophile receptor such as the methylene -γ-lactone group is able to exhibit seed germination activities. The above data also supported observations that the same substance can inhibit germination of one species and promote another (Fischer & Quijano, 1985). Furthermore, The two pseudoguaianolides confertiflorin and its desacetyl derivative, both isolated from Ambrosia confertiflora, showed considerable differences in activity. Sorghum (87%) and ryegrass (86%) are inhibited, and wheat (111%), clover (112%) and Palmer amaranth (111%) are promoted by confertiflorin. In contrast, ryegrass (114%) is promoted by the derivative, while most other seeds show little effects. Parthenin inhibited the germination of clover (89%) and carrot (92%), but promoted onion (109%), wheat (114%), cucumber (111%) and palmer amaranth (115%).

Sesquiterpene lactones have been found to have various other types of bioactivities as well. For instance, Jacobsson and Muddathir (1992) reported of four bioactive sesquiterpenes from the weed Polygonum glabrum Willd., which is heavily used as an anti-helmintic agent in Sudan and many other countries. In a related species, Polygonum hydropiper L. which appantly has antimicrobial, cytotoxic and insect-antifeedent activities and properties, the implicated sesuiterpenes are warburganal and polygodial (Jacobsson & Muddathir (1992).

A large part of the influence of substances known for their toxic allelopathic effects is postulated to be related to the presence of strong electrophilic or nucleophilic systems. The attack by these systems on specific positions of proteins or enzymes would change their configurations and destroy their activity. One of these systems, very common in many natural products is the α,β-unsaturated carbonyl group. This electrophilic system can undergo 1,4- Michael type addition as shown below, with electron donors such as sulphur atoms in the sulphydryl moiety, a group which is very abundant in proteins and nucleic acids (Marcias et al, 1992). The α-methylene-γ-lactone group which is present in many of the isolated natural sesquiterpenes, has been proposed as one of the factors which can determine their allelopathic activity in particular, as well as their biological activity, in general (Fischer, 1986).

Marcias et al (1992) propose that from the available literature, it is possible to relate the potential allelopathic activity of sesquiterpene-γ-lactones to two major factors: (a) the presence of certain functionalizations (where the α-methylene-γ-lactone moiety plays an important role) and (b) the different spatial arrangements that the molecules can adopt (Fischer, 1986).



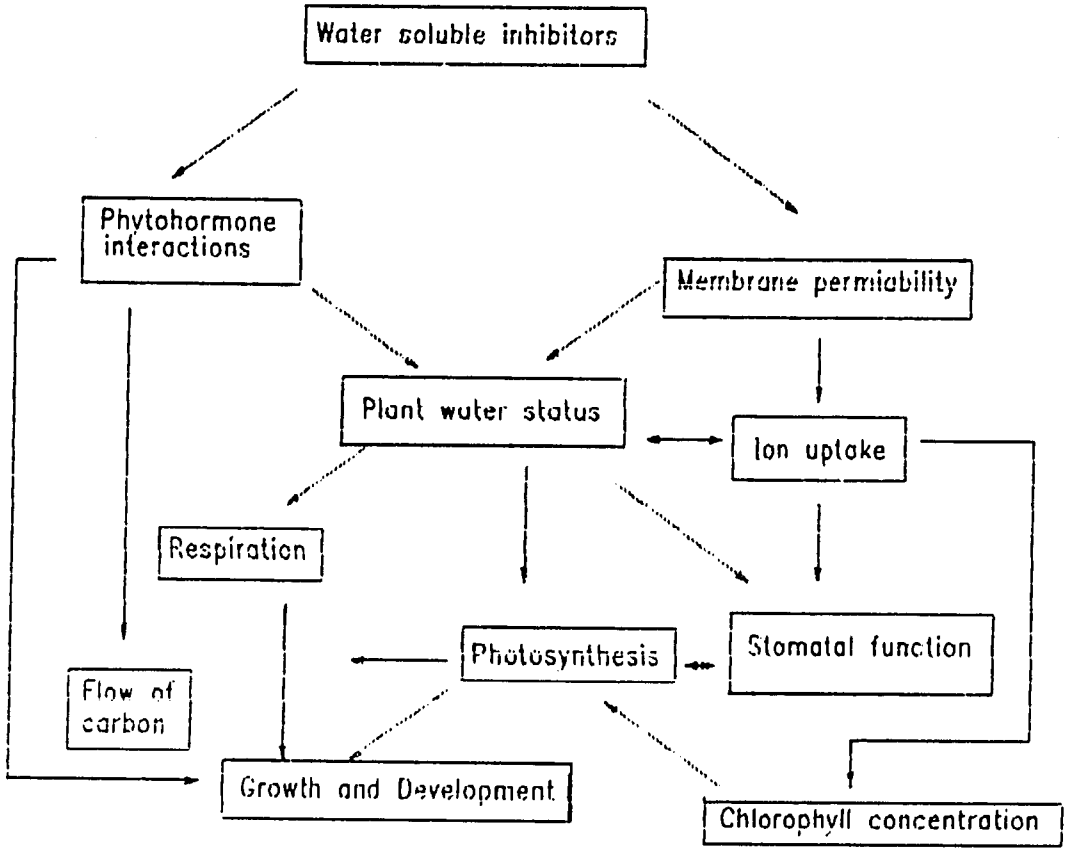
Tagitinin A, C and the other Tagitinins possess the unsaturated γ-lactone ring and in some cases the carbonyl group. Both of these functional groups are probably responsible for the demonstrable allelopathic activity of the compounds in Tithonia diversifolia. When one considers the structure of Tagitinin A and C, the major compounds in Tithonia, both can undergo the 1,4-Michael type addition with proteins or enzymes which are involved in seed germination and seedling growth. Our studies clearly indicate that the potential allelopathic influences Tithonia diversifilia can have on other plant species, could be implicated with the presence of sesquiterpene lactones, in particulat Tagitinin A, C and the new Tagitinin. The latter appears to be very highly active as a growth regulator, in much the same way as most other compounds reviewed by Marcias (1992) and Fischer and Quijano (1985). It is probable that these Tagitinins are inhibitory to seed germination and seedling growth of test species at high concentrations, but are stimulatory at low concentrations. In addition, as pointed out by many researchers, synergistic actions are also possible, and probably occur since the most purified active fraction needed a higher concentration to show 100% inhibition of test species, than partially purified fractions. Results of the present studies often indicated that active fractions became less active as the activity-directed fractionation progressed. Although the main Tagitinins (Tagitinin A and C) identified from the present studies have been known in the past, their direct involvement in allelopathic interactions are supported by our results.

Bioassays, no doubt, have been one of the most widely used tests to demonstrate allelopathic activity. Often, claims that a particular plant species inhibits the growth of another are based entirely on the seed germination response to solvent extracts of the suspected allelopathic plant; few of these test are of value in determining allelopathy under natural conditions (Leather & Einhellig, 1988). However, the veracity of the bioassay for evaluating naturally occurring compounds for phytotoxicity depends upon the physiological and biochemical response capacity of the bioassay organism, the mechanism(s) of action of the allelochemical.

Einhellig (1987) reported that allelopathic inhibition of germination and plant growth typically occurred as a result of joint action of several allelochemicals. In the same way, allelochemicals from Gliricidia or Tithonia may also interact in an additive or synergistic manner to produce the results that were obtained.

Both coumarin and soyasapogenol from Gliricidia and the sesquiterpene lactones from Tithonia are all compounds having low water solubility. Owing to their low water solubility, these compounds released from decaying residues, will diffuse only slowly into the soil solution. The process of allelopathy implies that allelochemicals of plant origin are released into the environment, subsequently modifying the growth and development of other plants. Definitive explanations of this process requires an understanding of how allelochemicals function. Some inhibitory or stimulatory allelochemicals indirectly influence the growth of the plants by their direct effects on nitrification, N-fixation, disease resistance, or susceptibility (Rice, 1984). Alternatively, more direct allelochemical effects may occur as stated previously. However, identifying the growth regulating mechanisms of the system or cellular level has been elusive. Allelochemicals appear to alter a variety of plant processes (Rice, 1984) and it is difficult to separate primary from secondary effects. Determination of mechanisms of allelochemical action in a particular situation is furtner complicated by the fact that several different compounds may be involved, which may act in concert to reduce or stimulate thegrowth of other species.

Allelopathy research has often implicated phenolic derivatives of coumarin, cinnamic acid and benzoic acid as growth inhibitors (Rice, 1984). While there has been no comprehensive assessment of physiological effects of a single substance, phenolic acids have been reported to alter seedling metabolism in a variety of ways, which include changes in photosynthetic rate, stomatal function, chlorophyll content, respiration rate, flow of Carbon, mineral uptake and membrane permeability. Einhellig et al (1985) proposed a hypothetical action sequence suggesting allelochemical involvement in plant processes, as shown below.



Numerous allelochemicals probably result from decomposition of shoot material of crop plants, non-crop plants, weeds, tree species and/or their dried/decaying residues; it is not likely that all have the same mode of action. Einhellig et al (1985) found evidence that allelochemical stress on another plant may act through a change in the plant water status. Alteration in water balance that does occur is one of the mechanisms of growth inhibition. The action of specific allelochemicals such as p-Coumaric acid and Ferulic acid lowers the water potential by reducing turgor and osmotic potential and cause partial stomatal closure in young seedlings. Any such interfence of allelochemicals on the water balance would be very damaging to a plant, particularly at a very young seedling stage. Effects on water balance are likely to impede other physical processes with the combined action causing growth reduction.

CHAPTER 6

GENERAL DISCUSSION & CONCLUSIONS

Evidence for allelopathy has been accumulating over many years, and as reviewed previously, plants influence each other's growth by means of (a) volatile compounds released from leaves, (b) exudates, from roots and rhizomes, (c) leachates from live plant parts or residues, and (d) decomposing residues incorporated in the growing medium or residues in natural, undisturbed condition. Although allelopathy has been long considered and investigated as a form of 'Chemical Warfare between plants', perhaps the most dramatic manifestation of this phenomenon is that all substances liberated by plants are not inhibitory to other plants, but rather, may be quite stimulatory. Some compounds released by plants may be both inhibitory and stimulatory, depending upon concentration, physiological activity and other factors. Since plants produce growth regulators which are both stimulatory and inhibitory to their own development, thus controlling their own growth, it is not too difficult to imagine that the same or similar compounds could equally well control the growth of other plants in natural communities.

Rice (187) recently stated as follows: " .....Our increasing knowledge of allelopathy is aiding greatly in our understanding of many ecological phenomena. Our increasing awareness of conditions under which certain crop residues cause allelopathic effects on subsequent crops should enable us soon to guard against detrimental effects and to manage rotations to take advantage of stimulatory effects of plant residues. Available evidence indicates that it will be possible through breeding or biotechnology, to develop crop cultivars that will inhibit growth of the chief weeds in a given area through allelopathic action and thus decrease the need for synthetic weedkillers. We are already able to use allelopathic companion crops or residues of allelopathic crop plants and weeds to control weed growth in some crops and orchards. Our understanding of allelopathic interactions between various plant species has been used advantageously in reforestation, and future developments are encouraging. Considerable information is available concerning movement of chemicals from and factors determining their effectiveness after egression from plants. Nevertheless, these areas of allelopathy are probably the ones that merit the strongest research emphasis in the near future..."

Without any doubt allelopathic interactions are complex. Putnam (1985) stated that there are no cases where one chemical has been

unequivocally proven to explain an entire allelopathic situation. Almost all allelochemical interactions involve not only products of higher plants, but also those of micro-organisms, either as enhancers or detoxifiers of activity.

The current research concentrated on the potential allelopathy phenomena or interactions involved with the use of foliage or leaf residues (fresh or dried) of Gliricidia maculata and Tithonia diversifolia in large amounts as 'green manures' or 'mulches' in many agricultural situations in Sri Lanka. The foliage of Gliricidia maculata is known to provide excellent green manure to a variety of upland and lowland crops including rice. As stated previously in Chapter 1, on a dry weight basis Gliricidia leaves contain approximately 3.7-4.2 % nitrogen, 0.38-0.4 % phosphorous, 2 % potassium, 1.4 % calcium and 0.4 % magnesium, while Tithonia leaves contain 3.9 % nitrogen. Both are recommended for the use as 'bulk' green manure in several agricultural systems.

The potential of Gliricidia leaves to improve the general soil fertility (i.e. bulk density, organic matter content, exchangeable K, Ca, Mg, Nitrate-N, Ammonium-N, earthworm activity), overall productivity of rain-fed uplands of the dry zone of Sri Lanka, as well as their weed control ability when applied as 'mulches', has been highlighted by Handawela and Kendaragama (1992). They stated that, at the modest level of yields of tree loppings estimated at 5000 kg/ha/year and N analysis of the foliage at 3 %, nitrogen supply to the fields receiving Gliricidia foliage was about 150 kg/N/ha/year, of which about 16 % was removed by the crops grown. The fact that Gliricidia leaf residues had a profound effect on the composition of the weed flora and weed density, was also highlighted. Grass weeds in particular, appear to be more suppressed by Gliricidia leaves, than broad-leaf weeds.

The results of our research are in general agreement with these findings. The fact that aqueous extracts of Gliricidia stimulate the seed germination, growth and development of certain plant species, and inhibit the growth of others, is proved through the variety of laboratory and field studies. The well-known allelochemical, coumarin, was found to be released readily from fresh leaves upto 0.1% when they were dipped in water, for 24 hrs. From the experimental evidence it is possible to recognize the benefits of applying 2- 4 kg of mature, fresh leaves of Gliricidia per squre meter; it could be expected that Gliricidia leaves would provide a good fertilizer-effect and also cause suppression of weeds.

After applying the leaves as a surface mulch, daily watering or rainfall could easily cause leaching of coumarin to the soil underneath. Gradual liberation of coumarin due to weathering may also take place. Coumarin undoubtedly inhibits, germination of weed seeds lying shallow-

buried close to or on the soil surface. Subsequent applications of fresh leaves as surface mulch may provide continuous suply of coumarin which will make continuing impacts on weed seed germination. With the decaying of Gliricidia leaf material, the release of the triterpene Soyasapogenol, could also be expected. Soyasapogenol, which is a potent seed germination inhibitor and haustorial inducer, will also contribute very strongly to suppress of at least certain weed seeds from germination.

As discussed previously (Chapter 5), basically coumarin and its relatives, interrupt the cell division, cell elongation, arrest mitoses, particularly spindle formation, and cause ultrastructural reactions of the organisms (Cornman, 1946; Jensen & Welbourne, 1962; Jankay & Muller, 1976). During the decomposition process of Gliricidia fresh leaves materials, all coumarin or at least part of the amount contained, is likely to undergo bacterial conversion to form toxic compounds called dicoumarol and pathol, which are known to possess rodenticidal properties.

Gliricidia is undoubtedly an excellent allelopathic plant to be promoted further in domastic agriculture. There is ample evidence to suggest that Gliricidia leaves, used as surface mulch at 2 kg/m^2 and/or 4 kg/m^2, will control a wide variety of shallow-buried broad-leaf or grass weeds without any risk of environmental pollution, and at the same time to suply adequate amounts of nutrients, particularly N.

Tithonia diversifolia, unlike Gliricidia maculata, is an aggresive weed found in large populations, especially in the hill country of Sri Lanka. However, both are equally popular as green manures in the island. The characteristic bitter taste of the leaves is apparently due to the sesquiterpene lactones contained in them. From a series of laboratory studies it was proved that these leaves possess phytotoxic components in their leaves. In addition, field experiments and greenhouse studies also proved the ability of Tithonia leaf mulches for providing weed control combined with their fertilizer value, probably through release of nutrients. Treatments with 4kg/m^2 of fresh leaves gave the best weed control and caused considerable biomass increment of crop plants.

The present research found evidence that the phytotoxic activity of Tithonia diversifolia leaves was due to the presence of the germacranolides Tagitinin A, C and the new compound, Tagitinin G. Evidence supports the view that Tithonia fresh leaves can be recommended for use as a 'green manure' or 'surface mulch' in agricultural practices. Without any doubt, the sesquiterpene lactones that are released during the decay of Tithonia leaves have the ability to inhibit the germination of certain weed seeds, without unduly harming the crop plants. Furthermore, published literature indicates that the sesquiterpene lactones may protect crops from certain fungal and

bacterial attacks, and also prevent the crop from herbivore dameges because of the bitter taste of the leaves.

There is also a pressing need for better understanding of production by micro-organisms, of allelochemicals in soil or water that affect the growth of plants. This should extend also to the partial decomposition of a allelochemical from plants which produces more active compounds or simply more compounds which can increase allelopathic effects through additive or synergistic action. Grodzinsky (1987) also drew attention to the fact that one can never talk about the action of a single allelochemical: ".... everywhere they occur, many compounds having different biological activity may act simultaneously, perhaps mutually increasing their activity. As a rule, such allelochemicals are the intermediate products of soil humus, synthesis or the ground detritus in aquatic systems. High concentrations of these substances are lethal, moderate concentrations inhibit growth processes and low concentrations stimulate them...." (Grodzinsky, 1987)

Rice (1987) emphasizes that it is important to identify the allelopathic compounds in the substrate (soil or water) of the allelopathic plant, and to determine whether these compounds have come from the plant, are produced by partial decomposition of other compounds, or are synthesized by microorganisms using carbon sources from the plant. It is important to keep in mind that allelopathic compounds produced by microorganisms are just as much a part of the science of allelochemicals on those produced by higher plants, directly.

The weakest link in the chain of information concerning allelopathy is undoubtedly the movement of allelochemicals from the donor plant or its residues, to the recepient. There is a large body of indirect evidence, but only a small body of direct evidence concerning the movement of allelochemicals from the donor plant and uptake and translocation of the same compounds by the neighbour. Potential allelochemicals therefore, need to be tagged (with isotopes) in suspected allelopathic plants, and paths of the compound traced out of donor plants intoand through affected acceptor plants. Such studies should include possible movement of allelochemicals drom donor to receiver, through natuarl roots or stem grafts, mycorrhizal fungi and haustorial connections of parasitic plants (Rice, 1987).

After allelochemicals have been identified in the substrate, the concentration needs to be calculated and threshold concentration for activity should be determined against test plants using combinations of compounds present in the substrate, in addition to individual ones. Undoubtedly, many important allelopathic effects have been overlooked because of the use of single allelochemicals in determining threshold concentrations for activity.

Rizvi and Rizvi (1987) and Rice (1987) drew attention to the ways by which crop production may be improved through allelopathy. They are of the view that allelopathy can be exploited (a) to develop new crop management systems, and (b) for improving existing ones. They suggest that research in the use of the allelopathy phenomenon or allelochemicals in biological weed control should be vigorously pursued. This should include the use of residues as 'mulches' of allelopathic plants; rotation of crops, in which one or more of the crops is allelopathic towards major weeds; use of allelopathic 'cover crops'; underplanting of allelopathic 'companion crops' in orchards, vineyards and plantations etc., and the development (through breeding or genetic engineering) of crop cultivars which can control major weeds in a given area through their own allelopathic activity. Much more research is needed on the possible use of certain allelochemicals as herbicides or as structural models for herbicide development. However, it is relatively easy to see how plant residues such as those of Gliricidia and Tithonia could be manipulated to benefit field-cropping situations.

It has been customary in many parts of the developing world for peasant farmers to grow 2 or 3 crops/year in the same field, the rotational sequence being based on maintenance of soil fertility, soil structure and plant nutrients etc., but these have been generally formulated with little or no consideration for allelopathy. Any crop may release allelochemicals either directly in the form of exudate or vapours, or its residues/litter can produce them afterwards. Irrespective of the source, allelochemicals may affect the next crop in a rotational sequence either positively or negatively. It is unlikely that when crops are continuously supplying the soil with allelochemicals, these would not affect a follow-on crop. Rizvi and Rizvi (1987) tested this possibility with 2 rotational sequences: tobacco and maize, and tobacco and rice. Since the average nicotine content of tobacco leaves was 4%, and the leaf litter from a tobacco crop added up about 133 kg/ha of organic matter into soil, 5.32 kg nicotine/ha could get into soil, even without considering input through roots. Nicotine significantly increased the radicle and plumule growth of maize, but decreased all parameters studied in rice, radicle length being the most affected at 5 mM concentration. Results demonstrated different effects of Nicotine and suggested that careful consideration of allelochemical information of crops of a rotation management can improve its productivity, either by eliminating detrimental interactions or by exploiting beneficial ones.

Any improvement or new development in crop management based on allelopathic studies could not only increase production, but reduce expenditures on farm labour and agrochemicals. A reduction in the use of synthetic agrochemicals would lead to an improved quality of life. If allelochemicals can be developed as 'botanical pesticides' they would be better than synthetic ones owing to their smaller non-target toxicity, easy

biodegradability and non-polluting nature. If allelopathic potentials can be introduced into major crop cultivars in the world, it would provide an inexpensive, safe and relatively more permanent form of biological weed control. Burke (1987) also draws attention to the positive aspects of allelopathy-based weed control, as follows: "....the use of allelochemicals may overcome one of the many long-term drawbacks of synthetic agrochemicals, namely the unnatural excessive application of these chemicals. Such an application can be counter productive at times, in that the process of exogenously applying a high selection pressure of chemicals can cause rapid selection for organisms resistant to these agrochemicals, and therefore defeat the objective !....". Burke (1987) recommends the following priority areas for allelopathy research:

* Characterizing novel structures leading to new herbicides

* Identifying old structures with novel applications

* Developing and enhancing allelopathic properties of agronomic crops

Rice (1987) recently identified the following priority areas:-

* much research is needed on quantitative effects on crop yields of interference by most of the serious weeds, and on the relative contribution of allelopathy and competition to the total interference by each weed spp.

* Crop-crop relationships need to be investigated much more thoroughly to determine which crops can follow others with the least inhibitory or most stimulatory effects.

* More emphasis should be placed in investigations of stimulatory allelochemical effects, because these effects have been largely ignored in the past.

* Possible autotoxicity should be investigated also to determine if it is unwise to cultivate the same crop continuously in a given area, without rotations.

The allelochemicals which appear to be involved in the weed suppression abilities of leaf residues of Gliricidia (Coumarin, Soyasapogenol) and Tithonia (Tagitinin A, C, G) are relatively simple and mostly known compounds. These have thus far, never attracted the attention of weed scientists or chemists for development in any direction. However, Burke (1987), discussing the example of allelochemicals from Kalancho daigremontana, presents an excellent case of how similar simple compounds could turn out to be useful.

Kalancho daigremontana has been examined for allelopathic activity associated with the inhibition of growth of plantlets in soil close to the parent plant. Chloroform washings from leaves yielded 1-triacontanol (TRIA) as a major component, together with a few sterols and other unidentified components. Acetone extracts of roots gave Ferulate esters of normal C_{22}-C_{30} alcohols. In addition, isolates from the vermiculite used as soil in which Kalancho plants grew, gave phenolics from which Ferulic acid has been identified. Despite the marginal growth inhibition activity shown by these extracts, the detection of TRIA and Ferulic acid early in the chemical analysis had prompted further study.

Phenolics in general, and Ferulic acid in particular, have ample evidence as allelopathic agents. On the other hand, TRIA has been reported as a plant growth regulator. Burke (1987) states that although their research on Kalancho extracts could not duplicate the growth-promoting activities of TRIA either with whole plants or suspension cultures of test species, the discovery of Ferulic acid and TRIA linked together as a conjugate ester in the roots of Kalancho, was a significant finding. It has been suggested that this conjugate ester could, through enzyme hydrolysis, give rise to TRIA, which may then travel to aerial parts/leaves of the plant and promote its general well-being; Ferulic acid would simultaneously be extruded into soil in order to inhibit the growth of other plants in the surrounding medium.

Burke (1987) summarizes the significance of all this as below:

"...Although the compounds are well known and their effects well studied, the finding (of TRIA and Ferulic acid in a combined form) gives rise to a new concept in agrochemical design. Such a design would include, in a single molecule, a plant growth regulator and a herbicide or a pesticide-somewhat like having one's vitamins and antibiotics in a single capsule ! This is an example where one reward of pursuing allelochemicals is the novelty of the idea that accompanies trivial chemical products...."

Perhaps the same attitude should prevail in our search for potential uses of bioactive allelochemicals from natural sources and their development for appropriate practical applications.

Rizvi and Rizvi (1987) have also discussed at some length another novel idea, involving allelochemicals. Exploring the possibility of using allelochemicals as herbicides, they had tested several monoterpenes and terpenoids for suppression of germination and growth of the test weed Amaranthus spinosus and found that the monoterpene geraniol was the most potent, inhibiting the radicle growth by 100% and seed germination by 90% at 2 mM and completely inhibiting plumule growth at 3 mM. The possibility of geraniol being a fungicide had also been considered, since geraniol

inhibited the mycelial growth of Alternaria solani the test fungus, by 92%, at a concentration of 2 mM, and proved totally fungicidal above 2 mM. The occurrence of more than one pest controlling property in a single allelochemical such as geraniol, persuaded Rizvi and associates to propose using allelochemicals as Multipurpose pesticides, with arguably several benefits, such as :

(a) reduction in the cost of production and research since the cost of a single multipurpose compound would probably be less than the total cost involved with the development of several pesticides;

(b) reduction in total expenditure of crop production with the use of a single compound for crop protection;

(c) Since most allelochemicals affect only fewer non-target organisms, an improvement in quality of farm produce and through that improved quality of life;

(d) Any multipurpose pesticide would be ideal for use in Integrated Pest Management Systems (IPMS) which aim to minimize losses of crop yields and quality due to pests, through integration of several pest control approaches and achieve maximum benefits with minimum disadvantages. Despite being hazardous, a synthetic pesticide is often the dominating component of the IPMS. Moreover, an IPMS often requires simultaneous use of several synthetic pesticides, which, harmless singly, may become poisonous through interaction among themselves or their metabolites. Therefore, a viable approach would be to discover and use multipurpose pesticides, and thereby reduce the amounts of synthetic pesticides used. An allelochemical-based multipurpose pesticide would not only reduce the chances of synergistic toxicity, but also, being plant-derived, have all the merits of natural products.

Rizvi and associates (Rizvi & Rizvi, 1987) point out that geraniol, which has an ability to control more than one agriculturally important pest, a weed and a pathogenic fungus, is a multipurpose allelochemical. Up to 2.5 mM geraniol exerted no visible ill effects on a test crop, tomato, in which both the test pests are a problem. Thus the possibility of exploring allelopathy to develop multipurpose pesticides has promise !

As stated previously, in order to utilize allelopathic influences/ interference successfully in weed management, it is necessary to find naturally occurring chemical compounds in plants which inhibit seed germination, seedling development, and general growth or prevent propagule/ fruit production of weed species. These naturally occurring chemicals could

be either non-specific in action or act specifically only towards certain major weeds. Such compounds can be useful in many ways, one of the best known being the manipulation of crops themselves in an agroecosystem, as has been discussed before. An impressive list of relatively recent research has clearly lent support to the view which Overland in 1966 presented when she demonstrated that living plants and roots of barley actually produced and released toxic quantities of organic compounds capable of inhibiting the growth of common weed species.

Where crop lines that have co-evolved with interference from weeds have been studied, it appears that some are potentially capable of chemical inhibition. Putnam & De Frank (1974) screened a large germ plasm collection of Cucumis sativus and related Cucumis species and found several which demonstrated allelopathic activity at least in sand culture, under certain field conditions some cucumber accessions inhibited the growth of several weed species (Lockerman & Putnam, 1979). A large number of accessions of Avena sativa assayed by Fay and Duke (1977) for allelopathic activity, also yielded some very strong lines for inhibiting weeds through active exudation of a mixture of compounds.

Non-crop plants, either associated with crop species or planted as 'companion crops' or in rotational sequence with them, offer possibilities for allelopathic weed control. By producing toxins effective against weeds, but not harmful to the crop, the association of such plants can be manipulated to our considerable advantage. Published litertaure deals with several good examples, as given below.

Several leguminous non-crop species are apparently customarily intercropped or cover-cropped in rotation with corn in Mexico (Gleissman & Garcia, 1979; Gleissman et al, 1981). Two legumes (i.e. velvet-bean, Stizolobium deeringianum and sword-bean (Canavalia ensiformis) were grown in an area that was suffering from invasion of aggressive weed species. When water extracts of the fresh green leaf material of the two legumes(leachate extracts; 100 g soaked for 2 h in distilled water) were tested against three indicator crop species, both extracts significantly reduced the seed germination of cabbage, but not of corn and bean. However, the growth of seedlings of cabbage was severely inhibited, and that of corn and bean also inhibited by Canavalia leaf extract. Stizolobium also decreased cabbage seedling growth very significantly, affected corn slightly and had no effect on bean (Gleissman, 1983). Both Stizolobium and Canavalia demonstrated significant abilities to reduce weed numbers and biomass when planted as cover crops. Dry weights of weeds were reduced by as much as 57% by Stizolobium and by 68 by Canavalia compared with controls without the legume cover crops. Such legumes are planted in rotation with corn and are effectively used to shorted fallow from 4-5 years to only 6 months, in parts of Mexico (Gleissman, 1983). Stizolobium has been used in rotation with corn

on some soils continuously for 20 years, maintaining yields at close to 3.0 tons/ha, without the need for additional fertilizer or weeding other than the initial ground preparation at the time of corn planting. The fact that both legumes severely inhibited germination and growth of cabbage seeds, and not as significantly those of corn and beans, points to the possibility of tolerance in the crop components which have co-evolved through time with other plants. Cabbage, on the other hand, being a relatively recent introduction to new world agricultural systems, displays much less tolerance. Cannavalia, although managed in rotation with corn also, because of its bushy rather than vining habit, can be associated as an intercropped species, thus aiding directly in weed control during the cropping season (Gleissman, 1983).

In other studies aimed at demonstrating the potential for allelopathic interactions in crop-weed mixtures, bioassays of aqueous extracts of fresh or air-dried plant material of several crop, non-crop and weed mixtures were used by Gliessman (1983). Apparently "interplanting" of squash (Cucurbita pepo) in corn-bean polyculture or in monoculture stands, is common in certain parts of Mexico. Gleissman (1983) tested the leached extract of air-dried squash leaves for activity against corn, beans (Vigna sinensis) and cabbage in laboratory bioassays. Squash extracts caused a dramatic reduction in radicle elongation of cabbage (43% of controls), but only slightly affected corn and beans, confirming the suitability of the practice of interplanting squash with corn and bean. Gliessman (1983) explained that Mexican peasant farmers grew squash more for weed control and any harvest of fruit was really an added bonus. The squash is known to form a continuous plant cover over low-lying weeds, eliminating them, as well as restoring productivity of sol by adding large quantities of biomass (8-10 tons dry matter/ha).

These examples demonstrate the tremendously exciting possibilities offered by allelopathic plants, for manipulation to benefit our needs. It is not too diffult to see how both Gliricidia (which is even now the most common "Alley-cropping" hedge-row species), and Tithonia can be manipulated to our advantage.

Sri Lanka occupies a land area of 6.56 metric hectare of which only 2.62 M ha or 39.9% of total area has the potential for cultivation. Rapid expansion in population at 1.7% per annum has led to serious problems of land availability in much the same way as in other developing countries. One of the biggest challenges faced by agriculturists is to maintain or improve the level of productivity of Sri Lanka's agricultural lands and enable the country to produce food sufficient for its people. Continuous cultivation with excessive tillage has resulted in rapid decline of organic matter, tilth, nutrients, water holding capacity and an overall loss of soil fertility in many areas of agricultural land. Serious weed interferences

also threaten agricultural production, at least from time to time, in all areas of Sri Lanka's agriculture, without exception.

The present challenge, is undoubtedly to develop systems of farming which are environmently sound, ecologically stable and sustainable. The role of potential allelopathic plants and allelopathy phenomena in this, requires little emphasis, after the subject has evolved to the extent it has, as reviewed in this Report. It is our view that both Gliricidia maculata and Tithonia diversifolia are important tropical plants, which can make a significant impact on Sri Lanka's agriculture, provided that the appropriate research and development is carried out.

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LITERATURE CITED

Abdul-Wahab, A. L. and Rice, E. L. (1967) Plant inhibition by johnsongrass and its possible significance in old- field succession. Bull. Torrey Bot. Club, 94, 486-497.

Achhireddy, N. R. and Singh, M. (1984) Allelopathic effect on Lantana (Lantana camara) on milkweedvine (Morrenia odarata). Weed Sci., 32, 757-761.

Ahmad, S. Abdul- Wahab, and Rice, E. L. (1967) Plant inhibition by Johnson grass and its possible significance in old-field succession. Bull. Torrey Bot. Club, 94, 486-496.

Ahmad, V. U. Ahmed, W. Usmanghani, K. (1992) Triterpenoid saponins from leaves of Castanospermum australe. Phytochem, 31 (8), 2805-2807.

Aliotta, G., Cafiero, G., Fiorentino, A. and Strumia, S. (1993) Inhibition of radish seed germination and root growth by Coumarin and Phenylpropanoids. J. Chem. Ecol., 19 (2), 175-183.

Aliotta, G., De Napoli, L., Giordano, F., Piccialli, G., Piccialli, V. and Santacroce, C. (1992) An oleanane triterpene from Anagallis arvensis. Phytochem., 31(3), 929-933.

AlSaadawi I. S. and Rice, E. L. (1982a) Allelopathic effects of Polygonum aviculare L. I. Vegetational patterning. J. Chem. Ecol., 8, 993-1009.

AlSaadawi I. S. and Rice, E. L. (1982b) Allelopathic effects of Polygonum aviculare L. II. Isolation, characterizations and biological activities of phytotoxins. J. Chem. Ecol., 8, 993-1009.

AlSaadawi, I. S., Rice, E. L. and Karns, T. K. B. (1983) Allelopathic effects of Polygonum aviculare L. III. Isolation, Characterization and Biological Activity of Phytotoxins other than Phenols. J. Chem. Ecol., 9 (6), 761-774.

Altieri, M. A. and Doll, J. D. (1978) The Potential of Allelopathy as a Tool for Weed Management in Crops. PANS, 24, 495-502.

Anaya, A. L., Ramos, L., Hernandez, J. G. and Cruz, R. (1987). Allelopathy in Mexico. in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 89-101, ACS Symposium Series 330, American Chemical Society, Washington.

Arntzen, C. J., Falkenthal, S. V. and Bobick, S. (1974). Inhibition of photophosphorylation by kaempferol. Plant Physiol., 53, 304-306.

Ashton, F. M., Di Tomaso, J. M. and Anderson, L. W. J. (1985) Spikerush (Eleocharis sp.): A Source of Allelochemicals for the Control of undesirable aquatic plants. in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 401-414, ACS Symposium Series 268, American Chemical Society, Washington.

Avers, C. J. and Goodwin, R. H. (1956) Studies on roots. iv. Effects of coumarin and scopoletin on the standard root growth pathogen of Phleum pratense. Am. J. Bot., 43, 612-620.

Balke, N. E. (1985). Effect of Allelochemicals on mineral uptake and associated Physiological processes, in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 161-178, ACS Symposium Series 268, American Chemical Society, Washington.

Bandara, G. D. (1991) Comparsion of biomass production of Gliricidia sepium and Leucaena leucocephala. Proc. Second Regional Workshop on Multipurpose Tree Species in Sri Lanka, 47-60.

Bandara, S. M. K., Kendaragama, A. M. K. and Sangakkara, U. R. (1992) Effect of the tree component in alley cropping on chemical characteristics of soil and growth and yeild of maize. Proc. Third Regional Workshop on Multipurpose Tree Species in Sri Lanka, 52-60.

Bewley, J. D. and Black, M. (1985) Seeds. Physiology of Development and Germination. Plenum Press, New York.

Bhandari, D. C. and Sen, D. J. (1983) Allelopathy-An insight to Indian Work. Indian Rev. Life Sci., 3, 207-245.

Barnes, J. P. and Putnam, A. R. (1983) Rye residues contribute to weed suppression in no-tillage croping systems. J. Chem. Ecol., 9, 1045- 1057.

Barnes, J. P. and Putnam, A. R. (1986) Evidence for allelopathy by residues and aqueous extracts of rye (Secale cereale). Weed Sci.,34, 384-390.

Barnes, J, P. and Putnam, A. R. (1987) Role of Benzoxazinones in allelopathy by Rye (Secale cereale L.). J. Chem. Ecol., 13, 889-906.

Barnes, J. P., Putnam, A. R. and Burke, B. A. (1986) Allelopathic activity of Rye (Secale cereale L.). in Putnam, A. R. & Tang, C.S. (Eds): The Science of Allelopathy, pp 271-286, John Wiley & Sons Inc, New York.

Barnes, J. P., Putnam, A. R., Burke, B. A. and Aasen A. J. (1987) Isolation characterization of allelochemicals in Rye herbage. Phytochem., 26, 1385- 1390

Baruah, N. C., Sharma, R. P., Madhusudanan, K. P., Thyagarajan, G., Herz, W. and Murari, R. (1979) Sesquiterpene lactones of Tithonia diversifolia. Stereochemistry of the Tagitinins and related compounds. J. Org. Chem., 44(11), 1831-1835.

Baskin, J. M., Ludlow, C. J., Harris, T. M. and Wolf, F. T. (1967) Psoralen, an inhibitor in the seeds of Psoralea subacaulis (Leguminosae). Phytochem., 6, 1209-1213.

Bell, T. D. and Koeppe, D. E. (1972) Noncompetitive Effects of Giant Foxtail on the Growth of Corn. Agron. J., 64, 321-325

Bendall, G. M. (1975) The allelopathic activity of californian thistle [Cirsium avense (L.) Scop.] in Tasmania. Weed Res., 15, 77-81

Bhowmik, P. C. and Doll, J. D. (1982) Corn and Soybean response to allelopathic effects of weed and crop residues. Agron. J., 74, 601-606

Bhowmik, P. C. and Doll, J. D. (1983) Growth analysis of corn and soybean response to allelopathic effects of weed residues at various temperatures and photosynthetic photon flux densities. J. Chem. Ecol. 9 (8), 1264-1280

Bhowmik, P. C. and Doll, J. D. (1984) Allelopathic Effects of Annual Weed Residues on Growth and Nutrient Uptake of Corn and Soybean. Agron. J., 76, 383-388

Bonner, J. (1950) The role of toxic substances in the interaction of higher plants. Bot. Rev., 16, 51-65

Borner, H. (1959) The apple replant problem: I. The excretion of phlorizin from apple root residues. Contrib. Boyce Thompson Inst., 20, 39-56.

Borner, H. (1971) German research on allelopathy. Biochemical interaction among plants. 55-56. National Academy of Science, U.S.A.

Bradow, J. M. (1985) Germination promotion in Dormant Shepherdspurse (Capsella bursa-pastoris) Seeds by Strigol Analogs and other Stimulants. Weed Sci., 34, 1-7.

Buchholtz, K. P. (1971) The influence of allelopathy on mineral nutrition in U.S. in Biochemical Interactions Among Plants, pp. 86-89, Nat. Acad. Sci., Washington D.C.

Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1964) Structure elucidation of natural products by mass spectrometry. Vol 2, Holden-day-Inc.

Burgess, J. and Linstead, P. J. (1977) Coumarin inhibition of microfibril formation at the surface of cultured protoplasts. Planta, 133, 267-273.

Burke, B. A. (1987) Allelopathy: A Biotechnological-Agrochemical Approach in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 147-155, ACS Symposium Series 330, American Chemical Society, Washington.

Bykov, B. A. (1970) Vvedenie v fitocenologiju. "Nakua" Alma-Ata. Introduction to Phytocoenology (In Russian)

Camm, L. E., Chi-Kit, W. and Towers, G. H. N. (1976) An assessment of the role of furanocoumarins in Heracleum lanatum, Can. J. Bot., 54, 2562-2566.

Caussanel, J. P. and Kunesch, G. (1979) Qualitative and quantitative study of growth inhibitors in root exudates of common lambsquaters (Chenopodium album L.) at the beginning of its flowering on hydroponic culture and under controlled conditions. Z. Pflanzenphysiolr , 93, 229-231.

Chatterji, U. N. (1975) Some aspects of plant-plant chemical interactions. Indian J. Plant Physiol, 18, 91-96.

Cheema, Z. A., Ahmad, S., Majeed, S. and Ahmad, N. (1988) Allelopathic Effects of Wheat (Iriticum aestivum L.) Straw on germination and seedling growth of two weed species and cotton. Pak. J. Weed Sci. Res., 1 (2), 118-126.

Cheema, Z. A., Ahmad, S., Khan, M. A. and Ahmad, N. (1990) Cotton and weeds response to allelopathic effects of wheat residues and herbicide applications under two fertility levels. Pak. J. Weed Sci. Res., 3 (2), 65-77.

Chou, C. H. (1982) Allelopathy in agrosystems in Taiwan. Proc. Seminar on Allelochemicals and Pheromones, Taipei, R.O.C, 27-64.

Chou, C. H. (1987). Allelopathy in Subtropical Vegetation and Soils in Taiwan. in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 102-117, ACS Symposium Series 330, American Chemical Society, Washington.

Chou, C. H. and Chiou, S. J. (1979) Autointoxication mechanism of Oryza sativa 11. Effects of culture treatments on the chemical nature of paddy soil and rice productivity. J. Chem. Ecol., 5, 839-859.

Chou, C. H. and Chung, Y. T. (1974) The allelopathic potential of Miscanthes floridulus. Bot. Bull. Acad. Sin., 15, 14-27.

Chou, C.H. and Kuo, Y. L. (1986) Allelopathic research of subtropical Vegetation in Taiwan 111. Allelopathic Exclusion of Understory by Leucaena leucocephala (Lam.). J. Chem. Ecol., 12 (6), 1431-1447.

Chou, C. H. and Leu, L. L. (1992) Allelopathic substances and interactions of Delonix regia (Boj) Raf. J. Chem. Ecol., 18 (12), 2285- 2303.

Chou, C. H. and Lin, H. J. (1976) Autointoxication mechanisms of Oryza sativa: phytotoxic effects of decomposing rice residues in soil. J. Chem. Ecol., 2 (3), 353-376.

Chou, C. H. and Young, C. C. (1975) Phytotoxic substances in twelve subtropical grasses. J. Chem. Ecol., 1 (2), 183- 193.

Chou, C. H. and Patrick, Z. A. (1976). Identification and phytoxic activity of coumpounds produced during decomposition of corn and rye residues in soil. J. Chem. Ecol., 2: 369- 387.

Chowdry, K. P., Baruah, C. N., Sharma, R. P., Thyagarajan, G. and Herz, W. (1980) Structure of Deacetylviguiestenin (Tagitinin E) and Addendum. J. Org. Chem., 45, 535-536.

Corcoran, M. R., Geissman, T. A. and Phinney, B. O. (1972) Tannins as gibberellin antagonists. Plant Physiol., 49, 323-330.

Colton, E. C. and Einhellig, F. A. (1980) Allelopathic mechanisms of velvetleaf (Abutilon theophrasti Medic., Malvaceae) on Soybean. Amer. J. Bot., 67 (10), 1407- 1413.

Cornman, I. (1946) Alteration of mitosis by coumarin and parasorbic acid. Ame. J. Bot. 33, 217.

Datta, S. C. and Sinha-Roy, S. P. (1974) Allelopathy and inhibitors. Sci. & Cult., 40, 47-59.

De Candolle, M. A. P. (1832) Physiologie. Tome 111, Bechet Jeune, Lib. Fac. Med., Paris, 1474-1484.

Duke, S. (1986) Naturally occurring chemical compounds as Herbicides. Reviews of Weed Science, Vol 2, Weed Sci. Soc. America, 15-44.

Duke, S. O., Williams, R. D. and Markhart, A. H. (1984) Interactions of moisture stress and three phenolic compounds on lettuce seed germination. Ann. Bot., 52, 923-926.

Einhellig, F. A. (1984) Allelopathy- A natural protection, Allelochemicals, in Mandava, N. B. (Ed.): Handbook on Natural Pesticides : Methods, CRC Press, Boca Raton, FL.

Einhellig, F. A. (1986) Mechanisms and Modes of Action of Allelochemicals, in Putnam, A. R. & Tang, C. S. (Eds): The Science of Allelopathy pp 171-188, John Wiley & Sons Inc, New York.

Einhellig, F. A. (1989) Interactions among allelochemicals and other stress factors of the plant environment. in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 343-357, ACS Symposium Series 330, American Chemical Society, Washington.

Einhellig, F. A. and Kuan, L. (1971) Effects of scopoletin on stomatal aperture in tobacco and sunflower. Bull. Torry Bot. Club. 98, 155-162.

Einhellig, F. A. and Ramussen, J. A. (1979) Effects of three phenolic acids on chlorophyll content and growth of soybean and grain sorghum seedlings. J. Chem. Ecol., 5, 815-824.

Einhellig. F. A., Rice, E. L., Risser, P. G. and Wender, S. H. (1970) Effects of scopoletin on growth, CO_2 exchange rates, and concentration of scopoletin, scopolin and chlorogenic acids in tobacco, sunflower and pigweed. Bull. Torrey Bot. Club., 97, 22-23.

Elmore, C. D. (1985) Assessment of the Allelopathic Effects of Weeds on Field Crops in the Humid Midsouth, in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 21-32, ACS Symposium Series 268, American Chemical Society, Washington.

Evenari, M. (1949) Germination inhibitors. Bot. Rev., 15, 153-194.

Evenari, M. (1961) Chemical influences of other plants (allelopathy). In Ruhland, W (Ed.) Handbook d. Pflanzenphysiologie, pp. 691-736. Springer-Verlag, Berlin-Gottingen-Heidelberg.

Fay, P.K. and Duke, W. B. (1977) An assessment of allelopathic potential in Avena garmplasm. Weed Sci., 25, 224-228.

Fischer, N. H. (1986) The function of mono and sesquiterpene as plant germination and growth regulators. in Putnam, A. R. & Tang, C. S. (Eds): The Science of Allelopathy pp 203-218, John Wiley & Sons Inc, New York.

Fisher, R. F. (1987) Allelopathy: A Potential cause of Forest regeneration failure in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 176-184, ACS Symposium Series 330, American Chemical Society, Washington.

Fischer, N. H. and Quijano, L. (1985) Allelopathic Agents from common Weeds: Amaranthus palmeri, Ambrosia artimisifolia and related Weeds, in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 133-148, ACS Symposium Series 268, American Chemical Society, Washington.

Fischer, N. R., Oliver, E. J. and Fischer, H. D. (1979) In Progress in Chemistry of Organic Natural Products, Vol. 38, pp. 47- 429, Springer, N.Y.

Fischer, N. H., Weidenhamer, J. D. and Bradow, J. D. (1989) Dihydroparthenolide and other sesquiterpene lactones stimulate witchweed germination. Phytochem., 28 (9), 2315-2317.

Fischer, N. H., Weidenhamer, J. D., Riopel, J. L. , Quijano, L. and Menelaou, M. A. (1990) Stimulation of witchweed germination by sesquiterpene lactone: A structure-activity study. Phytochem., 29 (8), 2479-2483.

Fowden, L. (1974) Nonprotein amino acids from plants: Distribution, biosynthesis and analog functions. Recent Adv. Phytochem, 8, 95-122.

Friedman, T. (1987) Allelopathy in Desert Ecosystem. in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 53-68, ACS Symposium Series 330, American Chemical Society, Washington.

Friedman, T. and Horowitz, M. (1970) Phytotoxicity of subterranean residues of three perennial weeds. Weed Res.. 10, 382-385.

Fuerst, E. F. and Putnam, A. R. (1983) Separating the competitive and allelopathic components of Interference: Theoritical Principles. J. Chem. Ecol., 9, 937-944.

Funke, G. L. (1941) Essai de phytosociologie. Experimental Bull. Soc. Hist. Nat. Toulouse 76, 19- 21.

Gabor, W. E. and Veatch, C. (1981) Isolation of a Phytotoxin from Quackgrass (Agropyron repens) rhizomes.Weed Sci., 29, 155-159.

Gerald, R. L. and Einhellig, F. (1988) Bioassay of naturally occurring allelochemicals for phytotoxicity. J. Chem. Ecol., 14, 1821-1827.

Geissman, T. A., Griffin, S, Waddell, T. G. and Chen, H. H. (1969) Sesquiterpene lactones. Some new constituents of Ambrosia species: A. psilostachya and A. acanthocarpa. Phytochem, 8, 145-150.

Glass, A. D. M. and Dunlop, J. (1974) Influence of phenolic acids on ion uptake. IV. Depolarization of membrane potentials. Plant Physiol., 54, 855-858.

Gleissman, S. R. (1983) Allelopathic interactions in Crop-Weed Mixtures: Applications for Weed Management. J. Chem. Ecol., 9 (8), 991-999.

Gleissman, S. R. and Garcia, R. (1979) The use of subtropical legumes in accelerating the recovery of productivity of soils in the lowland humid tropics of Mexico. in Tropical Legumes: Resources for the Future, National Academy of Science Publication No. 27, Washington DC.

Gleissman, S. R., Garcia, R. and Amador, M. (1981) The adoption of traditional agricultural technology in the management of tropical agroecosystems. Agro Ecosystems, 7, 173-185.

Gnecco, S., Poyser, J. P. and Silva, M. (1973) Sesquiterpene lqctones from Podanthus ovatifolius. Phytochem., 12, 2469-2477.

Goodwin, R. H. and Kavanagh, F. (1949) The isolation of scopoletin, a blue-fluorescing compound of oat roots. Bull. Torrey Bot. Club, 76, 255- 265.

Gressel, J. B. and Holm, L. G. (1964) Chemical inhibition of crop germination by weed seeds and the nature of inhibition by Abutilon theophratsii, Weed Res., 4, 44-53.

Grodzinsky, A. M. (1987) Allelopathy in the Soviet Union. in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 39-43, ACS Symposium Series 330, American Chemical Society, Washington.

Gross, D. (1975) Growth regulating substances of plant origin. Phytochem., 14, 2105-2112.

Grummer, G. (1961) The Role of toxic substances in the relationships of higher plants. in Milthorpe, F. L., (ed): "Mechanisms in Biological Competition", pp. 219-228. Academic Press, N.Y.

Guenzi, W. D. and McCalla, T. M. (1962) Inhibition of germination and seedling Development by crop Residues. Soil Sci. Soc. Proc., 26, 456-458.

Guenzi, W. D. and McCalla, T. M. (1966a) Phenolic Acids in Oats, Wheat, Sorghum and Corn residues and their Phytotoxicity. Agro. J., 58, 303-304.

Guenzi, W. D. and McCalla, T. M. (1966b) Phytotoxic Substances Extracted from Soil. Soil Sci. Soc. Amer. Proc., 30, 214-216.

Guenzi, W. D. McCalla, T. M. and Norstadt, F. A. (1967) Presence and Persistence of Phytotoxic Substances in Wheat, Oat, and Sorghum Residues. Agron. J., 59, 163-165.

Gunasena, H. P. M., Mapa, R. B., Pushpa Kumara, D. K. N. G., and Hitinayaka, H. S. G. (1991) Effect of alley cropping on soil physical and chemical properties in the mid country intermediate zone. Proc. Second Regional Workshop on Multipurpose Tree Species in Sri Lanka, 78-92.

Gunatilleke, H. A. J. and Liyanage. M. De S. (1992). Current status and future needs in weed research in coconut in Sri Lanka. Tropical Weeds 1, 30- 39.

Handawela, J. and Kendaragama, K. M. A. (1991) Effect of Gliricidia sepium on upland soil fertility in the Dry Zone of Sri Lanka. Proc. of 2nd Regional Workshop on Multi-purpose Tree Species in Sri Lanka, 50-60.

Harrison, H. F. Jr. and Peterson, J. K. (1985) Allelopathic effects of Sweet potatoes (Ipomoea batatas) on yellow nutsedge (Cyperus esculentus) and alfalfa (Medicago sativa). Weed Sci., 34, 623-627.

Harper, J. R. and Balke, N. E. (1980) Inhibition of Potassium absorption in excised oat roots by phenolic acids. in Spanswick, R. M., Lucas, W. J. & Dainty, J. (Eds.) Plant Membrane Transport: Current Conceptual Issues, pp. 399-400, Elsevier, North Holland, New York.

Hasegawa, K. Mizutani, J. Kosemura, S. and Yamamura, S. (1992). Isolation and identification of lepidimoide a new allelopathic substances from mucilage of germinated cress seeds. Plant Physiol. 100, 1059- 1061.

Hasegawa, K. Shigenori, T. Urashima, M. Mizutani, J. Kosemura, S. and Yamamura, S. (1992). An auxin- inhibiting substances from light-grown maize shoots. Phytochemistry, 31, 3673-3676.

Heisey, R. M., Mishra, S. K., Putnam, A. R., Miller, J. R., Whitenack, C. J., Keller, J. E. and Huang, J. (1988) Biologically active natural products: Potential use in agriculture. Cutler, H. G. (Editor): ACS Symposium Series No. 380, Ame Chem. Soc. 66- 78.

Herz, W. and Sharma, P. R. (1975) A trans-1, 2- cis-4, 5- Germacradienolide and other New Germacranoloides from Tithonia species. J. Org. Chem., 40, 3118- 3123.

Hogetsu, T., Shibaoka, H., and Shimokoriyama, M. (1974) Involvement of cellulose in actions of gibberellin and kinetin-coumarin interactions on stem elongation. Plant Cell Physiol., 15, 265-272.

Holm, L. G., Plucknett, D. L., Pancho, J. B. and Herberger, J. P. (1977) The World's Worst Weeds. Distribution and Biology. University Press of Hawaii, Honolulu, 609 pp.

Horowitz, M. and Friedman, T. (1971) Biological Acitivity of subterranean residues of Cynodon dactylon (L.) Pers., Sorghum halepens L. and Cyperus rotundu L. Weed Res., 11, 88-93.

Hostettmann, K. and Marston, A. (1991) Modern Separation Methods. Natural Product Reports, 391- 413.

Irons, S. M. and Burnside, O. C. (1982) Competitive and allelopathic effects of sunflower (Helianthus annus). Weed Sci., 30, 372-377.

Inou, M., Nishimura, H., Li, H-H. and Mizutani, J. (1992) Allelochemicals from Polygonum sachalinensis FR.SCHM. (Polygonaceae). J. Che. Ecol, 18 (10), 1833-1841.

Jankey, P. Muller, W. H. (1976) The relationships among umbelliferone, growth and peroxidase levels in cucumber roots. Ame. J. Bot 63, 126-136.

Jacobsson, U. and Muddathir, A. K. (1992) Four biologically active sesquiterpenes of the dimane type isolated from Polygonum glabrum. Phytochem., 31 (12), 4207-4211.

Jensen, T. E. and Welbourne, F. (1962) The cytological effects of growth inhibitors on excised roots of Vicia faba and Pisum sativum. Proc. S. D. Acad. Sci., 41, 131-136.

Johnson III, W. C. and Coble, H. D. (1986) Effects of three Weed Residues on Weed and crop Growth. Weed Sci., 34, 403-408.

Jarvis, B. B., Pena, N. B., Madhusudana Rao, M., Comezoglu, N. S., Comezoglu, T. F. and Mandava, N. B. (1985) Allelopathic agents from Parthenium hysterophorus and Baccharis megapotamica. in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 149-160, ACS Symposium Series 268, American Chemical Society, Washington.

Jayawickrama, S. P. and Thenabadu, M. W. (1989) Studies on minaralization of four green manures commonly occuring in mid-country wet zone of Sri Lanka. J. Soil Sci. Sri Lanka, 6, 1-14.

Jensen, T. E. and Welbourne, F. (1962) The cytological effects of growth inhibitors on excised roots ofVicia faba and Pisum sativum. Proc. S. D. Acad. Sci. 41, 131- 136.

Juntilla, O. (1976) Allelopathic inhibitors in seeds of Heracleum laciniatum. Plant Physiol., 36, 374-378.

Kakuno, T. Yoshikawa, K. Arihara, S. (1992) Triterpene saponins from fruit of Ilex crenata. Phytochem, 31 (8), 2809- 2812

Kanchan, S. D. and Jayachandra, A. (1979a) Allelopathic effects of Parthenium hysterophorus L. I. Exudation of inhibitors through roots. Plant & Soil, 53, 27-35.

Kanchan, S. D. and Jayachandra, A. (1979b) Allelopathic effects of Parthenium hysterophorus L. III. Inhibitory effects of the weed residue. Plant & Soil, 53, 37-47.

Kanchan, S. D. and Jayachandra, A. (1980) Allelopathic effects of Parthenium hysterophorus L. IV. Identification of Inhibitors. Plant & Soil, 55, 67-75.

Kil, B. and Yim, Y. (1983) Allelopathic Effects of Pinus densiflora on undergrowth of Red Pine forest. J. Chem. Ecol., 9 (8), 1135-1148.

Kimber, R. W. (1973a) Phytotoxicity of Plant Residues II. The effect of rotting of straw from some aggressive grasses and legumes on the growth of wheat seedlings. Plant & Soil, 38, 347-361

Kimber, R. W. (1973b) Phytotoxicity of Plant Residues III. The relative effect of toxins and Nitrogen immobilization on the germination and growth of wheat. Plant & Soil, 38, 543-555.

Kobayashi, A., Morimoto, S., Shibata, Y., Yamashita, K. and Numata, M. (1980) C_{10}-Polyacetylenes as allelopathic substances in dominants in early stages of secondary succession. J. Chem. Ecol, 6, 119-131.

Koepp, D. E. (1972) Some reactions of isolated corn mitochondria influenced by juglone. Physiol. Plant., 27, 89-94.

Kumar, N. and Seshadri, R. T. (1976) A new triterpene from Pterocarpus santalinus bark. Phytochem., 15, 1417- 1418

Kuo, Y. L., Chou, C. H. and Hu, T. W. (1982) In Waller, G. R. & Chou, C. H. (ed.) Allelochemicals and Pheromones, pp. 07-119, Inst. of Botany, Academia Sinica, Taipei, R.O.C.

Leather, G. R. (1983a) Sunflowers (Helianthus annus) are Allelopathic to Weeds. Weed Sci., 31, 37-42

Leather, G. R. (1983b) Weed control using Allelopathic crop plants. J. Chem. Ecol, 9 (8), 983-989.

Leather, G. R. (1987) Weed control using allelopathic sunflowers and herbicide. Plant and Soil, 98, 17-23

Leather, G. R. and Einhelling, F. A. (1985) Mechanisms of Allelopathic action in Bioassay. in Thompson, A. C. (ed.): The Chemistry of Allelopathy-Biochemical Interactions Among Plants, pp. 197-205, ACS Symposium Series 268, American Chemical Society, Washington.

Leather, G. R. and Einhellig, F A. (1986) Bioassays in the study of Allelopathy, in Putnam, A. R. & Tang, C. S. (Eds.) The Science of Allelopathy, pp. 133-145. John Eiley & Sons, N.Y.

Leather, G. R. and Einhelling, F. A. (1988) Bioassay of naturally occuring allelochemicals for phytotoxicity. J. Chem. Ecol., 14, 1821-1827

Lee, I. K. and Monsi, M. (1963) Ecological studies onPinus densiflora forest, 1. Effect of plant substances on the floristic composition of the undergrowth. Bot. Magazine (Tokyo), 76, 400-413

Lehle, F. R. and Putnam, A. R. (1983) Allelopathic potential of Sorgnum: Isolation of seed germination inhibitors. J. Chem. Ecol., 9,1223- 1234

Levitt, J. and Lovett, V. J. (1984) Activity of allelochemicals of Datara stramonium L. (thorn apple) in contrasting soil types. Plant & Soil, 79, 181-189

Levitt, J., Lovett, V. J. and Garlick, P. R. (1984) Datura stramonium allelochemicals: Longevity in soil and ultrastructural effects on root tip cells of Helianthus annus L. New Phytol., 97, 213- 218

Li, H. H., Nishimura, H, Hasegawa, K., and Mizutani, J. (1992) Allelopathy of Sasa crenua. J. Chem. Ecol., 18 (10), 1785-1796.

Liebl, R. A. and Worsham, A. D. (1983) Inhibition of pitted morning glory (Ipomoea lacunosa L.) and certain other weed species by phytotoxic compounds of wheat (Triticum aestivum L.) straw. J. Chem. Ecol., 9 (8), 1027-1043.

Liyanage, M. De S. and Marasinghe, R. (1991) Performance of some introduced Gliricidia provenances in the low country intermediate zone of Sri Lanka. Proc. Second Regional Workshop on Multipurpose Tree Species in Sri Lanka, 8-18

Liyanage, L. V. K. and Wijerathne, A. M. U. (1987) Uses and Management of Gliricidia sepium in coconut plantations of Sri Lanka. Nitrogen Fixing Tree Association. Special Publication, 87 (1), 95-101.

Lockerman, R. H. and Putnam, A. R. (1979) Evaluation of Allelopathic Cucumbers (Cucumis sativus) as an aid for weed control. Weed Sci. 27, 54-57

Lockerman, R. H. and Putnam, A. R. (1981) Mechanisms for differential interference among cucumber (Cucumis sativus L.) accessions. Bot. Gaz., 142 (3), 427- 430

Lodhi, M. A. K. (1976) Role of allelopathy as expressed by dominating trees in a lowland forest in controll ng productivity and pattern of herbaceous growth. Am. J. Bot., 63, 1-8.

Lodhi, M. A. K. and Johnson, L. (1989) Forest understory biomass heterogeneity: Is "Moisture complex" or associated Litter the cause?, J. Chem. Ecol., 15, 429- 431

Lodhi, M. A. K. and Nickell, G. L. (1973) Effects of leaf extracts of Celtis laevigata on growth, water content, and carbon dioxide exchange rates of three grass species. Bull. Torry Bot. Club. 100, 159- 165.

Lovett, J. V. (1987) Allelopathy in Australia: Bacterial Mediation in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 156-174, ACS Symposium Series 330, American Chemical Society, Washington.

Lovett, J. V. and Potts, W. R. (1987) Primary effects of allelochemicals of Datura stramonium L. Plant & Soil, 98, 137.

Lovett, J. V., Levitt, J., Duffield, A. M. and Smith, N. G. (1981) Allelopathic potential of Datura stramonium (thorn apples), Weed Res., 21, 165 -170

Lynch, J. M. (1987) Allelopathy involving Microorganisms-Case Histories from the United Kingdom. in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 44-52, ACS Symposium Series 330, American Chemical Society, Washington.

Lynn, D. G. (1985) The Involvement of Allelochemicals in the Host Selection of Parasitic Angiosperms. in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 55-81, ACS Symposium Series 268, American Chemical Society, Washington.

Mandava, N. B. (1979) Natural Products in plant growth regulation. in Mandava, N. B. (ed.): Plant Growth Substances, pp. 135-, ACS Symposium Series 111, American Chemical Society, Washington.

Mandava, N. B. (1985) Chemistry and Biology of Allelopathic Agents, in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 33-54, ACS Symposium Series 268, American Chemical Society, Washington.

Mandava, N. B., McCalla, T. M. and Haskins, F. A. (1964) Phytotoxic Substances from soil micro-organisms and crop residues. Bacteriological Review, 28, 181-207

Manners, G. D. and Galitz, D. (1985) Allelopathy of Everlasting (Antennria microphylla): Identification of constituents phytotoxic to leafy Spurge (Euphorbia esula). Weed Sci. 34 8-12

Marcias, F. A., Galindo, J. C. G. and Massanet, G. M. (1992) Potential allelopathic activity of several sesquiterpene lactone models. Phytochem., 31 (6), 1969-1977.

McCalla, T. M. and Duley. F. L. (1949) Stuble mulch studies 111: Influence of soil microorganisms and crop residues on the germination, growth and direction of root growth of corn seedlings. Proc. Soil Sci.Society of America, 14, 196- 199

McCalla, T. M. and Haskins, F. A. (1964) Phytotoxic substances from soil micro-organisms and crop residues. Bacteriol. Rev., 28, 181-207.

Melek, F. R., Gage, D. A., Gershenzon, J. and Mabry, T. J. (1985) Sesquiterpene lactone and Diterpene constituents of Helianthus annuus. Pnytochem., 24, 1537-1539

Menges, R. M. (1987) Allelopathic effects of Palmer Amaranth (Amaranthus palmeri) and other plant Residues in Soil. Weed Sci., 35, 339-347

Miller, H. E., Mabry, T. J., Turner, B. L. and Payne, W. W. (1968) Intraspecific variation in sesquiterpene lactones in Ambrosia psilostachya (Compositae). Am. J. Bot., 55, 316-324.

Molisch, H. (1937) Der Einfluss einer Pflanae auf die andere-allelopathie. Gustav Fisher Verlag, Jena.

Moreland, D. E., Egley, G. H., Worsham, A D. and Monaco, T. J. (1966) Regulation of plant growth by constituents from higher plants. Adv. Chem, 53, 112-141.

Muir, A. D. and Majak, W. (1983) Allelopathic potential of diffuse knapweed (Centaurea diffusa) extracts. Can. J. Plant Sci. 63,989-996

Mukherjee, M., Ghatak, K. L. Ganguly, S. N. and Antoulas, S. (1984) Rubinic acid, a triterpene acid from Rubus fruticosus. Phytochem., 23, 2581 - 2582.

Muller, W. H. (1965) Inhibitory terpenes volatilized from Salvia shrubs, Bull. Torrey Bot. Club, 92, 38-45·

Muller, C. H. (1966) The role of chemical inhibition (allelopathy) in vegitational composition, Bull. Torrey Bot. Club, 93, 332-351

Muller, C. H. (1969) Allelopathy as a factor in Ecological Process, Vegetatio, 18, 348-357

Muller, C. H. and Chou, C. H. (1972) Phytotoxins: an ecological phase in phytochemistry, in Harbone, J. B. (Ed.): Phytochemical Ecology, pp. 201-216, Academic press, London.

Muller, C. H. and Moral, R. D. (1966) Soil toxicity induced by terpenes from Salvia leucophylla. Bull. Torrey Bot. Club, 93(2), 130-137

Muller, C. H. and Muller, W. H. (1964) Volatile growth inhibitors produced by Salvia spp. Bull. Torrey Bot. Club, 91, 327-330.

Muller, C. H., Muller, W. H. and Haines, B. L. (1964) Volatile growth inhibitors produced by shrubs, Science, 143, 471-473.

Murray, R. D. M., Mendez, J. and Brown, S. A. (1982) The Natural Coumarins: Occurrence, Chemistry and Biochemistry. Wiley, Chichester, U.K.

Nair, M. G., Epp, M. D. and Burke, B. A. (1988) Ferulate esters of higher fatty alcohols and allelopathy in Kalanchoe daigremontina, J. Chem. Ecol., 14, 589- 602

Napoli, D. L., Piccialli, G., Piccialli, V., Santacroce, C. and Carolla, R. (1992) Two oleanane tritrepenes from Anagallis arvensis, Phytochem., 31, 3965 -3968

Nicollier, G. F. and Thompson, A. C. (1982) Phytotoxic compounds from Meliotis alba (white sweet clover) and isolation and identification of two new flavonoids. J. Agric. Food Chem., 30, 760-764.

Nicollier, G. F., Pope, D. F. and Thompson, A. C. (1985) Phytotoxic compounds isolated and identified from weeds. in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 207-218, ACS Symposium Series 268, American Chemical Society, Washington.

Nielson, K. F., Cuddy, T. and Woods, W. (1960) The influence of the extracts of some crops and soil residues on germination and growth. Can. J. Plant Sci., 40, 188-197.

Osvald, H. (1950) An antagonism between plants. Int. Congress Bot. (Stockholm), 7, 167-170.

Overland, L. (1966) The role of allelopathic substances in the 'smother crop' barley, Am. J. Bot., 53, 423-432

Papanov, G. Bozov, P. Malakov, P. (1992) Triterpenoids from Lavandula sepica. Phytochem. 31 (4), 1424-1426

Patrick, Z. A. (1955) The peach replant problem in Ontario. II. Toxic substances from microbial decomposition products of peach root residues. Can. J. Bot., 33, 461-486.

Patrick, Z. A. (1971) Phytotoxic substances associated with the decomposition in soil of plant residues. Soil Sci., 111, 13-18.

Patrick, Z. A. and Koch, L. W. (1958) Inhibition of respiration, germination and growth by substances arising during decomposition of certain plant residues in soil. Can. J. Bot., 36, 631-647.

Patrick, Z. A., Toussoun, T. A. and Koch W. L. (1964) Effect of crop residue decomposition products on plant roots. Ann. Rev. Phytopath., 2, 267-292

Patrick, Z. A., T. A. Toussoun, T. A. and Snyder, W. C. (1963) Phytotoxic substances in arable soils associated with decomposition of plant residues. Phytopathol., 53, 152-161.

Patterson, D. T. (1981) Effects of allelopathic chemicals on growth and physiological responses of soybean (Glycine max). Weed Sci., 29, 53-59.

Pepperman, Jr., A. B. and Blanchard, E. J. (1985) Improvement in the synthesis of Strigol and its analogues in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 415-425, ACS Symposium Series 268, American Chemical Society, Washington.

Perera, A. N. F. (1992) Gliricidia as a fodder in liverstock production- Sri Lanka experience, Proc. Third Regional Workshop on Multipupose Tree Species in Sri Lanka, 72-79

Peters, E. J. and Luu, K. T. (1985) Allelopathy in Tall Fescue, in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 273-284, ACS Symposium Series 268, American Chemical Society, Washington.

Pickering, S. V. (1917) The effect of one plant on another. Ann. Bot., 31, 181-187

Pickering, S. V. (1919) The action of one crop on another. Jour. Roy. Hort. Soc., 43, 372-380

Pope, D. F., Thompson, A. C. and Cole, A. W. (1985). Phytotoxicity of Root Exudates and Leaf Extracts of nine plant species, in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 219-234, ACS Symposium Series 268, American Chemical Society, Washington.

Prakash, N. E. and Tissera, K. M. E. P. (1992) Effect of multipurpose trees on soil microflora in agroforestry practices, Proc. Third Regional Workshop on Multipurpose Tree Species in Sri Lanka, 43-51

Pratish, K. C., Baruah, N. C., Sharma, R. P., Thangarajan, G and Herz, W. (1980). Structure of Deacetylviguiestenin (Tagitinin E). J. Org.Chem., 45, 535-536

Proebsting, E. L. (1950) A case history of a "peach replant" situation. Proc. Am. Soc. Hort. Sci., 56, 46-48.

Putnam, A. R. (1983a) Allelopathic Chemicals: Nature's herbicides in action. Chem. Eng. News, 4, 34- 45

Putnam, A. R. (1983b) Allelopathy: a breakthrough in weed control? American Fruit Grower, 103, 10

Putnam, A. R. (1985a) Weed Allelopathy in Duke, S. O. (Ed.): Weed Physiology, Vol.1: Reproduction and Ecophysiology. pp. 131-155, CRC Press Inc., Florida, USA

Putnam, A. R. (1985b). Allelopathy Research in Agriculture- past highlights and potential, in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 1-8, ACS Symposium Series 268, American Chemical Society, Washington.

Putnam, A. R. (1986) Allelopathy: Can it be managed to benefit Horticulture? HortSci., 21 (3), 411-413

Putnam, A. R. (1988a) Allelochemicals: Blessings or curses for agriculture? Comments Agric. & Food Chem., 1 (4), 183-206

Putnam, A. R. (1988b) Allelochemicals from plants as herbicides, Weed Tech., 2, 510-518

Putnam, A. R. and DeFrank, J. (1983) Use of phytotoxic plant residues of selective weed control, Crop Protec., 2, 173-181

Putnam, A. R. and Duke, W.B. (1974) Biological suppression of weeds: Evidence for allelopathy in accessions of cucumber, Science, 185, 370-372

Putnam, A. R. and Duke, W. B. (1978), Allelopathy in Agroecosystems, Ann. Rev. Phytopath., 16, 431-451

Putnam, A. R. and Tang, C. S. (1986) Allelopathy: State of the Science, in Putnam, A. R. & Tang, C. S. (Eds.) The Science of Allelopathy, pp. 1-17. John Eiley & Sons, N.Y.

Putnam, A. R. and Weston, L. A. (1986) Adverse Impacts of Allelopathy in Agricultural Systems. in Putnam, A. R. & Tang, C. S. (Eds.) The Science of Allelopathy, pp. 43-56. John Eiley & Sons, N.Y.

Putnam, A. R., De Frank, J. and Barnes, J. P. (1983) Exploitation of Allelopathy for Weed Control in Annual and Perennial Cropping Systems, J. Chem. Ecol., 9, 1002-1010

Raghwendra Pal, D., Kulshreshthra, D. K. and Rastogi, R. P. (1976) Chemical constituents of Withonia tagitiflora Desf. Part II. Structure of Tagitinin B by application of homonuclear INDOR Spectroscopy, Ind. J. Chem., 14B, 77-80

Raghwendra Pal, D., Kulshreshthra, D. K. and Rastogi, R. P.(1977) Chemical constituents of Withonia tagitifolia Desf. Part IV. Tagitinin C, D & F. Ind. J. Chem., 15B, 208-211

Rahman, A. (1986) Nuclear Magnetic Resonance. pp. 357, Springer- Verlag, New York.

Rahman, A. (1989) One and Two dimentional NMR Spectrocopy. Elsevir, New York.

Rahman, A. U. and Pervin, A. (1991) The Nuts and Bolts of Modern Two-Dimentional NMR Spectroscophy- A suggested Protocol for Structure Elucidation. J. Chem. Soc. Pak., 13, 127-151

Rahman, A. U. and Ahmad, V. U. (1992) ^{13}C- NMR of Natural products, I: Mono and Sesquiterpenes, pp. 281. Plenum press. New York.

Reher, G. and Budesinsky, M. (1992) Triterpenoids from plants of the Sanguisorbeae. Phytochem., 31 (11), 3909-3914

Rice, E. L. (1968) Inhibition of nodulation of inoculated legumes by pioneer plant species from abandoned fields. Bull. Torrey Bot. Club, 95, 346-358

Rice, E. L. (1971) Inhibition of nodulation of inoculated legumes by leaf leachates from pioneer species from abandoned fields. Am. J. Bot, 58, 368-371

Rice, E. L. (1974) Allelopathy. Academic press, New York. 353 pp.

Rice, E. L. (1979) Allelopathy- an update. Bot. Rev., 45, 15-109.

Rice, E. L. (1984) Allelopathy. 2nd ed. Academic Press, New York, USA. 422 pp.

Rice, E. L. (1986) Allelopathic Growth Stimulation, in Putnam, A. R. & Tang, C. S. (Eds.) The Science of Allelopathy, pp. 23-42. John Eiley & Sons, N.Y.

Rice, E. L. (1987) Allelopathy: An Overview. in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 8-22, ACS Symposium Series 330, American Chemical Society, Washington.

Rice, E. L. and Pancholy, S. K. (1972) Inhibition of nitrification by Climax ecosystems, Am. J. Bot., 59, 1033-1040

Rice, E. L. and Pancholy, S. K. (1973) Inhibition of Nitrification by climax ecosystems 11. Additional evidence and posible role of tannins, Am. J. Bot., 60, 691-702

Rizvi, S. J. H. and Rizvi, V. (1987) Improving Crop Productivity in India-Role of Allelochemicals. in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 69-75, ACS Symposium Series 330, American Chemical Society, Washington.

Rizvi, S. J. H., Mukerjee, D. and Mathur, S. N. (1981) Selective Phytotoxicity of 1,3,7 trimethylxanthine between Phaseolus mungo and some weeds. Agric. Biol. Chem., 45, 1255 - 1256

Robinson, T. (1980) The Organic Constituents of Higher Plants. Cordus Press. North Amherst. 352 pp.

Rodrigues, E. Towers, G. H. N. and Mitchell, J. C. (1976) Biological activities of sesquiterepene lactones. Phytochem., 15, 1573- 1580

Rovira, A. D. (1969) Plant Root Exudates, Bot. Rev., 35, 35-51

Rudiger, W. and Lohaus, E. (1987). Germination and Growth Inhibitors as Allelochemicals. in Waller, G. R. (ed): Allelochemicals: Role in Agriculture & Forestry, pp. 118-128, ACS Symposium Series 330, American Chemical Society, Washington.

Saggese, E. J., Foglia, T. A., Leather, G., Thompson, M. P., Bills, D. D. and Hoagland, P. D. (1985). Fractionation of Allelochemicals from Oilseed Sunflowers and Jerusalem Artichokes, in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 99-112, ACS Symposium Series 268, American Chemical Society, Washington.

Sangakkara, U. R. (1991) Early growth and nodulation patterns of Gliricidia sepium, Proc. Second Regional Workshop on Multipurpose Tree Species in Sri Lanka, 30-39

Sashida, Y. Ogawa, K. Mori, N. Yamanouchi, T. (1992) Triterpenoids from the fruit galls of Actinidia polygama. Phytochem., 31 (8), 2801-2804

Schreiner, O. and Reed, S. H. (1907) The production of deleterious excretions by roots. Bull. Torrey Bot. Club., 34, 279- 303

Schreiner, O. and Reed, H. S. (1908) The toxic action of certain organic plant constituents, Bot. Gaz. 45, 73 - 102

Schreiner, O. and Sullivan, M. X. (1909) Soil fatigue caused by organic compounds, J. Biol. Chem., 6 39-50

Schwimmer, S. (1958) Influence of popyphenols and potato components on potato phosphorylase. J. Biol. Chem., 232, 715-721.

Seo, S., Tomita, Y. and Tori, K. (1975) Carbon -13 NMR spectra of Urs- 12-Enes and application to structural assignments of components of Isodon japonicus Hara tissue cultures. Tetra. Lett., 1, 7-10.

Sen, D. N. (1981) Ecological Approaches to Indian Weeds. Geobios International, Jodhpur.

Seneviratne Banda, K. M. Kendaragama, K. M. A. Sangakkara, U. R. (1992). Effect of the tree component in alley cropping on chemical characteristics of soil and growth and yeild of maize. Proc. Third regional workshop on multipurpose tree species in Sri Lanka, 52- 60.

Shettel, N. L. and Balke, N. E. (1983) Plant growth responses to several allelopathic chemicals. Weed Sci., 31, 293-298.

Shilling, D. G., Leibl, R. A. and Worsham, A. D. (1985). Rye (Secale cereale L.) and Wheat (Triticum aestivum L.) Mulch: The suppression of certain broadleaf weeds and the Isolation and identification of Phytotoxins, in Thompson, A. C. (ed.): The Chemistry of Allelopathy-Biochemical Interactions Among Plants, pp. 243-272, ACS Symposium Series 268, American Chemical Society, Washington.

Singh, M., Tamya, V. R. and Nigg, H. N. (1989) HPLC identification of allelopathic compounds from Lantana camara, J. Chem. Ecol., 15, 81-89.

Smeda, R. J. and Putnam, A. R. (1988) Cover Crops Suppression of Weeds and Influences on Strawberry Yields. HortSci., 23 (1), 132- 134.

SSSA, (1979) Glossary of soil Science terms. Soil Science Society of America. Madisan, Wisconsin. 36.

Stevens, K. L. and Merril, G. B. (1985). Sesquiterpene Lactones and Allelochemicals from Centaurea spp, in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 83-98, ACS Symposium Series 268, American Chemical Society, Washington.

Svensson, S. B. (1972) Effect of coumarin on growth, production of dry matter, protein and nucleic acids in roots of maize and wheat and the interaction of coumarin with metabolic inhibitors. Physiol. Plant., 27, 13-24.

Szczepanski, A. J. (1977) Allelopathy as a means of biological control of water weeds. Aquatic Bot., 3, 193-197.

Tang, C. S. and Young, C. C. (1982) Collection and identification of allelopathic compounds from undisturbed root system of bigalta limpograss (Hemarthria altissima). Plant Physiol., 69, 155-160.

Tang, C. S. and Zhang, B. (1986) Qualitative and quantitative determination of the allelochemical sphere of germinating mungbeans, in Putnam, A. R. & Tang, C. S. (Eds.): The Science of Allelopathy, pp. 229-249, John Wiley & Sons, N.Y.

Theophrastus. (ca 300 B.C.) Enquiry into plants and minor Odours and Weather Signs: Vol;2 (Translated to English by A. Hort), W. Heinemann Publishers, London. (1916)

Toussoun, T. A., Weinhold, A. R. Linderman, and Patrick, Z. A. (1968) Nature of Phytotoxic Substances Prodiced during Plant Residue Decomposition in Soil. Phytopath., 58, 41-45

Tubbs, C. H. (1973) Allelopathic relationships between yellow birch and suger maple seedlings, Forestry Sci., 19, 139- 145

Tukey, H. B. Jr. (1969) Implications of Allelopathy in Agricultural Plant Science, Bot.Rev., 35 1-16

Ulubelen, A., Brieskorn, C. H. and Ozdemir. (1977) Triterpenoids of Salvia horminum constitution of a new diol, Phytochem.,16, 790-791

Van Aller, R. T. Pessoney, G. F., Rogers, V. A., Watkins, E. J. and Leggett, H. G. (1985) Oxygenated fatty acids: A class of allelochemicals from aquatic plants. in Thompson, A. C. (ed.): The Chemistry of Allelopathy- Biochemical Interactions Among Plants, pp. 387-401, ACS Symposium Series 268, American Chemical Society, Washington.

Van Sumere C. F. and Massart, L. (1959) Natural substances in relation to germination. Proc. Int. Cong. Biochem. 4th., 5, 20-32.

Van Sumere, C. F., Cottenie, J., De Greef, J. and Kint, J. (1972) Biochemical studies in relation to the possible germination regulatory role of naturally occurring coumarin and phenolics. Recent Adv. Phytochem, 4, 165-221.

Walters, D. T. and Gilmore, A. R. (1976) Allelopathic effects of fescue on the growth of sweetgum. J. Chem. Eco., 2. 469- 479

Waller, G. R. Kumari, D. Freedman, J. Freedman, N. and Chou, C. H. (1986) Caffeine autotoxicity in Coffea arabica L. in Putnam, A. R. & Tang, C. S. (Eds.): The Science of Allelopathy, pp.243- 269, John Wiley and Sons, New York.

Wang, T. S. C., Yang, T. K. and Chuang, T. T. (1967) Soil phenolic acids as plant growth inhibitors, Soil Sci., 103, 239-246

Weerakoon, W. L. (1982) Organic fertilizer (Recycling of Organic matter), Country Report. Proc. Asian Productivity Organization Symposium, Tokyo. 197-216.

Weston, L. A. and Putnam, A. R. (1986) Inhibition of legume seedling growth by residues and extracts of quackgrass (Agropyron repens) Weed Sci., 34, 366- 372

Weston, L. A., Burke, A. B., Putnam, A. R. (1987) Isolation Characterization and activity of phytotoxic compounds from Quackgrass [Agropyron repens (L.) Beauv.], J. Chem. Ecol., 13, 403- 421

Whitlon, D. S., Sadowski, J. A. and Suttie, J. W. (1978) Mechanisms of coumarin action: Significance of Vitamin K epoxide reductase inhibition. Biochemistry, 17, 1371-1377.

Whittaker, R. H. and Feeny, P. P. (1971) Allelochemics: Chemical interactions between species. Science, 171, 757- 770

Wijewardene, R. and Waidyanatha, P. (1984) Conservation Farming: Systems, Techniques & Tools. Department of Agriculture, Sri Lanka.

Wilson, E. R. and Rice, E. L. (1968) Allelopathy as expressed by Helianthus annuus and its role in old- field succesion. Bull. Torrey Bot. Club 95(5) 432- 448

Winter, A. G. (1961) New physiological and biological aspects in the interrelationships between higher plants. Symp. Soc. Expt. Biol., 15, 229-244.

Wu, M. M. H., Liu, C. L. and Chao, C. C. (1976) Identification and purification of the phytotoxin produced by Fusarium oxysporum Schl. in relation to yeild decline of ratoon cane. J. Chinese Agric. Chem. Soc., 14 (3,4), 160-165

Yamane, A., Fujikura, J., Ogawa, H. and Mizutani, J. (1992a) Isothiocyanates as allelopathic compounds from Rorippa indica Hiern. (Cruciferae) Roots. J. Chem. Ecol., 18 (11), 1941-1954.

Yamane, A., Nishimura, H. and Mizutani, J, (1992b) Allelopathy of Yellow Field cress (Rorippa sylvestris): Identification and Characterization of Phytotoxic constituents. J. Chem. Ecol., 18 (5), 683-691.

Yoshioka, H., Mabry, T. J. and Timmermann, B. N. (1973) Sesquiterpene lactones: Chemistry, NMR and Plant Distribution. pp. 2-21, University of Tokyo press, Tokyo.

Young, C. C. (1986) Autointoxication of Asparagus officinalis in Putnam, A. R. & Tang, C. S. (Eds.): The Science of Allelopathy, pp. 101-112, John Wiley and Sons, New York.

Xue, Z. H., Lu, Z. Z., Konno, C., Soejarto, D., Cordell, G. A., Harry, H. S., Fong, and Hodgson, W. (1988) 3-- (3,4- Dihydroxy cinnamoyl)- Erythrodiol and 3--(4- Hydroxycinnamoyl)- Erythrodiol from Larrea tridentata. Phytochem., 27(1), 233- 235

Zuniga, G. E., Argandona, V. H. Niemeyer, H. M. and Corcuera, L. D. (1983) Hydroxamic acid content in wild and cultivated Gramineae. Phytochem., 22, 2665-2668.

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APPENDIX I

Plate 1: Gliricidia leaf mulch applied to field plots (2 kg/m^2), one month after application, showing considerable weed control

Plate 2: Gliricidia leaf mulch applied to field plots (4 kg/m^2), one month after application, showing considerable weed control

Plate 3: Tithonia leaf mulch applied to field plots (2 kg/m^2), one month after application, showing considerable weed control

Plate 4: Tithonia field trial (Field Trial III) control-weeded plots, three month after initiation of trial, showing vigorous crop growth in absence of weeds

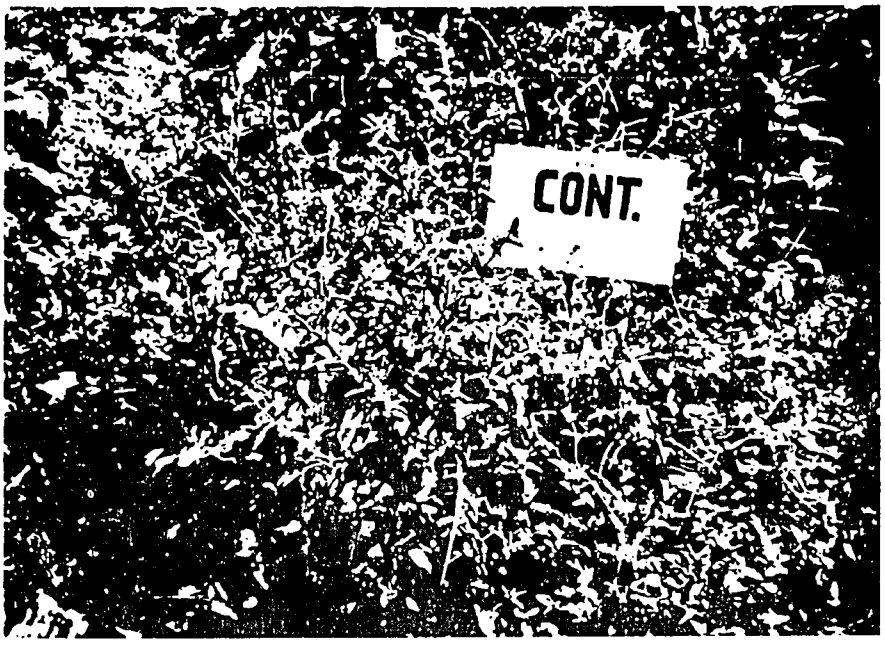

Plate 5: Tithonia field trial (Field Trial III) control-unweeded plots, three month after initiation of trial, showing rampant weed growth and very poor crop growth

Plate 6: Tithonia field trial (Field Trial III) Treatment plots which received 2 kg/m^2 leaf residues, three month after initiation of trial

Plate 7: Tithonia field trial (Field Trial III) Treatment plots which received 4 kg/m^2 leaf residues, three month after initiation of trial

Plate 8: Tithonia field trial (Field Trial III) Treatment plots which received 6 kg/m^2 leaf residues, three month after initiation of trial