EFFECT OF CURCUMINS AND OIL OF TURMERIC (*CURCUMA LONGA*) ON THE PHOTOSTABILITY AND EFFICACY OF AZADIRACHTIN AND AZADIRACHTIN RICH NEEM OIL

HEMANTA CHOWDHURY

DIVISION OF AGRICULTURAL CHEMICALS
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI - 110 012
1996

Dedicated

To My Beloved Parents

EFFECT OF CURCUMINS AND OIL OF TURMERIC (*CURCUMA LONGA*) ON THE PHOTOSTABILITY AND EFFICACY OF AZADIRACHTIN AND AZADIRACHTIN RICH NEEM OIL

by

HEMANTA CHOWDHURY

A Thesis
submitted to the Faculty of the Post-Graduate School
Indian Agricultural Research Institute, New Delhi,
in partial fulfilment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

IN

AGRICULTURAL CHEMICALS

1996

Approved by:

Chairman : _____
(Dr. Suresh Walia)

Members : _____
(Dr. B.S. Parmar) 23/12/96

_____
[Dr. (Ms.) P. Dureja]

_____
(Dr. P.J. Rao)

_____
(Dr. S.C. Dutta)

Dr. Suresh Walia
Senior Scientist

Division of Agricultural Chemicals
Indian Agricultural Research Institute
New Delhi - 110 012.

CERTIFICATE

This is to certify that the thesis entitled, **Effect of Curcumins and Oil of Turmeric (*Curcuma longa*) on the Photostability and Efficacy of Azadirachtin and Azadirachtin Rich Neem Oil**, submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in **Agricultural Chemicals**, embodies the results of a *bona fide* research work carried out by **Mr. Hemanta Chowdhury**, under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

All the assistance and help received during the course of the investigation have been duly acknowledged by him.

(**Dr. Suresh Walia**)
Chairman
Advisory Committee

Date : November 18, 1996.
Place : New Delhi

ACKNOWLEDGEMENT

It is a great privilage for me to express my esteemed and profound sense of gratitude to the chairman of my advisory committee, Dr. Suresh walia, Senior Scientist, Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi, for suggesting me a challenging problem, judicious and precious guidance, constant encouragement and constructive criticism during the course of investigation.

I feel immense pleasure to convey my heartfelt thanks to the Head, Dr. S.K. Handa, and Professor and member of my advisory committee, Dr. B.S. Parmar, of the Division of Agricultural Chemicals for providing necessary facilities and encouragement throughout this study.

I express my sincere bouquet of gratitude to the esteemed members of my advisory committee Dr. (Ms) Prem Dureja, Dr. S.C. Dutta Senior Scientists of the division of Agricultural Chemicals and Soil Science and Agriculture Chemistry respectively and Dr. P.J. Rao, Principles Scientist, Division of Entomology. A special word of thanks to Dr. (Mrs.) S. Dhingra for providing the necessary facilities, guidance and suggestions during the bioassay studies.

My heartfelt thanks to Dr. V.S. Saxena, Senior Scientist, Division of Entomology for his invaluable suggestion and inspiration during the course of study.

I take this opportunity to acknowledge Neem Mission, Pune, for providing good quality neem seeds and Dr. R.P. Singh (Entomolgy) for providing standard azadirachtin sample.

I am greatfuly indebted to Dr. R.C. Gupta, Department of Chemistry, State University, New York, USA, for recording mass spectra, as well as Mr. Subrato Choudhury and Mr. Joginder Singh, Bruker India, for recording ^1H NMR spectra (400 MHz) of the compunds. Mr. Somyajulu, Mr. A.P. Singh Verma and Mr. B.D.

Gupta need a special word of mention for the help and assistance rendered to me by them.

Candid thanks to Parthada, Tapanda, Kuntal, Anup, Ranjan, Kali, Apurba, Shishir, Chapu, Gour, Manish, Anil, Manjree and Sujoy whose concerted efforts have enable me to reach this milestone. I thankfully acknowledge the help rendered by Sridevi and Sudhakar in the Bioassay studies.

Special thanks to Garg Type and Electrostat constituting Mr. and Mrs Garg, Surender and Pramod for typing the manuscript neatly and correctly.

I convey my heartiest thanks and deepest sense of respect to my parents and my brother and sisters who prayed to the All mighty for my success.

This endeavour would have remained a dream without the constant love and inspiration of a person who was there whenever I needed him the most - Amrit, just a thought away.

Finally, the Senior Research Fellowship awarded by the Indian Agricultural Research Institute, New Delhi is gratefully acknowledged.

IARI, New Delhi ***(HEMANTA CHOWDHURY)***
Dated : 18th November, 1996

CONTENTS

Chapter	Title	Page No.
1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	5
3.	MATERIALS AND METHODS	51
4.	RESULTS AND DISCUSSIONS	80
5.	SUMMARY AND CONCLUSIONS	127
	BIBLIOGRAPHY	i - xix

INTRODUCTION

, Owing to extensive chemicalization of agriculture, pesticides science over the past five decades has progressed tremendously. The role of chemicals in pest control has remained unchallenged, however, following the publication of Rachel Carson's "Silent Spring" thirty five years ago, the use of synthetic pest control chemicals has become the focus of controversies. Over a period of time, excessive use of synthetic insceticides has led to the development of insect resistance to insecticides, insecticide-induced resurgence of pests, and has inflicted adverse effects on non-target organisms such as natural enemies of insects, parasites and predators, honey bees, pollinators, fish, cattle and above all human beings. Some pesticides and their degradation products/ metabolities cause serious damage to the environment and the agricultural produce. As a result of the excessive use of insecticides in crop protection and public health, the general population is also subjected to the risk of their toxic residues.

Botanicals and biopesticides being environmentally benign are considered as potential alternative to synthetic pesticides. The plant kingdom which is a rich storehouse of biologically active compounds can thus be exploited to develop pest control chemicals materials of economic value.

Plant chemicals or botanicals are often referred to as either primary or secondary metabolites. Primary metabolites are substances widely distributed in nature, occurring in one form or

another in virtually all organisms. In higher plants such compounds are mainly concentrated in seeds and vegetative storage organs and are needed for physiological development because of their roles in basic cell metabolisms. These include products namely vegetable oils, fatty acids, and carbohydrates such as sucrose, starch, pectin and cellulose etc. Secondary metabolites have no apparent function in the plant's primary metabolism but often have an ecological role. They are pollinator attractants, represent chemical adaptations to environment stress, or serve as chemical defences against micro-organisms, insects and higher predators. Some of the secondary metabolites are merely the end products of bio-synthetic pathways and other excretory products. The toxic principles in plants are perhaps produced as a result of the defence mechanism evolved by plants against hervibore onslaught to enhance the chances of its survival. The remarkable diversity of natural insecticides evolved in plants is the result of adaptation due to intense herbivore pressure (Spencer, 1988). Examples of commonly useful secondary metabolites are nicotine, the pyrethrins and rotenone, which are used in limited quantities as pesticides. Secondary natural products often have highly complex structures with many chiral centers which may determine biological activity. A good example of a secondary metabolite having a high degree of structural complexity is the naturally occurring neem based insecticide azadirachtin (Bilton *et al.*, 1987).

Out of 2,50,000 angiosperms so far documented, over 2000 existing species of higher plants have been reported to possess insecticidal activity (Crosby, 1971). The plants that have shown

potential in pest control during past few years are neem- *Azadirachata indica* A. Juss.; China berry - *Melia toosenden* L. and *M. azedarach* L. (Chiu, 1984); West Indian mahogany - *Swietenia mahagony* Jacq. (Chiu, 1984); custard apple - *Annona squamosa* L. (Mariappa and Saxena, 1983); African marigold - *Tagetes erecta* L. (Morallo-Rejesus and Eroles, 1978); French marigold - *Tagetes patula* L. (Morallo - Rejesus and Decena, 1982); thunder god - vine - *Tripterygium wilfordii* Hook (Chiu and Zhang, 1982); *Ryania speciosa* (Jefferies *et al.*, 1991); *Vitex rotundifolia* (Watanabe *et al.*, 1995) and *Artemisia annua* (Duke *et al.*, 1987; Brown, 1993).

In recent years, products of neem (*Azadirachta indica*) have come under close scrutiny of scientists around the world as the most promising source of natural insecticides. Since 1980, four major international conferences (Two in Germany and one each in Kenya and Australia) and one world neem conference in India have been held to focus attention on the neem tree. Being environment friendly, neem based products are increasingly sought after throughout the world and in the foreseeable future, its share in global pesticide market is going to increase dramatically. However, like other botanicals, neem products have the problem of limited stability and less persistence under field conditions. Because of the negative influence of temperature, UV and sunlight, pH, rainfall, humidity and other environmental factors on the active principles, the residual effect of neem based products is generally restricted to less than 7 days. Among these abiotic factors, light is perhaps the most important in bringing about the degradation of

neem products and consequently the decreased residual life. Under these circumstances, it is necessary to photostabilize azadirachtin based neem products so that their residual life is increased sufficiently to provide better insect control.

Since synthetic stabilizers are not likely to be safe, stabilization by natural anti-oxidants seems to be a method of choice for their environmental suitability. The crude extract of *Curcuma longa* rhizome turmeric oil and curcuminoids are well known for their insect repellant and anti-oxidative properties. The anti-oxidative potential of curcumins , therefore, offer an opportunity to explore the possibility of stabilizing azadirachtin and azadirachtin spiked neem oil with curcuminoids and other turmeric products. With this objective in mind, studies were conducted to investigate the effect of curcumins and turmeric oil on the photostability and efficacy of azadirachtin rich neem oil.

REVIEW OF LITERATURE

2.1 *Azadirachta indica A. Juss* (Neem)

Azadirachta indica A. Juss. (Syn. *Melia indica* Brandis, *Melia azadirachta* Linn., and *Melia parviflora* Moon.) belongs to the family Meliaceae. The commercial use of neem is known from the vedic period in India over 4000 years B.C. (Lauridsen *et al.*, 1991) and the domestic uses have been mentioned by Kautilya in his Arthashastra (4th century B.C) (Ray, 1956). Neem is known to grow wildly in the Shivalik hills and the dry forests of Andhra Pradesh, Tamil Nadu and Karnataka. The tree occurs widely in Pakistan, Bangladesh, Srilanka, Malayasia, Indonesia, Thailand, Middle East, Sudan and Niger. In Ayurveda the neem tree is regarded as 'Sarva Roga Nivarini'. Neem also has various environmentally beneficial attributes (Govindachari, 1992). On account of the large canopy, it releases high amount of oxygen during the day time as compared to other trees. Hence, it has a reputation as an air purifier. The tree increases soil fertility and soil water-holding capacity.

Neem is an undemanding tree and thrives successfully on dry, stony, clayey and shallow soils. Its root system has an unusual ability to glean nutrients and moisture even from highly leached sandy soils. It can flourish on calcarious soils with pH upto 8.5. Neem can stand shade and temperature upto 49°C but cannot survive frost, however, neem trees in Dade County, Florida, have occasionally withstood temperature below 0°C (Anonymous, 1980).

The neem fruit is an ovoid drupe 1.4-2.4 cm long. They are green when young and turn yellow to brown when ripe. The epicarp is thin and endocarp is hard and bony. The mesocarp is pulpy and is eaten by humans, birds and animals. The seed is ovoid or spherical and the weight of seed kernel accounts only for about 10% of that of the whole fruit.

India having wide variations in soil and climate has a very large population of neem trees. It is estimated that in India 14 to 25 million neem trees are in existance. (Ketkar, 1976). A fully grown tree is expected to yield about 50 kg fruits and 350 kg leaves annually depending on rainfall and soil conditions. Assuming the lower figure of 14 million neem trees in India, these are expected to produce 0.7 million metric tonnes of fruits and about 5 million metric tonnes of leaves annually.

2.1.1 Chemical Constituents of Neem

Neem is a storehouse of chemically diverse and structurally complex bioactive molecules. It has been a subject of several reviews (Warthen, 1979; Hegnauer, 1983; Jones *et al.*, 1990; Schmutterer, 1990; Devakumar and Sukhdev, 1993; Mordue and Blackwell, 1993). Influenced by its folk-lore medicinal values, the chemists took up the task of isolating active principles from various parts of neem tree in general and neem fruits in particular. In the year 1942, Siddiqui was successful in isolating major bitter principle nimbin in a crystalline form (mp 205°C, $[\alpha]_D$ + 170°) for the first time along with two other crystalline compounds nimbinin and nimbidin from neem oil.

Fruits are the most important source of the ingredients of neem that affect insects in various ways. Several derivatives are obtained from neem fruits. Besides the fruit; leaves, bark, fruit coat and root also contain a variety of bioactive principles. Chemical components isolated from various parts of the neem tree are described as follows.

2.1.2 Neem Leaves and Flowers

The steam distillate of the fresh matured leaves yield a number of cyclic tri and tetrasulfides (Pant *et al.*, 1986). Besides, the long chain alkanes consisting of octadecane, nonadecane, hexacosane, nonacosane, tetratriacontane, *n* - hexacosanol, *β*-carotene and xanthophyll were identified form leaves. Polyphenolics like quercetin, isorhamnetin, isoprenylated flavanone, nimba flavone (Garg and Bhakuni, 1984) flavanoglycosides of quercetin and myrecetin were also detected from leaves. The flowers have been reported to contain flavonoides such as kaempferol, myricetin, quercetin and their flavonoglycosides (Subramanian and Nair, 1972).

The fresh winter leaves of neem have been reported to contain protomeliacins (Table I), nimbocinone (I) (Siddiqui *et al.*, 1986b), nimocinolide, isonimocinolide (Siddiqui *et al.*, 1986a) and isonimbocinolide (Siddiqui *et al.*, 1986d). Among these compounds, nimocinolide showed mild insect growth regulating property and affected fecundity of house fly (*Musca domestica*) and mosquito (*Aedes aegypti*) at a dosage of 100-500 ppm. In another study, two limonoids of azadirone (II) group, nimocin (Siddiqui *et al.*, 1986a),

Table 1. Chemical components from neem leaves and flowers/twigs

S.No.	Name	Molecular formula	Melting point (°C)	Chemical nature
1.	Nimbocinone	$C_{30}H_{46}O_4$	78	Protomeliacin
2.	Nimocinolide	$C_{28}H_{36}O_7$	160	Limonoid with all rings intact and containing γ-hydroxybutenolide side chain
3.	Isonimocinolide	$C_{28}H_{36}O_7$	165	-do-
4.	Isonimbocinolide	$C_{32}H_{42}O_9$	-	-do-
5.	Nimocin	$C_{33}H_{38}O_4$	195	Limonoid of *azadirone* group.
6.	Meldenindiol	$C_{26}H_{34}O_4$	-	-do-
7.	Isomeldenin	$C_{28}H_{36}O_5$	-	-do-
8.	4α, 6α -Dihydroxy-A-homoazadiradione	$C_{28}H_{36}O_6$	180	-do-
9.	Vilasinin	$C_{26}H_{36}O_5$	255	Limonoid with all rings, intact and A-ring carrying 1, 3-dioxygen functions, *vilasinin* group.
10.	Azadirachtanin-A	$C_{32}H_{40}O_{11}$	225	-do-
11.	Dihydronimbic acid	-	-	C-secomeliacin, *nimbin* group
12.	Nimbolide	$C_{27}H_{30}O_7$	245-247	-do-
13.	28-Deoxynimbolide	$C_{27}H_{32}O_6$	170	-do-
14.	Nimbandiol	$C_{26}H_{32}O_7$	121	-do-
15.	6-0-Acetyl nimbandiol	$C_{28}H_{34}O_8$	178	-do-
16.	Isoazadirolide	$C_{32}H_{42}O_{10}$	130	C- seco limonoids containing γ-hydroxy butenolide side chain.
17.	Margosinolide	$C_{27}H_{32}O_8$	130	-do-
18.	Isomargosinolide	$C_{27}H_{32}O_8$	125	-do-
19.	Desacetyl nimbinolide	$C_{28}H_{34}O_{10}$	196	-do-
20.	Desacetyl isonimbinolide	$C_{28}H_{34}O_{10}$	180	-do-

meldenin diol from fresh leaves, isomeldenin (Pachapurkar *et al.*, 1974) from yellow leaves and 4 α, 6 α-dihydroxy A - homoazadiradione (Bruhn *et al.*, 1984) from dried leaves have also been reported. Vilasinin (Pachapurkar *et al.*, 1974) and azadirachtanin -A (III), two pro-C-seco-meliacins were also isolated from fresh green leaves. Whereas, vilasinin was formed as a result of oxidation of C_{28} followed by C_{28}-C_6 linkage through ether bridge instead of B-D ring fusions, Azadirachtanin-A contains a C_{19}-C_{29} ether linkage (Rao *et al*,1977). Among the C-secomeliacins with or wihtout modified side chain, three analogues of nimbin namely, 2,3-dihydronimbic acid and nimbolide (Ekong, 1967), 28-deoxo-nimbolide and isoazadirolide have been isolated from neem leaves. From the green twigs margocinolide and its three congeners, 23-oxo isomer, two isomeric desacetyl nimbinolide and desacetyl isonimbinolide (Siddiqui *et al.*, 1986c) have also been isolated.

2.1.3 Root and Stem Bark

Among the twenty four tricyclic diterpenoids (Table-2) isolated from root and stem bark, sugiol (12-hydroxy-13-methyl, podacarpa-8, 11, 13-trien-7-one) was the first to be isolated from each series respectively (Sengupta *et al.*, 1960). Other structurally related compounds have different substituents at 3, 12, and 13 positions of podacarpanoid and abietanoid nucleus. In addition to the co-occurrence in seed oil, gedunin and deacetyl gedunin have also been isolated from neem bark. In this group of compounds D-ring has undergone oxidative expansion. Gedunin has been reported to possess both antifungal and antimalarial properties. Whereas, nimbolin - A was isolated from trunk bark/heart wood,

Table 2. Chemical component in neem root and stem bark

S.No.	Name	Molecular formula	Melting point (^{0}C)	Chemical nature
1.	Sugiol	$C_{16}H_{20}O_2$	297	Phenolic diterpenoids
2.	Nimbisonol	$C_{18}H_{24}O_3$	179	-d0-
3.	Nimbinone	$C_{18}H_{22}O_3$	125	-d0-
4.	Nimbiol	$C_{18}H_{24}O_2$	250	-d0-
5.	Nimosone	$C_{20}H_{26}O_3$	73	-d0-
6.	Methylnimbiol	$C_{19}H_{26}O_2$	143	-d0-
7.	Margocin	$C_{20}H_{26}O_2$	134	-d0-
8.	Margocilin	$C_{20}H_{28}O_3$	127	-d0-
9.	Margocinin	$C_{20}H_{26}O_4$	144	-d0-
10.	Nimbidiol	$C_{17}H_{22}O_3$	223	-d0-
11.	Nimbionol	$C_{18}H_{24}O_4$	129	-d0-
12.	Nimbionone	$C_{18}H_{22}O_4$	79	-d0-
13.	Demethyl nimbionol	$C_{17}H_{22}O_4$	135	-d0-
14.	Methyl nimbionone	$C_{19}H_{22}O_4$	119	-d0-
15.	Nimbione	$C_{18}H_{22}O_3$	103	-d0-
16.	Nimbonone	$C_{20}H_{28}O_2$	69	-d0-
17.	Nimbosodione	$C_{19}H_{24}O_3$	135	-d0-
18.	Nimbosone	$C_{20}H_{28}O_2$	139	-d0-
19.	Nimbonolone	$C_{20}H_{28}O_2$	77	-d0-
20.	Margosone	$C_{20}H_{28}O_3$	173	-d0-
21	Margosolone	$C_{18}H_{24}O_3$	170	-d0-
22.	Nimbilicin*	$C_{20}H_{24}O_3$	-	-d0-
23.	Nimbocidin*	$C_{19}H_{30}O_2$	-	-d0-
24.	Nimolinin*	$C_{20}H_{28}O_3$	114	-d0-
25.	Nimbilin*	$C_{32}H_{50}O_{10}$	149-150	C-seco meliacin, *salannin* group

contd...../2

Table 2. (contd...)

S.No.	Name	Molecular formula	Melting point (°C)	Chemical nature
26.	Nimbandiol	$C_{26}H_{32}O_7$	121	C-seco meliacin, *nimbin* group
27.	6-0-Acetyl nimbandiol	$C_{28}H_{34}O_8$	178	-do-
28.	Isonimbinolide	$C_{30}H_{36}O_{11}$	114	C-seco Limonoid containing γ- hydroxy butenolide side chain
29.	Gedunin	$C_{28}H_{34}O_7$	220	Limonoid with oxidatively expanded ring D, *gedunin* group
30.	7 - Desacetyl gedunin	$C_{26}H_{32}O_6$	259-62	-do-

* Root bark and heart wood.

nimbolin - B and nimbilin were obtained from trunk wood (Fujiwara *et al.,* 1984) and root bark (Ara *et al.,* 1989b) respectively. These compounds are considered as precursors to C-secomeliacins and contained C_7 cinnamoyloxy moiety instead of acetoxy/tigloyl substituent in other vilasinin derivatives. Two new polyacetate derivatives, margosinone and margosinolone have also been reported from stem bark (Ara *et al.,* 1989a).

Aqueous extract of bark contained gallic acid, (+) gallocatechin, (-) epicatechin, (+) catechin and epigallocatechin. Gallic acid, (-) epicatechin and (+) catechin have been reported to inhibit the generation of chemiluminiscence by activated human polymorphonuclear leukocytes (Van der Nat *et al.,* 1991). Condensed tannins to the extent of 15% were reported to occur in bark (Hegnauer, 1983). Stem bark contains bicyclic carbohydrates such as polyscaccharides GIa and Glb (Fujiwara *et al.,* 1982), polysaccharides GIIa and GIIIa (Fujiwara *et al.,* 1984) and GIII Do' Ia and IIa (Van der Nat *et al.,* 1987).

2.1.4 Fruit Coat

When activity of neem fruit coat extractive (RB-b) and neem seed extractive (RB-a) was evaluated (Siddiqui *et al.,* 1996) against *M. domestica*, it was noted thant RB-b fraction was 200 times more effective (LC_{50} 1.00 ppm) than RB-a fraction (LC_{50} 200 ppm). When formulated with PBO and triton-X, RB-b fraction was found superior (LC_{50} 6.28 $\mu g/cm^2$) to methoprene (LC_{50} 18.85 $\mu g/cm^2$) against the test insect *Sitophilus oryzae.* Their observation led to the isolation and characterization of three known

triterpenoids epoxy azadiradione, azadiradione (Lavie *et al.*, 1971) and kulactone (Siddiqui *et al.*, 1991) and eleven new compounds listed in Table 3.

2.1.5 Neem Seed/Kernel

Neem seed is the storehouse of neem oil and a large number of tetraterpenoids therein. Neem kernels contain about 20-45% fixed oil constituting normal glycerides of oleic (53%), stearic (19%), palmitic (16%), linoleic (11%) and minor amounts of azachidic, behenic, lignoceric and myristic acids (Devakumar and Mukerjee, 1983; Rukmini, 1987). About 43 headspace volatiles/ steam volatile constituents of neem seed/oil have been identified by GC-MS analysis (Balandrin *et al.*, 1988; Mubarak and Kulatilleke 1990; Riar *et al.*, 1990). The steam volatiles of neem oil have been shown to possess contraceptive action (Sinha *et al.*, 1984; Devakumar *et al.*, 1990; Riar *et al.*, 1990).

The bitterness of neem is due to the occurrence of limonoids which are tetranortriterpenoids based on apo-euphol (or apo-tirucallol) skeleton. The term 'limonoid' is derived from limonin, the first tetranortriterpenoid obtained from citrus bitter principles (Taylor, 1984). The limonoids occurring in Meliacae are also known as meliacins. Out of over 300 limonoids known from various sources, about one third is accounted by neem (*Azadirachta indica*) and Chinaberry (*Melia azedarach*) alone. Structure of most of the complex meliacins isolated from neem seeds have been elucidated on the basis of spectroscopic studies particularly ^{1}H NMR, 2D ^{1}H NMR, ^{13}C NMR, and NOE studies. Among the various

Table 3. Chemical constituents in neem fruit coat

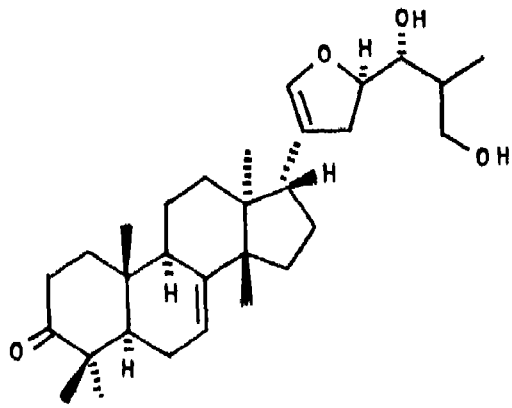
S.No.	Name	Molecular formula	Melting point (°C)	Chemical nature
1.	Kulactone	$C_{30}H_{44}O_3$	164	Protomeliacin
2.	Limocinol	$C_{30}H_{50}O$	-	-do-
3.	Limocinone	$C_{30}H_{48}O$	-	-do-
4.	Azaditol	$C_{32}H_{46}O_6$	112	-do-
5.	Limocin-A	$C_{29}H_{42}O_5$	-	-do-
6.	Limocin-B	$C_{29}H_{42}O_5$	-	-do-
7.	Limocinin	$C_{34}H_{44}O_6$	-	-do-
8.	Azadiradione	$C_{28}H_{34}O_5$	168	Limonoid of *azadirone* group.
9.	Nimolinone	$C_{30}H_{44}O_3$	190	Protomeliacin
10.	Azadirachtol	$C_{32}H_{46}O_6$	112	-do-
11.	Azadirachnol/ Naheedin	$C_{32}H_{48}O_6$	171	-do-
12.	Isonimolicinolide	$C_{30}H_{36}O_9$	102	Limonoid with all rings intact and containing γ- hdroxybutenolide side chain
13.	Nimocinol	$C_{28}H_{36}O_5$	130	Limonoid of *azadirone* group
14.	17-α-Hydroxy azadiradione	$C_{28}H_{54}O_6$	-	-do-
15.	Nimbocinol	$C_{26}H_{32}O_4$	161	-do-
16.	Nimolicinolic acid	$C_{26}H_{34}O_6$	94	-do-
17.	Nimolicinol	$C_{28}H_{34}O_7$	270-274	Limonoid with oxidatively expanded ring D, *gedunin* group.

groups of meliacins which differ from each other in basic nuclear structure and pattern of oxygenation, C-secomeliacins are most important as azadirachtin, salannin and nimbin are part of it. List of the chemical constituents present in neem seed kernels is given in Table 4a and 4b.

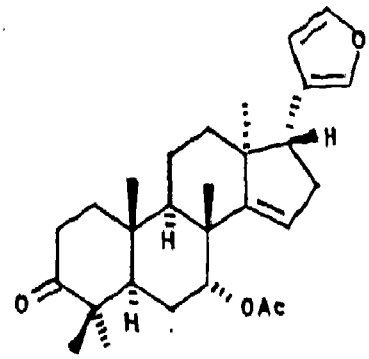
2.1.5.1 C-seco meliacins of nimbin and salannin group

C-Secomeliacins are the most complex triterpenoids confined largely to the neem and the Persian lilac, *Melia azedarach*. Most of these compounds belong to nimbin (13 compounds), salannin (9 compounds) and azadirachtin (14 compounds) group of compounds. Atleast seven C-secomeliacins containing γ-hydroxy butenolide as the side chain in place of furan ring have been isolated from neem seeds.

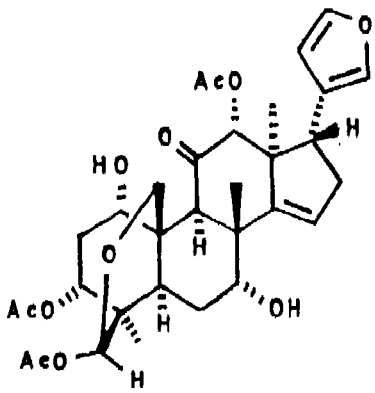
The isolation of nimbin (IV) in 1942 marked the beginning of the chemistry of neem meliacins (Siddiqui, 1942). Once the structure of nimbin became known by spectroscopic and chemical methods, assignment of structures for its analogues became fairly easy. Some of the important analogues of nimbin include, 6-desacetyl nimbin, 4-epinimbin (V), nimbidinin (VI), nimbinal, nimbinol, nimbolide, nimbene and nimbandiol. During the isolation of nimbin from neem oil, a new related tetranortriterpenoid - salannin (VII) was isolated whose structure was elucidated on the basis of ^{1}H NMR spectrum of its diacetate and its evident relationship with nimbin. Extensive chemical modification studies of salannin (Henderson *et al.*, 1968) led to conversion of salannin to its desacetyl derivative and nimbidic acid



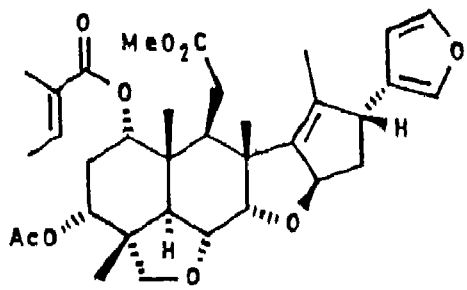
Nimbocenone
(I)



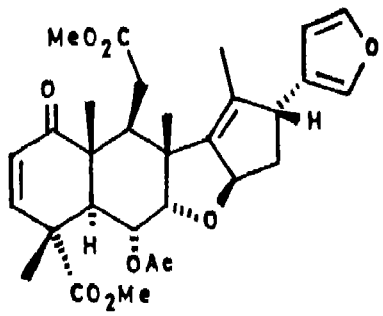
Azadirone
(II)



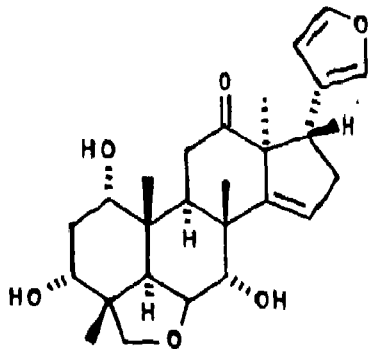
Azadirachtanin A
(III)



Salannin
(VII)



(IV) Nimbin (4 α -COOMe)
(V) Epinimbin (4 β -COOMe)



Nimbidinin
(VI)

Table 4a. Chemical components in neem seed kernel

S.No.	Name	Molecular formula	Melting point (°C)	Chemical nature
1.	Meliantriol	$C_{30}H_{50}O_5$	176	Protomeliacin
2.	Azadirone	$C_{28}H_{36}O_4$	Amorph.	Limonoid of *azadirone* group
3.	Meldenin	$C_{28}H_{36}O_5$	244	-do-
4.	7-Acetylneo-trichilenone	$C_{28}H_{36}O_5$	208	-do-
5.	Azadiradione	$C_{28}H_{34}O_5$	168	-do-
6.	17-Epi-azadiradione	$C_{28}H_{39}O_5$	205	-do-
7.	17-β-Hydroxy azadiradione	$C_{28}H_{34}O_5$	177	-do-
8.	17-β-hydroxy mmbocinol	$C_{26}H_{32}O_5$	-	-do-
9.	7-Benzoyl nimbocinol	$C_{33}H_{36}O_5$	Amorph.	-do-
10.	Nimbinin	$C_{28}H_{39}O_6$	203	-do-
11.	1-α-methoxy 1,2-di-hydronimbinin	$C_{29}H_{38}O_7$	235-236	-do-
12.	1β, 2β-Epoxy nimbinin	$C_{28}H_{34}O_7$	110-11	-do-
13.	7-Deacetyl- 7 - benzoyl nimbinin	$C_{33}H_{36}O_6$	Amorph.	-do-
14.	Dihydronimbinin	$C_{28}H_{36}O_6$	-	-do-
15.	7 - desacetyl -7-benzoyl gedunin	$C_{33}H_{36}O_7$	278	Limonoid with oxidatively expanded ring D, *Gedunin* group
16.	Mahmoodin	$C_{30}H_{38}O_8$	Amorph.	-do-
17.	Vepinin	$C_{28}H_{36}O_5$	Amorph.	Limonoid with all rings intact and A ring carrying 1,3-dioxygen functions, *vilasinin* group

contd.....2/

Table 4a. (contd...)

S.No.	Name	Molecular formula	Melting point (^{0}C)	Chemical nature
18.	Nimbidinin	$C_{26}H_{34}O_6$	282-284	Limonoid with all rings intact and A ring carrying 1,3-dioxygen functions, *vilasinin* group
19.	1, 3 -Diacetyl vilasinin	$C_{30}H_{40}O_7$	157-158	-do-
20.	Vilasinin triacetate	$C_{32}H_{42}O_8$	228	-do-
21.	1-Tigloyl - 3-acetyl vilasinin	$C_{33}H_{46}O_8p$	-	-do-
22.	3-Acetyl-7-tigloyl vilasinin lactone	$C_{33}H_{46}O_8$	243	-do-
23.	Limbocinin	$C_{35}H_{42}O_{11}$	-	-do-
24.	Limobocidin	$C_{35}H_{42}O_{13}$	-	-do-
25.	Ohchinolide B	$C_{35}H_{44}O_{10}$	211-212	C-seco meliacin *salannin* group
26.	Salannin	$C_{34}H_{44}O_9$	168	-do-
27.	3-Desacetyl salannin	$C_{32}H_{42}O_8$	214-215	-do-
28.	Salannol	$C_{32}H_{44}O_8$	208	-do-
29.	2',3',-Dehydro salannol	$C_{32}H_{42}O_8$	-	-do-
30.	Salannol Acetate	$C_{34}H_{36}O_9$	Gummy	-do-
31.	Nimbin	$C_{30}H_{36}O_9$	205	C-seco meliacin, *nimbin* group
32.	6-Desacetyl nimbin	$C_{28}H_{34}O_8$	208	-do-
33.	4-Epinimbin	$C_{30}H_{36}O_9$	196-197	-do-
34.	Nimbinene	$C_{25}H_{34}O_7$	134	-do-
35.	6-Desacetyl nimbinene	$C_{26}H_{32}O_6$	141	-do-

contd.....3/

Table 4a. (contd...)

S.No.	Name	Molecular formula	Melting point (^{0}C)	Chemical nature
36.	Salannolide	$C_{34}H_{44}O_{11}$	>320	C-seco limonoid containing γ- hydroxy butenolide side chain
37.	Salanno-lactam-I	$C_{34}H_{45}N\ O_9$	213	-do-
38.	Salanno-lactam-II	$C_{34}H_{45}N\ O_9$	-	-do-
39.	Nimbocinolide	$C_{32}H_{42}O_9$	-	Limonoid with all rings intact and containing γ-hydroxy butenolide side chain
40.	7-Deacetyl azadirone	$C_{26}H_{34}O_3$	-	Limonoid of *azadirone* group
41.	Dihydro nimbinin	$C_{28}H_{36}O_6$	-	-do-
42.	Nimbidic acid	$C_{26}H_{34}O_7$	230	C-seco meliacin, *salannin* group
43.	2', 3'-Dehydro-salannol	$C_{32}H_{42}O_8$	-	-do-
44.	Nimbanol	$C_{29}H_{34}O_8$	195-197	C-seco meliacin, *nimbin* group
45.	6-Desacetyl nimbinal	$C_{27}H_{32}O_7$	Amorph.	-do-
46.	Nimbinol	$C_{29}H_{36}O_8$	Amorph.	-do-

Table 4b. Azadirachtins and related C-secomeliacins in neem seed kernels

S.No.	Name	Molecular formula	Melting point (°C)
47.	Azadirachtin A	$C_{35}H_{44}O_{16}$	165
48	3-Desacetyl-3-cinnamoyl azadirachtin	$C_{42}H_{48}O_{16}$	-
49.	Azadirachtin B	$C_{33}H_{42}O_{14}$	204-6
50.	Azadirachtin D	$C_{34}H_{44}O_{14}$	-
51.	Azadirachtin E	$C_{30}H_{38}O_{15}$	-
52.	Azadirachtin H	$C_{33}H_{42}O_{14}$	248
53.	Azadirachtin I	$C_{32}H_{42}O_{12}$	200
54.	Vepaol	$C_{36}H_{48}O_{17}$	-
55.	Isovepaol	$C_{36}H_{42}O_{14}$	-
56.	Azadirachtin F	$C_{33}H_{44}O_{14}$	-
57.	1,3-Diacetyl-11, 19-deoxa- 11-oxo-meliacarpin	$C_{31}H_{40}O_{13}$	Amorph.
58.	Azadirachtin G	$C_{33}H_{42}O_{14}$	-
59.	Azadirachtin K	$C_{34}H_{40}O_{15}$	260
60.	11-Methoxy-azadirachtinin	$C_{36}H_{46}O_{16}734$	Amorph.

which were later isolated from neem. The occurrence of other analogues namely salannol, dehydrosalannol and salannol acetate indicates the versatility of neem in esterifying the two hydroxyl groups present in salannin (Harris *et al.,* 1968).

Among the seven C-secomeliacins containing γ-hydroxy butenolide as the side chain in place of furan ring, atleast three compounds namely salannolide, salannolactam and margocinolide have been isolated from seeds. Margocinolide has a structure intermediate between azadirone and salannolide. Thus it has A-ring similar to azadirone with a difference only of ether bridge between C_{28} and C_6 in salannolide.

2.1.5.2 Azadirachtins

The isolation of azadirachitin from neem seed kernel extract by Butterworth and Morgan (1968) by careful chromatographic fractionation monitored antifeedant assay with *Schistocerca. gregaria* led to the isolation of azadirachtin as a microcrystalline compound with profound bioactivity against insects. It has been found to inhibit feeding in over 200 species of insects at a concentrations of 10-100 ppm. It exhibits ecdysis inhibition activity at much lower concentration of 1-10 ppm. This prevents the insect larvae form developing into mature insect. Several other isolation procedures were subsequently reported (Ubel *et al.,* 1979; Yamasaki *et al.,* 1986; Schroeder and Nakanishi, 1987; Rembold, 1988). The isolation of these compounds is tedius involving partition between solvents, column choromatography monitored by TLC, prep-TLC and prep-HPLC. The extraction of

azadirachtin is best achieved with 95% ethanol followed by 85% aqueous methanol (Warthen *et al.,* 1984), wherein the second cycle offered maximum quantity of azadirachtins. Among various other solvents, methyl-*tert*-butyl ether and azeotropic mixture of methanol and methyl-*tert*-butyl ether were efficient for the extraction of active neem components (Feurhake, 1984).

The most outstanding isolation procedure has been reported by Schroeder and Nakanishi (1987) which yielded excellent yield of azadirachtin. It involved first defatting of neem seed kernels with hexane before ethanol extraction. The ethanolic extract was partitioned sequentially with hexane and water to get rid of adhering fats, sugars, and proteins respectively. The resultant residue in ethyl acetate was first filtered through a bed of silica gel followed by vacuum liquid chromatography, crystalisation from CCl_4 and finally flash chromatography to yield azadirachtin of 70-80% purity. A preparative reversed phase liquid chromatography isolation of azadirachtin from neem seed kernels have also been developed for large scale isolation of more than 90% pure azadirachtin (Ubel *et al.,* 1979). Another method involved sequential normal phase and reverse phase flash and preparative HPLC to obtain 98% pure sample with 29 per cent overall yield efficiency (Yamasaki, *et al.,* 1986). Recently an HPLC techniques has been utilised to obtain pure azadirachtin (mp 160°C, $[\alpha]_D$ = - 66°) (Govindachari *et al.,* 1990; 1991). A preparative HPLC method has also been reported for the isolation of azadirachtin A, B, D, H and I.

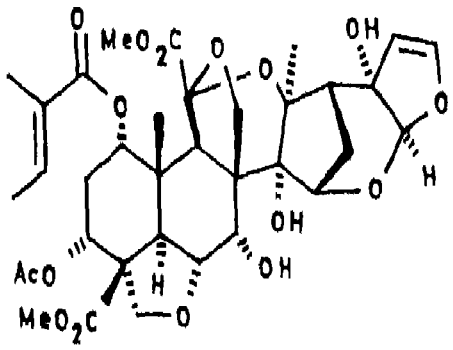
Elucidation of azadirachtin structure was challanging and formidable task because of the fact that the molecule contains 16 oxygen atoms and several chiral centres. Moreover, it could not be obtained in a crystalline form owing probably to co-occurance of its analogues. It took seventeen years for the complete structure determination of azadirachtin molecule. Its complex structure is embodied with diverse oxygen functionality, comprising an enol ether, acetal, hemiacetal, tetra-substituted oxirane, a variety of carboxylic esters, secondary and tertiary hydroxyl groups and a tetrahydrofuran ether. The molecule has sixteen stereogenic centres seven of which are quarternary.

Initially, Morgan could determine its correct molecular formula and indentify most of the functional groups and assign the partial structure. Nakanishi in 1975 (Zanno *et al.*, 1975) made a landmark in the elucidation of its complex structure. Their interpretations, were based primarily on partially relaxed Fourier transformed (PRFT/CWD) ^{13}C NMR spectroscopy which brought out the structural similarity with salannin and nimbin. The position of tiglate and acetate were correctly assigned to C_1 and C_3 respectively. Nakanishi's azadirachtin (VIII) silenced the scientific community until 1984 when Kubo *et al.*, reported the isolation and NMR structure determination of a new azadirachtin congener named deacetyl azadirachtinol (IX). The proposed structure was the first example of an azadirachtin to have a single C_8 - C_{14} bond joining two halves. Subsequently Ley and co-workers submitted evidences in favour of structure (X) in which tetrahydrofuran bridge was located in between C_{19} and C_{11} and

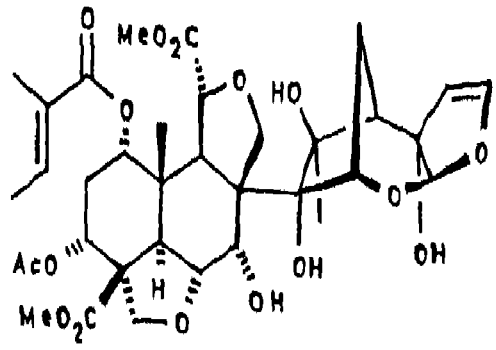
the configuration at C_{13} has been reversed. In 1985, Kraus (Kraus *et al.,* 1985) came out with altogether different revision of its structure (XI) which is currently accepted. His group established the location of oxygen bridge between C_{11} and C_{19}, the presence of an epoxide between C_{13} and C_{14}, relocation of hydoxyl group at C_{11} as a hemiacetal and conformation of α-orientation of C_{13} Me.

X-ray diffraction studies with crystalline detigloyldihydroazadirachtin (Broughton *et al.,* 1986) confirmed the Kraus structure. The highly hindered nature of 11-OH was reasoned to be due to the existance of strong intramolecular H-bonding between C_{11} hydroxy and the oxirane oxygen (2.66 A^0) and significantly weaker intramolecular H-bonding between C_{20} and C_7 hydroxyl oxygen. These two H-bonds were stated to act as main stabilizing factor for the structure. Ley and co-workers (Bilton *et al.,* 1987) have shown the existence of a strong H-bonding between 11-OH and epoxide oxygen both by X-ray crystallography and NMR studies. Nakanishi and co-workers (Turner *et al.,* 1987) produced evidences to show that azadirachtin has a free rotation around C_8 and C_{14} single bond and exists as two rotamers at 180 K. Kraus *et al.* (1987) also observed the presence of two rotamers in the ratio of 3:2 at 183 K supporting certain restriction of the rotation around C_8/C_{14} bond.

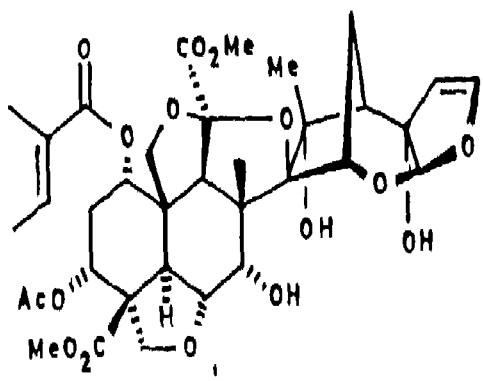
Besides azadirachtin A (XII), Rembold (1989) described other structurally related compounds e.g. azadirachtin B (XIII), C, D (XIV), and E (XV) etc. (Table-4b). 3-Tigloylazadirachtinol isolated by Klenk *et al.* (1986) was described as being identical with azadirachtin B isolated following its activity in the *Epilachna*



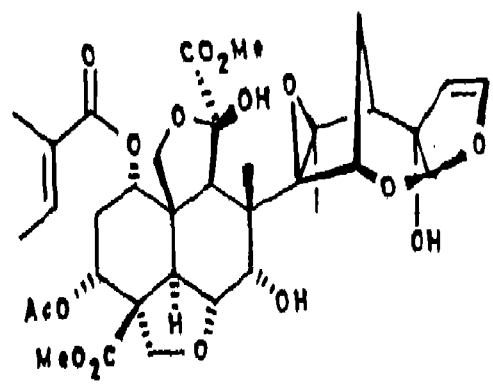
Nakanishi's azadirachtin
(VIII)



Kubo's deacetylazadirachtinol
(IX)

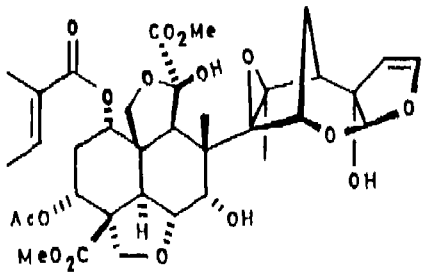


Ley's azadirachtin
(X)

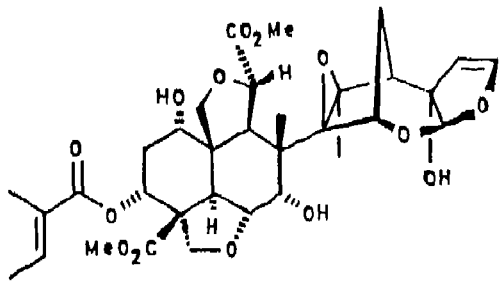


Kraus's azadirachtin
(XI)

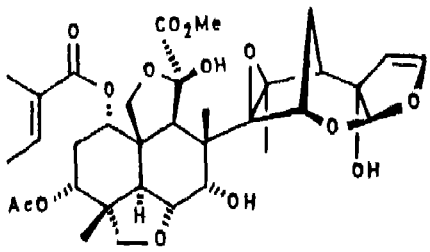
bioassay. It is probably the active compound of lower polarity than azadirachtin and was not investigated further. There has been some confusion as to wheather 3-tigloyl azadirachtinol corresponds to a compound described earlier by Kubo and co-workers (1986) and named as deacetylazadichtinol. Azadirachtin C described by Rembold (1989) could be designed only the partial structure comprising a *trans* - decalin substituted azadirachtin. Azadirachtin D differs from azadirachtin A in that C_{29} is not oxidised and is present as an angular methyl group. A compound with a similar oxidation level, 1- cinnamoyl-3-feruloyl-11-hydroxymeliacarpin has been isolated from the Persian lilac *Melia azedarch* (Kraus, 1986). Whereas, Azadirachtin E (Rembold, 1989) corresponded to detigloylazadirachtin, Azadirachtin F (XVI) and G (XVII) are structural congeners of 3- tigloylazadirachtinol. For azadirachtin F, unoxidised C_{19} angular methyl group is accompanied by a pendent methyl glycolate side chain at C_{19} whilst for azadirachtin G, C_{13} - C_{14} oxirane is absent and replaced by a double bond and a C_{17} hydroxyl group. Azadirachtin H (XVIII) and I (XIX) have recently been isolated from neem seed kernels by an unique preparative HPLC method (Govindachari *et al.*, 1991) which lack C_{12} carbomethoxy group. The compound corresponding to the peak centred at 16 min. during the preparative run on RP-18 column furnished a white residue (50 mg) which was resolved onto 2 peaks centred at 8.9 min and 11.1 min. on an analytical RP-8 column eluted with CH_3CN-H_2O (28:72) at a flow rate of 1 ml/min. Azadirachtin H and I were then separated on preparative RP-8 column at a flow rate of 10 ml/min with retention times of 48 and



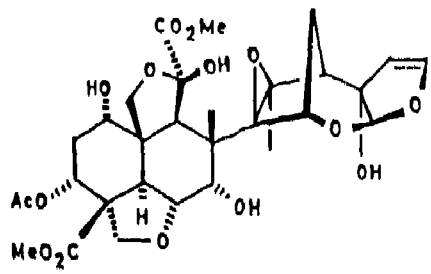
Azadirachtin A
(XII)



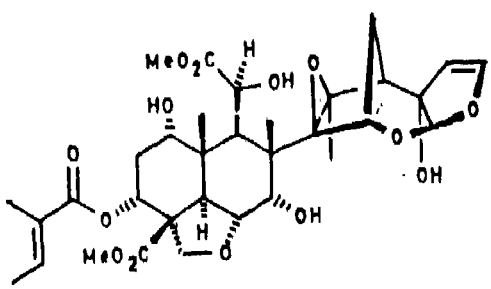
Azadirachtin B
(XIII)



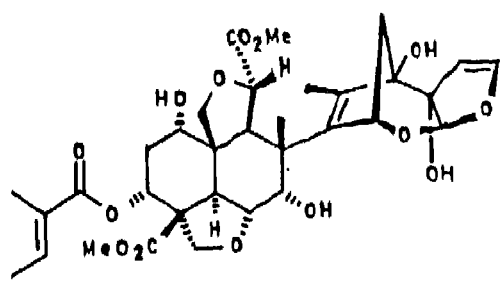
Azadirachtin D
(XIV)



Azadirachtin E
(XV)



Azadirachtin F
(XVI)

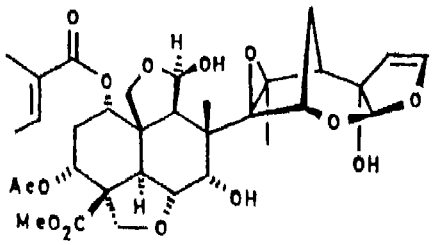


Azadirachtin G
(XVII)

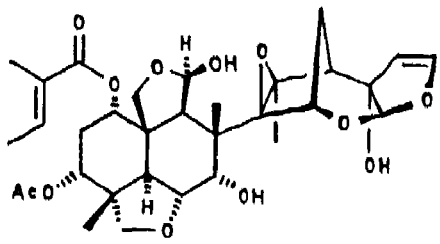
37.8 min respectively. Whereas, azadirachtin H differs from azadirachtin A by substitution of a hydrogen in place of the carbomethoxy group at C_{11}, azadirachtin I differs from H by the further replacement of the carbomethoxy group at C-4 by a methyl group. Kraus and co-workers (1985) isolated another azadirachtin analogue, 22, 23-dihydro-23-β methoxyazadirachtin [Vepaol (XX)] which along with a 23-α methoxy analogue [Isovepaol (XXI)] was also reported by Shankaram *et al.*, (1987). Most recently, Schmutter and co-workers have reported the isolation and structure determination of a new azadirachtin congener named marrangin (XXII) (Ermel *et al.*, 1991). This compound is even more effective as an insect antifeedant than azadirachtin A.

2.2 Structure-Activity Relationships

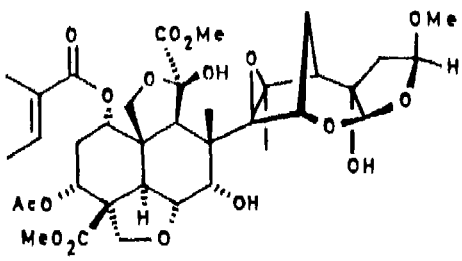
Studies conducted on insect antifeedant activity of azadirachtin and its derivatives revealed that neither hydrogenation of Δ^{22} double bond nor deacetylation caused any change in activity, however, blocking of hydroxyl group affected the feeding inhibitory activity. Thus, acetylation of azadirachtin resulted in 75% decrease in activity, whereas, esterification with bulky trimethylsilyl group eliminated it altogether suggesting that stereochemical environment around hemiacetal regions is critical for its activity. In another study, screening azadirachtin and its seven derivatives for insect growth regulatory effect on larvae of tobacco budworm (*Heliothis virescens*) again indicate that free hydroxyls are essential for IGR activity. The LC_{50} values (ppm) of azadirachtin A-G against larvae of Mexican beetle (*Epilachana varivestis*) were found to be 1.66, 1.30, 12.97, 1.57-2.80, 1.15 and



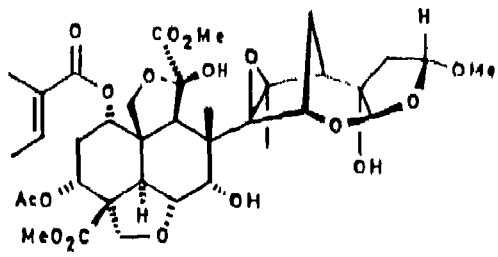
Azadirachtin H
(XVIII)



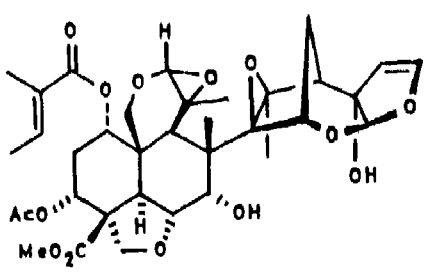
Azadirachtin I
(XIX)



22, 23 - dihydro - 23 - β - methoxyazadirchtin
(Vepaol)
(XX)



22, 23 - dihydro - 23 - ∝ - methoxyazadirachtin
(isovepaol)
(XXI)



Marrangin
(XXII)

7.69 respectively (Rembold 1990a; 1990b). Interestingly, 3-detigloylazadirachtin B and hydrogenated derivatives were more active than the parent molecule. Another study for insect feeding deterrant action against African leaf worm *Spodoptera littoralis* B, revealed that hydroxyfuranacetal moiety is important for high levels of potency. It was suggested that stereochemistry at C_7 is crucial and that the bridging oxygen substituent at C_6 play an important role (Ley *et al.*, 1993).

In neem research, a great feat was accompalished when Ley and his group at London's Imperial College succeeded in synthesizing the two chemical structures, the decalin portion and hydroxyfuran fragment that together constitute the azadirachtin molecule. Interestingly, these two azadirachtin portions have different insecticidal effects. Whereas, decalin portion disrupts insect growth and development, the hyroxyfuran moiety provides antifeedant properties. However, when these two compounds were individualy tested, they were found to be less effective than the parent azadirachtin molecule.

2.3 Bioactivity of Neem Products and its Relationship with Azadirachtin content

The antifeedant property of neem seed kernel was first described by Pradhan *et al.* (1962) for *Schistocerca gregaria*. Morgan and Butterworth (1968) later demonstrated that azadirachtin was active as an antifeedant against the desert locust at low concentration. Its ability to act as a growth inhibitor was first discovered by Schmutterer and Rembold (1980). These reports aroused worldwide interest in neem as possible source of

pest control chemicals. A large number of reviews and reports are now available describing the bioactivity of neem against a broad spectrum of insect pests, phytonematodes, and plant pathogens (Sexena *et al.*, 1988; Kaul *et al.*, 1990; Schmutterer, 1990; Mordue and Black Well, 1993; Parveen and Alam, 1993; Singh *et al.*, 1993; Singh and Raheja, 1996). Inspite of the vast information available on bioactivity spectrum of neem, emphasis in this review has been laid on some recent reports describing bioactivity of neem products and their relationship with azadirachtin content.

Growth disruption by azadirachtin has been shown to be primarily due to its effect on neuroendrocrine centres leading to changes in morphogenetic hormones rather than to the altered feeding behaviour. (Sieber and Rembold, 1983). It exhibited not only the juvenilizing effect on the last instar larvae of *Spodoptera litura* but also showed inhibitory effect on larval-larval and pupal-adult transformations (Gujar and Mehrotra, 1983). Following application of azadirachtin @ 2 µg/g body weight to *Schistocerca gregaria,* ovarian development was found to be completely inhibited (Subrahmanyam and Rao, 1986) due to possible delay in the synthesis and release of neurosecretory hormones in the brain.

The growth disrupting potential of azadirachtin was also demonstrated against the final instar larvae of two major pests namely castor semilooper *Achaea janata* and tobacco leaf caterpillar *Spodoptera litura* (Rao and Subrahmanyam, 1987) by inhibiting processes like egg maturation, mating or egg laying leading to reduced fecundity. Histological investigations have revealed that in addition to the sensitive tissues, insects treated

with azadirachtin have degenerated ovaries (Dorn et al, 1987; Schluter, 1987). Depending on the stage of development, oocytes develop abnormally and vitellogenesis inhibited (Koul, 1985; Schmutterer, 1987; Rembold, 1989). Studies conducted on insect growth regulating effects of neem seed kernel extracts (NSKE) and crude and pure azadirachtin showed a pronounced growth regulating effect during imaginal development. The results point out the strong interference of azadirachtin with hormonal balance most probably with ecdysteroid titre in insects (Zebitz, 1986).

Azadirachtin injected into newly moulted last instar larva of milk weed bug *Oncopeltus faciatus* induces a variety of effects which are dose dependent. Whereas, low doses retarded and delayed ecdysis (the peak of ecdysteroid titre is reduced and delayed by two days), the medium dose suppressed ecdysis (the peak of ecdysteroid titre is reduced and delayed by seven days). The high azadirachtin doses prevent apolysis as well as ecdysis and ecdysteroid titre peak was further reduced as compared to medium doses (Dorn *et al.*, 1986). The average longivity of permanent larvae increased unexpectedly with rising doses being 31 days at an azadirachtin dose of 8 µg/larva. This is more than four-fold the life span of 5th instar larvae between last larval and adult ecdysis.

Azadirachtin and its three derivatives 22, 23-dehydroazadirachtin, 2', 3', 22, 23-tetrahydroazadirachtin and 3-deacetylazadirachtin at 2 and 4 µg dose levels were effective causing 100% larval mortality of *Heliothis virescens* F. At a dose of 1.0 µg, all four compounds prevented pupation of fifth instar

and caused larval mortality. At the lowest dose of 0.5 μg, all the compounds except 3-deacetylazadirachtin were significantly active (*Barnby et al.*, 1989).

The recent studies on effect of neem seed oil and azadirachtin on aphid reporduction demonstrated that both the products inhibited aphid reproduction and produced large number of dead offsprings (Lowray and Isman, 1996). It also delayed the growth of embryos and various stages of development leading to higher mortality values of embryos. Thus because of tremendous reproductive ability of aphids, a decrease in fertility would enhance the control of aphids by natural enemies.

The overlapping of antifeedant effects with growth regulatorry activities account for the disturbance of metamorphosis and reproduction of fecundity. It is believed that the release of neuropeptides disrupts the control of insect metamorphosis and behaviour on the level of moulting leading to inhibition of ecdysis. Another view is that azadirachtin blocks receptors for ecdysteroids which are needed for larval development (Schmutterer, 1990).

Nematicidal principles from neem have been earlier screenced against root knot nematode *Meloidogyne incognita*. Different concentrations (50 to 250ppm) of eight C-secomeliacins including azadirachtin , nimbin, epinimbin, salannin when evaluated *in vitro* against *M. incognita* recorded that all the eight meliacins were highly nematicidal with LC_{50} values ranging between 55 and 157 ppm (Devakumar *et al.*, 1985).

2.4 Variation of Azadirachtin Content in Neem Ecotypes

Neem like other botanical preparations vary considerably with respect to concentration of its active principles particularly azadirachtin depending on the genome of the tree(s) from which seed is collected, the geographic area of origin, and yearly variations in environmental conditions (Ermel *et al.*, 1987). Isman *et al* (1990) reported that azadirachtin content of neem oil varies widely and that 72 to 90% of the variation in bioactivity of the oils can be accounted for by variation in azadirachtin content. Thus azadirachtin content is an useful quality control criterion for neem oil as a precursor for insecticide production. In another study on neem ecotypes of India (Rengasamy *et al.*, 1996), azadirachtin content varied depending on climate, soil type and altitude. Ecotypes growing in regions with moderate climate with red laterite and black soil and altitudes less than 500 m above mean sea level were rich in azadirachtin content, whereas, ecotypes growing in high altitude alluvial soils with extreme hot and cold climates had very low azadirachtin content. Although azadirachtin content ranged from 0.14 to 1.66 per cent, it was not the only component responsible for the bioactivity of neem extracts. Variation of azadirachtin content during growth and storage of neem have also been studied (Yakkundi *et al.*, 1995). Results indicated that azadirachtin appears only after 9th week and gradually reaches the maximum of 0.38 - 0.44% (w/w on a dry weight basis). Interestingly, it was found to decrease to 0.29 - 0.32% (w/w) by the 19th week. It was thus, recommended that for better azadirachtin content, fruits should be harvested in the 17th week of development

when neem fruits turn green to yellow. Further, under normal conditions of storage of seeds, azadirachtin content was reduced to about 6.8% and 55% in dark and day light respectively in a period of four months.

While confirming the relationship between bioactivity of neem materials and their azadirachtin content, it was established that azadirachtin was largely responsible for both repellent (behavioural) and toxic (physiological) actions of neem on stored product insects. However, neem extracts are slightly more active than pure azadirachtin when applied at equivalent azadirachtin concentrations, indicating that azadirachtin is not the only active compound in neem (Xie *et al.*, 1995).

2.5 Neem Formulations

Interest in neem pesticides has grown over the last ten years as more of the synthetic pesticides are phased out due to environment and food safety problems. The chemical complexity of azadirachtin and the diverse structural requirements for insect bioactivity restricts the synthesis of the molecule and therefore, commercial neem products will depend upon neem seed extracts. Since azadirachtin content is highly correlated with both behavioural and physiological effects (Isman *et al.*, 1990) it was suggested that azadirachtin content should be used as a quality control criterion for the analysis of active ingredient in formulations of neem based botanical insecticides.

In United States, two neem insecticides namely Margosan-O and *Azatin* were developed by W.R. Grace and Company and

Agridyne Technologies Inc. have received an exemption from residue tolerence on food crops by the U.S. EPA. Whereas, Margosan-O (MO) has 0.25% azadirachtin content and 3-5% neem oil, Azatin has 3% azadirachtin but no neem oil. RH-9999, another neem insecticide in the experimental stage produced by Rohm and Haas Co. is a wettable powder that contains chemically modified azadirachtin (20% a.i.) and no neem oil. Neemguard, again a product of W.R. Grace and Co. is a formulated neem oil product produced from neem seed kernels (90% a.i.) which has insecticidal activity for some species. NeemAzal, a product of Trifolio- M-GmbH, Germany (Kleeberg, 1996) is prepared as a concentrate of the insecticidally active neem ingradients. The concentrate powder form called NeemAzal usually contains 30% azadirachtin-A and about the same amount of other azadirachtins. The formulated product "NeemAzal-F" or "NeemAzal-T" contains 5% aza-A and has shelf life of about 2 years at about 20°C.

In India, nearly three dozen neem based products listed by Parmar and Ketkar have been either marketed or are awaiting commercialization (Parmar and Ketkar, 1993). At least five products namely Godrej Achook (Godrej Agrovet Ltd.), Margocide Ck and Ok (Monofix Agroproducts Ltd.), Nimbicidine (T. Stanes and Co. Ltd) and RD-9, Repelin (ITC Ltd.) have been registered with Central Insecticide Board. Most of the products are either oil-based or based on various extractives. RD-9, Repelin is available as an EC formulation containing azadirachtin concentration of 3000 ppm and is effective in managing several key pests of rice, cotton, vegetables and oil seed crops at a

concentration of 1.0% either alone or its combination with recommended chemical pesticides (Subramaniam *et al.*,1996). Godrej Achook on the other hand is an enriched formulation containing azadirachtin, azadiradione, nimbocinol and epinimbocinol (totalling 2800 ppm) in the form of water soluble powder. It has antifeedant and growth disrupting properties and is capable of controlling insect pests of major crops.

Recently technical know-how for a neem oil based EC formulation developed in Indian Agricultural Research Institute has been transfered to Jay Agrochem Ltd., New Delhi through NRDC based on this technology, the company launched its product "Jayneem" in the country in August, 1996. Several more applications are stated to be awaiting clearance from the Registration Committee. Under section 9 of Indian Insecticides Act. 1968, neem kernel extract and "neem oil" based formulations based formulations should contain not less than 1500 and 300 ppm of azadirachtin respectively.

2.6 Degradation and Persistence of Neem Products

Under the impact of light (UV/sunlight) pesticide molecule gets degraded and the efficacy of the molecule is considerably reduced. Of the total solar radiation received on earth, only 5-6 per cent is contributed by the UV portion (between 290 and 400 nm) is resposible for photo-chemical transformations. After absorption of light, pesticidal molecule may undergo bond breaking and bond making processes leading to variety of photo transformations. The products of direct

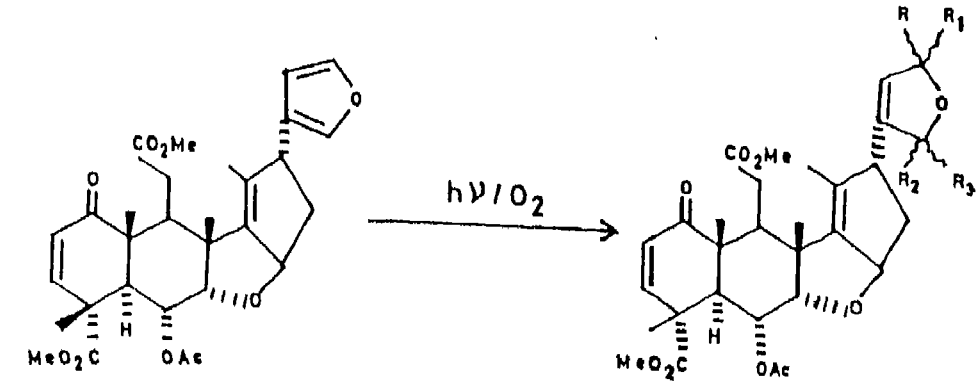
photoreactions are considerably more susceptible to biodegradation than the parent xenobiotic itself (Hwang *et al.*, 1987). In some other cases, the presence of short-lived reactive chemical transients (even in minute quantity) produced on the absorption of sunlight by organic and inorganic chromophores in the environment enhances the degradation of pesticides. The most important naturally occurring chromophores are humic acids, fulvic acids and humins (Choudhry, 1984). Acetone, a well known triplet sensitizer reportedly present in various natural aquatic systems is also responsible for enhanced degradation of pesticides (Choudhry *et al.*, 1979).

2.6.1 Effect of UV Light

Azadirachtin and related meliacins are sensitive to light temperature, pH, rainfall, organic slovents and gets degraded readly in the environment. Among these factors, light is perhaps the most important one. When applied in the form of formulations, neem products containing azadirachtin are exposed to UV/sunlight. In azadirachtin and related molecules, the two major UV-absorbing chromophores - vinyl ether group and the α, β-unsaturated ester [(*E*)-7-methyl but -2-enoate] group are major targets for various photochemical reactions leading to their degradation. Salannin and nimbin having structural similarity to azadirachtin under dye-sensitized photochemical conditions in methanol or chloroform solution produced corresponding α, β- unsaturated γ-lactones. Whereas, salannin yielded salannolide(XXIII) and isosalannolide(XXIV), nimbin offered nimbinolide(XXV) and isonimbinolide(XXVI) respectively. Studies conducted on biological

activity of azadirachtin, 2', 3', 22, 23- tetrahydroazadirachtin and 3-deacetyl azadirachtin as well as their ultra-violet degradation products against tobacco budworm, *Heliothus virescens* F. (Barnby *et al.*, 1989) revealed that of the four compounds, 2',3',22,33-tetrahydroazadirachtin was the most stable to UV-radiation. It retained biological activity even after 200 h of exposure and after 400 h of exposure only 15% of the compound was found to degrade. Interestingly, the remaining three compounds namely, azadirachtin, dihydroazadirachtin, and 3-deacetyl azadirachtin though degraded completely under the impact of UV-light retained their biological activity indicating that one or more of the photodegradation products are atleast as biologically active as the native molecule.

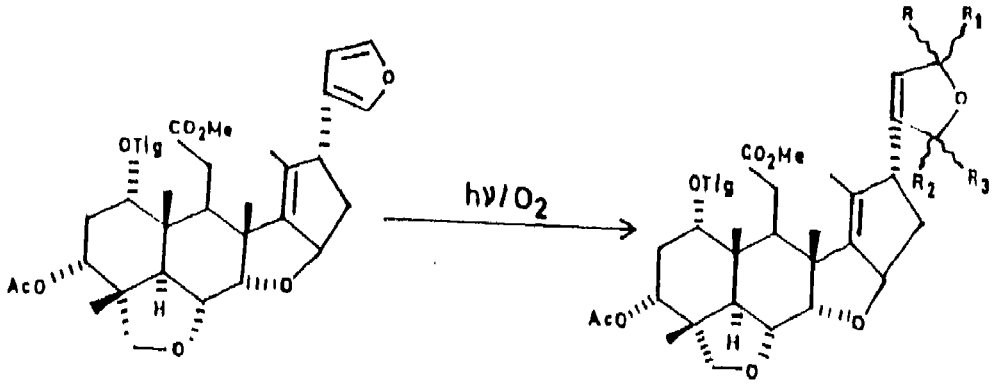
The use of supercritical fluid chromatography (SFC) has emerged as a simple and fast technique for the analysis of azadirachtin (Huang and Morgan, 1990). Recently, photoisomerisation of azadirachtin has been studied employing liquid chromatography coupled with high field ^{1}H NMR spectroscopy. The photoreaction was studied in benzene solvent under both nitrogen and oxygen atmosphere (Johnson *et al.*, 1994) followed by its monitoring with super critical fluid chromatography. The HPLC- ^{1}H NMR data of the photoproduct obtained in nitorgen atmosphere indicated that (*E*)-2- methylbut -2-enoate ether group in azadirachtin-A(XXVII) had been converted into (*Z*)-2-methyl but -2-enoate ether group(XXVIII). On the other hand, in the presence of UV-radiation and oxygen atmosphere, two major UV-absorbing chromophores-vinyl and tigloyl group possibly undergo



Salanin

R = H ; R_1 = OH ; R_2,R_3 = O (XXIII)

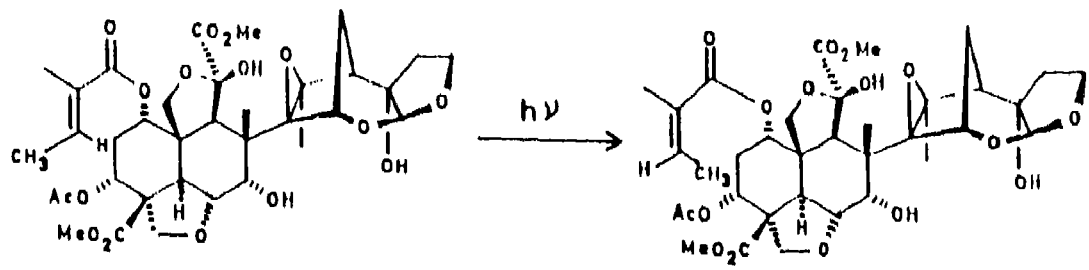
R, R_1 = O ; R_2 = H ; R_3 = OH (XXIV)



Nimbin

R = H ; R_1 = OH ; R_2,R_3 = O (XXV)

R, R_1 = O ; R_2 = H ; R_3 = OH (XXVI)



Azadirachtin A (*E*)
(XXVII)

Azadirachtin A (Z)
(XXVIII)

photochemical reactions mediated by peroxides and hydroperoxides leading to formation of large proportion of polar products (dimer and polymer etc.) from which no photoproduct could be isolated. It was further shown that when oxygen was excluded from the reaction, there was an improved yield of the photoproduct (21.8%) and a large amount of azadirachtin was recovered (56.7%) with none of the polar products. Very recently, Yakkundi *et al.* (1996) reported that when azadirachtin was exposed to UV-light for 10 h in presence of alcoholic solvents the C'_2-C'_3 double bond of tigloyl moiety was missing, possibly due to the formation of alcohol adduct accross the double bond. Their studies thus indicated that azadirachtin is highly sensitive to light.

2.6.2 Effect of pH

Besides light, pH is another important factor influencing stability of azadirachtin. Very recently, the hydrolysis of azadirachtin was studied in several aqueous buffers (pH 4.1-8.1) and four natural waters (pH-6.2, 7.3, 8.0 and 8.1) at 20-45°C (Szeto *et al.*, 1996) to determine the kinetics of azadirachtin degradation over normal pH ranges, so that its stability in water-based spray mix and its fate in the aquatic environment could be predicted. It was concluded that azadirachtin hydrolysed readily at 35°C, its disappearance followed pseudo-first order kinetics and that the degradation rate was faster in basic than in acidic pH. Depending on the pH, severel unidentified conversion products were detected in the incubated solutions. Based on the comparision of rate constants, azadirachtin appeard to be more susceptible to hydrolysis than synthetic organophosphorus group of pesticides.

The azadirachtin was expected to degrade more rapidly in water and hence less persistant in the aquatic environment than synthetic insecticides. In yet another study Ruch *et al*, 1996 investigated the degradation of NeemAzal (an oil free, stable EC containing 1% azadirachtin and several other limonoids) and its formulations in water and soil. Detailed HPLC investigations indicated that NeemAzal is degraded hydrolytically, thermally and/ or microbially under different conditions with a half-life period of a few days.

2.7 Photostabilization of Botanical Products

Botanical pesticides can be stabilized in two ways, firstly, by using photostabilizers including antioxidants and ultraviolet screens as in the case of photolabile chrysanthemates (Miskus and Andrews, 1972; Ueda *et al.*, 1974; Piper and Rappaport, 1982) and secondly, by the replacement of the photolabile sites in the molecule with stable moieties. In case of pyrethroids second approach has been successful wherein incorporation of halogen groups (Cl, Br) alongwith other structural modifications had yielded a large number of photostable pyrethrods (Miyamoto *et al*, 1981; Ruzo, 1983). The photostabilizer apprroach has the potential advantages in improving the cost effectiveness of stabilized compounds, in maintaining the favourable toxicology of molecule, and in controlling persistence as desired for specific control situations by varying the type of photostabilizer or its concentration. Several attempts have also been made to stabilize pyrethroids (natural and synthetic) to prolong their effective life. A few examples are the addition of trialkylphenols (Smith and Hill, 1974),

The azadirachtin was expected to degrade more rapidly in water and hence less persistant in the aquatic environment than synthetic insecticides. In yet another study Ruch *et al*, 1996 investigated the degradation of NeemAzal (an oil free, stable EC containing 1% azadirachtin and several other limonoids) and its formulations in water and soil. Detailed HPLC investigations indicated that NeemAzal is degraded hydrolytically, thermally and/ or microbially under different conditions with a half-life period of a few days.

2.7 Photostabilization of Botanical Products

Botanical pesticides can be stabilized in two ways, firstly, by using photostabilizers including antioxidants and ultraviolet screens as in the case of photolabile chrysanthemates (Miskus and Andrews, 1972; Ueda *et al.*, 1974; Piper and Rappaport, 1982) and secondly, by the replacement of the photolabile sites in the molecule with stable moieties. In case of pyrethroids second approach has been successful wherein incorporation of halogen groups (Cl, Br) alongwith other structural modifications had yielded a large number of photostable pyrethrods (Miyamoto *et al*, 1981; Ruzo, 1983). The photostabilizer apprroach has the potential advantages in improving the cost effectiveness of stabilized compounds, in maintaining the favourable toxicology of molecule, and in controlling persistence as desired for specific control situations by varying the type of photostabilizer or its concentration. Several attempts have also been made to stabilize pyrethroids (natural and synthetic) to prolong their effective life. A few examples are the addition of trialkylphenols (Smith and Hill, 1974),

4-aminoazobenzene (Smith and Templin, 1956), Yellow 10 (2,4-dihydroxyazobenzene) and 4-substituted 2,6-dinitroanilines (Dureja *et al*, 1984).

For optimum stabilization of pyrethroids, a combination of antioxidant, solvent and ultroviolet absorbant provided greater stabitily than if each agent was used singly (Miskus and Andrews, 1972). The solvent used in a formulation has an important role to play in its stabilization. Mineral oil e.g. saturated paraffin is preferred one as it is quite non-reactive and does not produce peroxides as do ketones or ethers. The most useful of the UV-screening agents are the derivatives of benzophenone and esters of substituted benzoic acids. The greatest stabilization was achieved with those compounds possessing an hydroxyl group attached directly to an aromatic nucleus and having 14 or more carbon atoms; e.g., 4-methyl -2, 6-di-*tert*-butylphenol or 2,6-dioctadecyl-paracresol. (Mason, 1970).

Among the various antioxidants studied, pyrogallic acid and hydroquinone were found to be superior in stabilizing pyrethrins. Butylated hydroxy toluene (BHT) was not effective with pyrethrins against *T. castaneum*. However, when pyrogallol, hydroquinone and BHT was used in pyrethrum dust formulation could stabilize the pyrethrin content for above 9 months when assayed chemically.

Aqueous extracts of neem cake and sitaphal seed in general enhanced the residual action of pyrethrin upto 35 days when applied at 1:10 and 1:20 ratios respectively, whereas, solvent

extract of deoiled neem cake could not enhance the residual toxicity of pyrethrins. Ahmed *et al.* (1973a) developed formulations consisting of selective ingredients such as pyrethrum extract, aqueous extract of de-oiled neem seeds, trichloro ethylene, pyrogallic acid, sesame oil, beewax etc., in different ratios. As evident from chemical assays, the two pyrethrin formulations with and without neem extract and that with PBO retained large amount of pyrethrins to the extent of 82.1, 74.2 and 76.0 per cent respectively at the end of 56 days. The better result with the formulation containing neem extract could be attributed to possible stabilization of pyrethrins with neem ingradients.

Bioactivity evaluation of various organic solvents against *T. castaneum* indicated that solvents such as acetone, alcohol, methanol, petrol and spirit did not have significant effect after 3 days, whereas, kerosene, mineral terpentine and ethylacetate were marginally better in retaining residual toxicity for upto 5 days (Ahmed *et al.*, 1973).

Among the various solvents, trichloromethylene performed the best as it is non-inflammatory had mild fumigation action and is suitable for use on large scale (Munrao, 1964).

Inspite of the several reports on stabilization of pyrethrins, not much work has been done on stabilization of neem products. Since turmeric constituents are well known for their anti-oxidant properties they can be used as potential photostabilizer for neem based agrochemicals.

2.8 *Curcuma longa*(Turmeric)

Curcuma longa (Turmeric) referred to in ancient Indian Vedic texts as Haridra and commonly known as Haldi is known for its colouring, flavouring and digestive properties. It acquired additional importance because of medicinal properties and use in Ayurvedic system of medicines.

Curcuma, a genus in the family Zingiberaceae (Sub-order Zingiberoidae) consists of several species of rhizomatous herb distributed in india, China, Indonesia, Siam, Malay Archipelago. and Northern Australia. Among the various species *Curcuma longa* L., (Syn. *C. domestica*) yields commercial grade turmeric. To a smaller extent, *C. aromatica* Salisb. *C. amada* Roxb. and *C. zedoaria* Rose in India and China, and *C. xanthorrhiza* Roxb. in Indonesia are also cultivated for marketing.

Curcuma longa is a perennial herb with thick and fleshy rhizomes and leaves in sheaths. The underground rhizome, which is processed into the spice, consists of two distinct parts, the egg-shaped primary or mother rhizome an extension of the stem, and several long cylindrical multi-branched secondary rhizomes growing downward from the primary rhizome. Based on their shape, the two forms are differentiated in the western trade- the bulbs as *C.rotunda* and the finger like cylindrical forms as *C. longa* though both are from the same plant. Huge quantities of leaves obtained after harvesting turmeric remain largely under- utilized though a part of it is used as fuel. The dried or fresh leaves yield a pale yellow essential oil (1.5-1.7 per cent) having pleasant spicy and therapeutic odour.

2.8.1 Chemical Compositioin

Apart from common plant constituents such as starch, protein, fat and fiber, the components that are characteristic of the spice turmeric are the deep yellow pigments, the curcuminoids, and the volatile oils. The yellow pigment is present to the extent of 2 to 5% depending on location, variety and maturity of the rhizomes. The dried rhizomes of *C. longa* yield about 2-6% essential oil and about 58% of the oil is composed of turmerones. Gas chromatography of this oil showed the presence of *p*-cymene, β-sesquiphellandrene, turmerone (XXIX), *ar*-turmerone(XXX) etc. Turmerone and *ar*-turmerone were reported to be in the ratio of 50:40 (Rupe *et al*, 1934). Thin layer chromatography of turmeric oil has been performed with a series of developing solvents of increasing polarity1.5-1.7 per cent . It was seen that turmerones, terpenes and other oxygenated compounds merged into a bigger band with high R_f value.

The strong yellowish colour of the crude turmeric oil is due to the presence of curcuminoids having mainly biphenylheptadienedione nucleus. In addition to curcumins, as many as thirty different ingredients have been identified from turmeric extract some of which include: i) curcuma lactone; ii) curcumanolide A&B; iii) curcumenes; iv) curcumenols; v) curcumic acid; vi) curcumone; vii) curcuquinone; and viii) curcusone A, B, C and D. Besides these, d-α-phellandrone, d-sabinene, zingiberene, borneol, 1,8-cineole, turmerone and *ar*-turmerone, sesquiterpene alcohols, α- and γ-alantone, bisabolene (Shankaracharya and Natarajan, 1974; Govindarajan, 1980) and

two monoterpenes, α-pienene and β-pienene (Visan *et al.*, 1989) have also been reported in the essential oil.

2.8.1.1 Isolation of Curcumins and Related Products

An efficient way of isolating total curcuminoids is to extract turmeric powder with hot ethanol and precipitating the concentrate with kerosene. The separated mass after washing with pet ether was crystallised from ethanol. Other reports of separating pure curcumins in higher yields (1.1%) involved prior extraction of the rhizome powder by hexane to remove much of the volatiles and fatty compounds and then extracting the colouring matter with benzene (Janaki and Bose, 1967). The concentrate is readily crystallized on cooling and is further purified by crystallization from ethanol. This product is a mixture of mainly three components namely curcumin-I, II and III. curcumin-I being the dominat product.

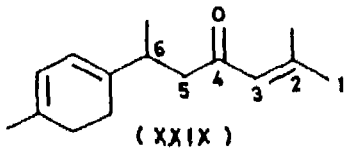
Curcumin-I [1, 7-bis (4-hydroxy-3-methoxy phenyl)-1, 6-heptadiene-3, 5-dione(XXXI)] is the major curcuminoid present in rhizome along with other two minor curcuminoids, curcumin-II (1-(4-hydroxy phenyl)-7-(4-hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5-dione (XXXII) and curcumin-III (bis (4-hydroxy cinnamoyl) methane)) (XXXIII) (Srinivasan, 1953; Shankaracharya and Natarajan, 1974; Krishnamurthy *et al.*, 1976). The total coloured components can be separated into three clearly visible yellow components by column chromatography. The dominant component eluting first with benzene was shown to be curcumin-I as reddish-orange prisms. The remaining two amorphous orange-

yellow product were identified as curcumin-II and III. In micro-quantities, curcumins can also be separated by preparative thin layer chromatography. The absorption spectra of these components vary slightly with maxima at 429 nm for curcumin-I, 424 nm for demethoxy curcumin and 419 nm for bisdemethoxy curcumin. Synthesis of curcumin from acetylacetone and vanillin had been reported earlier by Pavolini has been further improved by Pabon (1964) to get 80% yield.

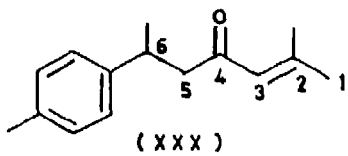
Recently, two new curcumin related natural phenolics have been isolated from rhizomes of *Curcuma domestica* (syn. *C. longa*) alongwith known curcuminoids (Masuda *et al.*, 1993). Their sturctures were determined to be 1, 5-bis (4-hydroxy - 3-methoxyphenyl) -hepta - (1*E*, 2*E*) -1, 4-dien-3-one(XXXV) and 1-(4-hydroxy - 3-methoxyphenyl)-5-(4-hydroxyphenyl) -penta -(1*E*, 4*E*)-1, 4-dien-3-one (XXXVI) respectively. From fresh rhizomes, three other pigments namely 1-(3,4,5-trimethoxyphenyl)-7-3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione (XXXIV), 1-(4-hydroxy -3-methoxyphenyl)-7-(3,4-dihydroxyphenyl)-1,6-heptadiene-3,5-dione(XXXVII) and 1,7-bis (4-hydroxyphenyl)-1,4,6-heptadien-3-one(XXXVIII) have been isolated and characterised spectroscopiclly. Interestingly, these compounds showed stronger anti-oxidative activity than curcumin-I indicating that diketo moiety is not only responsible for activity.

2.8.2 Analysis of Curcumins

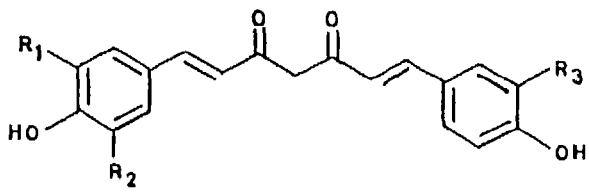
A variety of methods have been developed for the determination of turmeric products. Usually the separation of



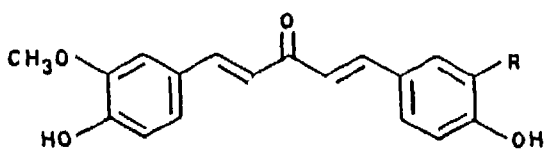
(XXIX)



(XXX)

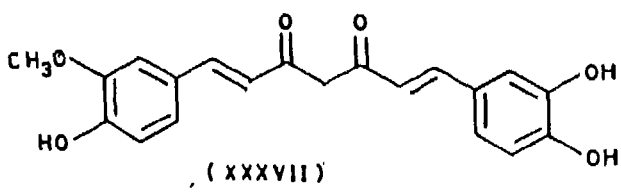


	R_1	R_2	R_3
(XXXI)	OCH_3	H	OCH_3
(XXXII)	OCH_3	H	H
(XXXIII)	H	H	H
(XXXIV)	OCH_3	OCH_3	OCH_3

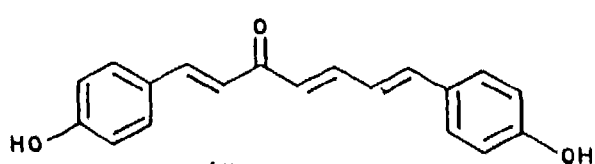


(XXXV) R = OCH_3

(XXXVI) R = H



(XXXVII)



(XXXVIII)

curcuminoids is achieved by thin layer or paper chromatography (Govindrajan, 1980) often in combination with spectroscopy for quantitative examination. The boric acid test is one of the official indentification tests for curcumins (FAO/WHO 1971; 1976). TLC determination of curcumins in spices is possible by direct measurement of the fluorescence intensity of rubrocurcumine on the layer obtained by treating curcumins with boric acid reagent. The best developing solvent systems include i) benzene - chloroform - ethanol (45:45:10); chloroform - acetic acid (90:10), chloroform-acetic acid (80:90) and chloroform-ethanol (90:10). Separation of the methanolic extract of turmeric on TLC plate with chloroform-acetic acid (80:10) gave the best results. For accurate determination of curcumins, the described methods are often unsatisfactory due to interference caused by co-extractives.

An HPLC method for the determination of the curcumins was earlier reported (Asakawa *et al.*, 1981) which could not separate the three curcuminoids. A simple HPLC system equipped with flourescence detector was subsequently developed (Tonnesen and Karlsen, 1982) which separates curcumin and its structural isomers as well and makes it possible to determine with high accuracy, the absolute curcumin content of a sample. To improve the separation conditions for curcumins and to avoid interference from other constituents, another LC method was subsequently developed using electrochemical detector for better selectivity (Smith and Witowska, 1984). The analysis was best done using an ultraviolet detector at 254 nm and acetonitrile - buffer (pH 4.4) (64:40) as eluent.

2.8.3 Turmeric in Pest Control

Turmeric has various uses in animal and agricultural pest control. A chloroform extract (100%) of *Curcuma longa* has been reported to be effective in treatment of *Trichophyton verrucosum*, a ringworm of cattle (Thakur *et al.*, 1983). When *C. longa* rhizome powder mixed with the extracts of *Allium cepa*; *A.sativum*, *Citrus limonum* and powdered seed of *Abrus precatorius* in sesame oil base, was applied to piglets, infection created by *Sercoptes scabies* sub, sp. *Suis*, was completely eliminated. The essential oil from *C. longa* at 3000 ppm have been reported to inhibit mycelial growth of agriculturally important fungus *Aspergillus flavus* (Singh *et al.*, 1984). The insect repellent action of turmeric was reported as early as 1959 by Sreenivasamurthy and Krishnamurthy. Turmeric powder is commonly mixed with Basmati rice for protection against insects in India and Pakistan. Whereas, turmeric powder repelled the granary weevil *Sitophylus granarius* L. and grain borer *Rhyzopertha dominica*, its petorleum ether extract was repellent against *Tribolium castaneum* (Jilani and Su, 1983). From the petroleum ether extract Su *et al.* (1982) isolated two insect repellent components identified as turmerone and *ar*-turmerone. These componds @ 200 $\mu g/cm^2$ gave an average 43.1% and 62.9% repellency respectively to *Tribolium castaneum* after eight weaks. Turmerone was thermally unstable and yielded its dimer or more stable form, *ar*-turmerone in the presence of air. When repellent action of turmeric oil, sweetflag oil (*Acorus calamus* L.), neem oil and a commercial neem formulation Margosan-O was investigated, Margosan-O at 1000 ppm was found to be most active followed by

neem oil, turmeric oil and sweetflag oil (Jilani *et al.*, 1988). No adult progeny was recorded in rice treated with 500 or 1000 ppm of turmeric oil or 1000 ppm of Margosan-O. In a paper strip method of the same experiment, turmeric oil was the most repellent followed by neem oil, sweetflag oil and Margosan-O. During 8 hr. of test period, repellency of turmeric oil and sweetflag oil decreased more rapidly than that of neem oil or Margosan-O indicating that neem oil and Margosan-O are relatively more persistant. The decreased persistence of turmeric and sweetflag oil was attributed to higher volatilization of active components-turmerone or *ar*-turmerone from turmeric oil and asarone from sweetflag oil. Insect control property of essential oils isolated from three spices namely, turmeric, black pepper, (*Piper nigram*) and cardamom (*Eleffaria cardamomum*) against *Lasioderma serricornae* important pest of stored products especially tobacco and spices, revealed that coating or spraying its oil to the respective spices caused 100% adult mortality at a dose of 0.1 mg/10 g spice (Samuel *et al.*, 1984). It was subsequently observed that turmeric was effective in the control of bruchid, *Callosobruchus maculatus*, a stored grain pest of green gram *Vigna radiata*. The adult females were more tolerant than adult males. The LD_{50} and LD_{99} for males were 0.1 and 1.5 ppm. Though as compared to synthetic insecticides, the extracts were less active, they were much more effective than the other plant origin insecticides such as black pepper and neem.

Laboratory studies conducted to evaluate the efficacy of turmeric powder (TP) and Mustard oil (MO) alone and in different combinations as protectant for milled rice against *Sitophilus oryzae*

revealed that 4 ml/kg dosage of MO in combination with 2 to 2.5 g TP/kg of rice offered best protection through complete suppression of the progeny (Chander *et al.*, 1991). Though mustard oil dosage exhibited excellent protection of rice, its use may not be desirable as problem of rancidity may develop. During prolonged storage, TP at the effective concentration can prevent the mustard oil from becoming rancid. Similarly, against *T. castaneum*, TP and unrefined mustard oil (MO) alone did not cause significant adult mortality. The progeny of the treated insect was suppressed by only 60 and 80% at doses of 2% TP and 8 ml/kg MO respectively. On the other had mustard oil at 8 ml/kg + 1-10 g of turmeric/kg of rice gave complete protection (Chander *et al.*, 1992).

Feeding toxicity studies conducted on turmeric and its alcoholic extracts revealed that turmeric is nontoxic even at very high dose level of 2.5 g/kg body weight of experimental rats, guineapigs and monkeys which corresponds to a maximum consumption of 5 g/day for an adult human with an average weight of 70 kg.

2.8.4 Therapeutical and Anti-oxidative Activity of Curcuminoids

Curcuminoids have been reported to possess bactericidal and *in vitro* antifungal activity (Thakur *et al.*, 1983), in experimental animals. It is also known to act as topical agent in anti-cancer therapy and has anti-inflammatory, antimalarial (Pradhan *et al.*, 1983), antiarthritic, antithrombolic and antirheumatic antigastric antitumour antihepatotoxic and anticoagulative (Toda *et al.*, 1985) activity. Most of the activities

of curcuminoids are mainly due to its being a very strong antioxidant.

The anti-inflammatory action of curcumin and related compounds when tested for their *in vitro* inhibition of lipid peroxide formation revealed that: i) the caffeic acid moiety is very potent as an *in vitro* inhibitor of lipid peroxidation, ii) two caffeic acid molecules when joined together by a methylene bridge lead to bis-demethoxy curcumin which is the most potent antioxidant, iii) methylation of curcumin decreases the antioxidant property. and iv) demethylated derivatives of curcumin and ferulic acid viz. 3, 4-dihydroxy cinnamoylmethane and caffeic acid are the most potent inhibitors of lipid peroxidation.

In an atempt to identify the antioxidative components in the methanol extract of crude drugs Toda *et al.* (1984, 1985) detected several antioxidative components such as caffeine, d-catechin and l-epicatechin in the leaves of *Thea sinensis* L. Geniposidic acid was identified in seeds of *Plantgo asiatica* L. The activities of these components were found to be comparable to those of butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT). Earlier, antioxidant property of *C. longa* constituents was evaluated by air oxidation of linoleic acid (Tanizawa *et al.*, 1983). Curcuminoids (curcumin-I, II & III) were identified as the active principles responsible for anti-oxidative property. The 50% linoleic acid oxidation inhibitory concentration of each samples showed that LC_{50} of curcumin, demethoxy curcumin and bis-demethoxy curcumin were higher than that of the standard anti-oxidant BHA or BHT and lower than that of dl-α-tocopherol. While studying

the structure-activity relationships, it was revealed that cinnamic acid showed weaker antioxidative activity than *p*-coumaric acid. Thus phenolic hydroxy group was considered to be important for imparting antioxidative property. Presence of methoxy group next to phenolic hydroxy group also coutributed to the antioxidative activity of curcuminoids. A phenolic hydroxy group instead of methoxy group adjacent to phenolic hydroxy group is preferable. Further, the double bonds in curcuminoids also contribute to the antioxidative activity. Eugenol and zingerone which possess the partial chemical structure of curcumin were also found to be stronger antioxidative agents (Hasegawa, 1979; Fujio and Hiyoshi, 1964).

Antioxidative studies conducted on recently isolated two new curcumin type pigments having only one keto group instead of two in curcumins revealed that these compounds have better anti-oxidative potential than curcumin-I (Masuda *et al.*, 1993). The study concluded that the effect of the diketone system in curcumins on the anti-oxidant activity was low. In another study (Jitoe *et al.*, 1992), acetone extract of nine tropical ginger including five *curcuma* species exhibited better anti-oxidant activity which can replace α-tocopherol as a naturally occuring anti-oxidant. Since ginger extract had a low curcuminoid content inspite of their strong activity, it indicated the possibility of finding a new effective anti-oxidant other than curcumins in the tropical ginger.

2.9 Stablization of Azadirachtin with Turmeric Products

Azadirachtin has some inherent disadvantages such as

increased biodegredability, photolability and sensitivity to acid and base. Such undesirable traits can be overcome by using anti-oxidants and UV- sereens in their formulations to increase their residual toxicity and shelf-life. Since synthetic UV-screens and anti-oxidants may not be environment safe due to their possible toxic residues, natural anti-oxidants from *Curcuma longa* (Turmeric) being environmentally benign may provide an alternative. The present study is thus aimed at investgating the potential of turmeric products in photostabilizing azadirachtin based products for increased residual life and better efficacy.

MATERIALS AND METHODS

3.1 Chemicals

Neem seed kernels were purchased from Neem Mission, Pune, India, and were ground before extraction. Ground turmeric powder used for culinary purpose was purchased from local market. Laboratory grade reagents and solvents were locally procured, purified and then used. All the solvents were distilled and dried before use. HPLC grade solvents were used for the analysis of azadirachtin and curcumin in the test samples. Azadirachtin (45%) isolated from neem seed kernels and curcumin-I (≈95%) isolated from *Curcuma longa* was used in photodegradation studies. Azadirachtin sample (50%) obtained from Australia (Courtesy Dr. R. P. Singh, Division of Entomology, IARI) served as standard reference.

3.2 Apparatus

3.2.1 Glass apparatus

Besides common glass apparatus, few special-wares such as quartz tubes (254-360 nm) and pyrex tubes (290-360 nm) were used in photochemical experiments.

3.2.2 Light sources

Experiments were conducted under sunlight in open space so that samples to be irradiated could receive maximum direct sunlight during day time (average 7-8 hour a day). Due care was taken to protect samples from rain, storm, dust, as well as morning

and night dew and fog. The temperature at the test surface varied between 25-35^{0}C. Sunlight intensity at wavelengths between 300-900 nm was approximately 720, 780 and 350 mw/cm^2 at the beginning, middle and end of the day, respectively.

3.2.2.1 High pressure mercury lamp

An indigenous photoreactor was used for photochemical reactions in solutions. Filament of the high pressure mercury vapour lamp 125 W (Phillips) was kept inside a quartz tube (4 cm id) and the tube containing UV-lamp was placed in a steel container and water was circulated to maintain the temperature around 25^{0}C (approx). Quartz tubes (2 cm i.d.) containing sample solution for irradiation were kept around the central tube containing UV lamp.

3.2.2.2 Germicidal lamp

Germicidal lamp of wave length 254 nm was used for irradiation of samples kept in the form of thin film in petri-plates.

3.2.3 Chromatography

3.2.3.1 Thin-layer chromatography (TLC)

Silica gel TLC plates were prepared by spreading a slurry of silica gel G containing 10 per cent binder (gypsum) in water on 6 cm x 20 cm glass plates uniformly maintaining a thickness of 0.25 mm with a TLC applicator. The plates were activated at (120^{0}C) for 2 h. The sample solutions were spotted on the TLC plates using capillary tubes. Plates were developed in suitable solvent system, air dried and visualized in iodine vapour or sprayed with dil. H_2SO_4 followed by heating. Azadirachtin spots on TLC

plates were visualized with 3% (W/V) vanillin in absolute ethanol containing 1% (V/V) sulfuric acid followed by gentle heating. For the preparative TLC, 20 cm x 20 cm glass plates were coated (0.25 mm) with silica gel slurry containing 10% binder (gypsum). Spots of curcumins were self indicating hence there was no need of visualization for preparative TLC. Spots were marked and scrapped. However, for routine TLC analysis of curcumin products spots were developed with iodine vapours. The scrapped silica gel was extracted with suitable solvent.

3.2.3.2 High performance liquid chromatography

Waters HPLC system equipped with 600 series pump and controller, 996 PDA detector and Rheodyne injector was used for analysis of azadirachtin and cucurmins in samples. The analysis was carried out on Navapack phenyl 16 A^{o}, 4 μm, 3.9x150 mm cartridge column containing dimethylphenylpropylsilyl bonded amorphous silica. Separation of azadirachtin-cucurmin test mixture was performed under isocratic conditions at a flow rate of 0.75 ml^{-1} using a mobile phase of methanol : water (65 : 35, V/V). The PDA detector was operated at a wavelength of 217 nm for azadirachtin and 254 nm for curcumins with 4.8 nm resolution. Separation of curcumins was achieved by Waters HPLC system consisting of 501 pump, 484 tunable detector and Lichrosorb RP-8 column (250 mm x 4 mm i.d.) at 254 nm absorption. Acetonitrile (0.5 ml/min.) was used as mobile phase. A 20 μl volume of sample was injected each time via a rheodyne injector (20 μl loop) for a run time of 10 to 20 min.

3.2.4 Spectroscopy

3.2.4.1 Nuclear magnetic resonance spectroscopy (^{1}H-NMR)

^{1}H-NMR spectra of curcumins were recorded on a Bruker 300 AC (300 MHz) instrument. Deuterio-dimethyl sulfoxide (deuterated -DMSO) was used as solvent and tetramethyl silane (TMS) was used as internal standard. Rest of the samples were analysed by Varian EM-360L, 60 MHz instrument. Deuterio-chloroform ($CDCl_3$) was used as solvent with TMS as an internal standard.

3.2.4.2 UV-Vis spectroscopy

The ultraviolet-visible (UV-Vis) spectra of curcumins were recorded with an Hitachi Model V-2000 double beam specrtophotometer in methanol using a quartz cuvette (1 cm path length).

3.2.4.3 FT-IR spectroscopy

Infra-red (IR) spectra were recorded in nujol and KBr Disc with Nicolet Impact 700 FT-infra-red spectrophotometer.

3.2.4.4 Gas chromatography-mass spectroscopy (GC-MS)

The GC-MS data were recorded on a HRGC-MEGA 2 series GC-coupled with Fisons TRIO-1000 ion trap mass spectrometer at an ionization potential of 70 ev. GC was equipped with an SE-54 capillary column (15 m x 0.25 mm i.d, film thickness, 0.1-0.15 μ). The initial temperature was kept at 60°C for 1 min. and then heated @ 15°C min^{-1} upto 250°C (10 min). Oven temperature was maintained at 270°C. Helium was used as the carrier gas.

Mass spectra of curcumin-I, II and III were recorded on Hewlett Packard 5980 mass spectrometer in Chemistry Department of State University of New York, USA.

3.2.5 Calibration of HPLC instrument

In order to check the linearity and detection limit of the instrument, the solution of azadirachtin (45%) in different concentrations (250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.95 ppm) were injected and a standard curve was prepared by plotting concentration on X-axis against area of the peak on Y-axis.

3.2.6 Tobacco seedlings

For conducting experiment on leaf surface, tobacco seedlings (*Nicotiana tabacum cv. Xanthi*) were grown in earthern pots (6" dia. with 6" height). One seedling was grown per pot. Leaves of nearly equal size and equal age were selected for the experiment. Samples were applied on an area equivalent to an area of a circle having 5 cm internal diameter. Plants were protected from storm, dust, night and morning dew and fog. Tobacco plants were selected for this experiment as tobacco leaves are tolerant to methanol and are oppositely arranged ensuring each leaf an equal exposure to the sunlight and there is no mutual shading in the leaves.

3.3.1 Extraction of Neem Seed Kernels

Azadirachtin enriched fraction was separated from the neem seed kernels by following Schroeder and Nakanishi method (1987).

Seed kernels from *Azadirachta indica* (2.0 kg) were placed in a mixer-cum grinder along with hexane (2.0 litres) and ground to a fine powder for fifteen minutes. In each lot, 500 gm seeds and 500 ml of hexane was taken. After completion of grinding, total material was taken in a 5 litre conical flask and was allowed to stand for 12h after which the mixture was agitated once again for 5 minutes. The resulting suspension was filtered through large Büchner funnel under vacuum. After solvent removal, the resulting seed cake (500 gm lot) was returned to the blender along with hexane (500 ml) and the above process repeated four times. The combined hexane extract was concentrated to obtain neem oil.

The seed cake was extracted in the same manner with five 2-liter (500 ml lot) volumes of 95% ethanol. Total material was transferred to a 5 liter conical flask and allowed to stand for 12h. Solvent was filtered through Büchner funnel under pressure and after solvent removal, a dark viscous extract was obtained (162 g). The ethanolic extract was then subjected to two quick, efficient partitioning between petroleum ether and 95% aqueous methanol to remove any remaining oils and other non-polar materials, followed by partitioning between water and ethyl acetate to remove water soluble proteins and sugars, if any. The ethyl acetate extract was vacuum filtered over silica gel bed and solvent distilled off under vacuo to obtain viscous concentrate (65 g).

The residue (65 g) was chromatographed in five portions, and ten 200 ml fractions were collected for each portion. The first 3-4 fractions probably consisting of least polar compounds which

were discarded. Fraction 5-8 were collected and concentrated in vacuo to obtain partially purified azadirachtin rich extract. The partially purified material was then dissolved in CCl_4, warmed on water bath and filtered through suction while warm. It was then stored in the fridger. An off-white powdery material (4.5 g) was separated out in freeze within two days and was collected by suction filtration. TLC was accomplished on 5 cm x 10 cm Silica gel G (Merck) coated plates.

3.3.2 LC-Determination of Azadirachtin Content

Azadirachtin sample obtained after extraction was dissolved in methanol and analysed by HPLC at 217 nm, following BIS, 1995 specifications with some modification to the prescribed method. A Waters HPLC unit equipped with Rheodyne injector (20 µl loop), Novapck phenyl column, PDA detector and printer-plotter-cum-integrator was used for the analysis. Methanol-water (65:35) with a stable flow rate of 0.75 ml/min was maintained as mobile phase. Retention time for azadirachtin was observed at 3.81 min.

10 mg of standard azadirachtin (50% pure) was weighed accurately into a 10 ml volumetric flask and dissolved in methanol and the volume was made up to the mark with methanol. 1 ml of this stock was pipetted out into 10 ml volumetric flask and volume was made up. Similarly, 10 mg of the sample was accurately weighed into another 10 ml volumetric flask. About 7 ml of methanol was added to it and shaken for 5 minutes and volume was made up with methanol. 1 ml of this solution was pipetted

into 10 ml volumetric flask and volume was made up with methanol.

20 µl of standard azadirachtin and sample solution were injected respectively to get area reproducibility for two consecutive injections. The variation in area of two consecutive injection remained within 2 per cent. From the HPLC chromatogram, percentage of azadirachtin in the sample was calculated by the following formula:

$$\text{Azadirachtin content (per cent by mass)} = (A_1/A_2) \times (m_2/m_1) \times P$$

Where,

A_1 = peak area of azadirachtin in sample solution;

A_2 = peak area of azadirachtin in reference standard solution;

m_1 = mass, in g, of the sample taken for the test;

m_2 = mass, in g, of the reference standard azadirachtin; and

P = purity of reference standard azadirachtin.

3.4.1 Extraction of Turmeric Rhizomes

Powdered air-dried rhizomes of turmeric (1 kg) were extracted with petroleum ether (40-60°) in a Soxhlet apparatus for 24 hr. The petroleum ether extract on removal of the solvent gave turmeric oil residue (18.3 g).

The de-oiled powder was further extracted in the same Soxhlet apparatus with benzene for 64 hrs. Evaporation of the benzene extract gave an orange powder (8.30 g). Thin layer chromatography (TLC) examination (benzene-ethanol 9:1) indicated presence of three components.

This mixture (4 g) comprising of three components was subjected to coloumn chromatography over silica gel with benzene as eluting solvent. The mixtgure was separated into three clearly separable yellow components. The dominant component eluting as raddish orange crystals (mp 184°C) was identified as curcumin-I. The second orange-yellow product (mp 169°C) and third yellow coloured compound (mp 210 °C) were identified (TLC) as curcumin-II and III respectively.

2.1.4.2 Separation and Identification of Turmeric Products

To obtain sufficiently pure curcumins, curcumin mixture (1 g) subjected to preparative TLC using 20 cm x 20 cm plates, each carrying 0.25 mm thick layer of silica (100 g). The extract (1 g) was applied to 20 plates, and eluted with benzene-ethanol (9:1). Three orange-yellow bands separated on the plate were marked, removed and extracted with acetone.

The top band after evaporation gave 1,7-bis 3-methoxy-4-hydroxy cinnamoyl (feruloyl) methane, (curcumin-I) (355 mg), m.p 184°C R_f 0.4 (Benzene-ethanol, 9:1). Found C, 68.48; H, 5.42; O,26.1%. Calc. for $C_{21}H_{20}O_6$: C, 68.5; H, 5.45; O, 26.05%. MS (m/z, rel.int.) 368(2.55%, M^+), 350(9.88%, M^+-H_2O), 272(6.06%), 232(5.68%), 217(13.73%), 190(44.97%), 177(100%), 161(12.15%), 160(7.49%), 149(10.71%), 145(79.46%), 137(94.67%), 117(39.26%). ^{1}H NMR (DMSO): δ 3.897 (s, 6H, 2x-0CH_3), 5.88 (s, 1H,-C(O)C<u>H</u>=C-), 6.50, 6.56 (d, 2H, *J*=16 Hz, -C<u>H</u>-C(O)-CH_2-C(O)-C<u>H</u>-), 6.85, 6.88 (d, 2H, J= 8Hz, 9,9'-H aromatic), 7.04, 7.07 (dd, 2H, J=2 & 8 Hz, 10, 10'-H_2), 7.08, 7.12 (d, 2H, J=2Hz 6, 6'-H aromatic), 7.51,

7.56 (d, 2H, J=16Hz, 4, 9'-H), 9.25 (2H, Ar-O<u>H</u>). IR (ν^{KBr}) cm^{-1} : 3427 (O-H str); 2950-3000 (α,β-unsaturated and aryl C-H str); 1611 (C=O str); 1509 (ring C⎓Cstr); 1287, 1207 (C-O str).

The middle band provided 4-hydroxy cinnamoyl (feruloyl) methane (Curcumin-II) (145 mg) m.p. 169°C R_f 0.30 (Benzene-ethanol, 9:1). Found C, 71.18; H, 5.64; O, 23.18. Calc. for $C_{20}H_{18}O_5$: C, 71.0; H, 5.35 O, 23.65. MS (m/z, rel.int.) : 338(0.6%, M^+), 320(14.08%, M^+-H_2O), 242(6.64%), 217(14.27%), 202(7.58%), 190(43.29%), 177(50.63%), 150(28.6%), 147(100%), 137(47.24%), 119(40.19%), 107(36.92%). ^{1}H NMR (DMSO): δ 3.897 (s, 3H, -OCH_3), 5.86 (s, 1H, -C<u>H</u>-C-), 6.47, 6.51 (d, 1H, J = 19 Hz, 3'-H), 6.52, 6.56 (d, 1H, J = 16 Hz, 3-H), 6.82, 6.85 (d, 1H, J= 8Hz, 9-H aromatic), 6.87, 6.90 (d, 1H, J= 8Hz, 9'-H aromatic), 6.87, 6.90 (d, 1H, J= 8Hz, 7'-H aromatic), 7.04, 7.00 (dd, 1H, J=2 and 8Hz, 10-H), 7.06, 7.1 (d, 1H, J= 2Hz, 6-H), 7.44 (d, 2H, J= 8Hz, 6' and 10'-H), 7.51, 7.56 (d, 2H, J= 16Hz 4, 4'-H), 9.27 (1H, Ar-OH), 9.71 (IH, Ar-OH). IR (ν^{KBr}) cm^{-1} : 3315 (broad O-H str); 2920(α,β-unsaturated and aryl C-H str); 1625 (C=O str); 1600 (ring C⎓ Cstr); 1136(C-O str).

The lowest band yielded bis (4-hydroxycinnamoyl) methane (curcumin-III) (130 mg) m.p. 210°, R_f (0.25, benzene-ethanol, 9:1) Found : C, 74.03; H, 5.19, O 20.79. Calc. for $C_{19}H_{16}O_4$, C = 74.02, H = 5.19, O, 20.77. MS (m/z, rel.int.) : 308(4.58%, M^+), 290(7.25%, M^+-H_2O), 212(6.93%), 202(8.10%), 187(10.99%), 174(4.6%), 160(40.1%), 147(100%), 131(18.47%), 119(38.04%), 115(17.79%), 107(42.17%). ^{1}H NMR (DMSO) : δ 5.97 (s, 1H, -C<u>H</u>=C-), 6.58-6.70 (d, 2H, J= 16Hz,3, 3'-H), 6.83, 6.85 (d, 4H, J= 8Hz, 7,

7' and 9, 9'-H), 7.46, 7.49 (d, 4H, J= $8H_2$, 6, 6' and 10, 10'-H), 7.52, 7.58 (d, 1H, J= 16Hz, 4'-H), 9.96 (2H Ar-OH). IR (ν^{KBr}) cm^{-1} : 3200-3300 (broad O-H str); 1600, 1626(C=O str); 1595, 1510 (ring C ⋯ Cstr); 1168, 1139(C-O str).

3.4.3 Synthesis of Curcumin-I Derivatives

3.4.3.1 Trimethyl curcumin-I

Curcumin-I (0.86 g) in dry acetone (50 ml) was treated with methyl iodide (1.0 ml) and the mixture refluxed over anhydrous potassium carbonate (1g) for 24 h. After filtration, solvent was evaporated and the residue extracted with ether. Evaporation of the ether extract gave trimethyl curcumin-I (1,7-bis (3, 4-dimethoxyphenyl) -4 methylhepta-1, 6-diene -3, 5-dione) (0.72 g), m.p. 150-151°C R_f (0.78, benzene-ethanol, 95:5). Found C, 70.5; H, 6.71; O, 22.79% Calc., for $C_{24}H_{26}O_6$, C, 70.35; H, 6.37; O, 23.28. ^1H NMR ($CDCl_3$) : δ 2.3 (s, 3H, (CH_3)-C=), 4.0 (s, 6H, 2x-OCH_3), 4.10 (s, 6H, 2 x-OCH_3), 7.10 (d, 2H, J= 18Hz, 2Ha proton). 7.20 (d, 2H, J=16Hz, 2x Ar-CH=C<u>H</u>-C-), 7.22 (d, 2H, J= 8Hz, 2Ha proton), 7.9 (d, 2H, J= 16 HZ 2x Ar-C<u>H</u>=CH-C(O)-). IR (ν^{KBr}) cm^{-1} : 2934(α,β-unsaturated and aryl C-H str); 1617, 1596 (C=O str); 1510 (ring C⋯ Cstr); 1260, 1167(C-O str).

3.4.3.2 Di-butyl Curcumin-I

Curcumin-I (0.86 g) in dry acetone (50 ml) was treated with *n*-butyl bromide (1.0 ml) and the mixture refluxed over anhydrous potassium carbonate (1 g) for 24h. After filtration, the solvent was evaporated and the residue extracted with ether. Evaporation of the ether extract gave dibutyl curcumin-I [1,7-bis

(3-methoxy-4-*n*-butoxyphenyl) - hepta - 1,6-diene -3,5-dione] (0.95 g) m.p. 121-123°C (methanol), Found C, 72.51; H, 7.49; O, 20% Calc. for $C_{29}H_{36}O_6$, C, 72.5; H, 7.5; O, 20%. ^{1}H NMR ($CDCl_3$): δ 1.3 (t, 6H, 2x - $OCH_2CH_2CH_2C\underline{H}_3$), 1.3-1.8 (m, 8H, 2 x- $OCH_2C\underline{H}_2C\underline{H}_2CH_3$), 3.95, (s, 6H, 2x- OCH_3), 4.25 (t, 4H, 2x- $OC\underline{H}_2CH_2CH_2CH_3$), 6.95 (d, 4H, 2 x-CO-CH=CH, J=16 Hz), 7.2 (m, 6H, aromatic), 7.65 (d, 2H, 2x-COCH=C$\underline{H}$-, 16 Hz). IR (ν^{KBr}) cm^{-1} : (α,β-unsaturated and aryl C-H str); 1620, 1600 (C=O str); 1509 (ring C⋯ Cstr); 1239, 1306, 1137(C-O str).

3.4.4 Photolysis of Curcumin-I under UV-Light

Pure curcumin-I (50 mg) obtained from preparative TLC was dissolved in methanol (50ml) and was irradiated in a water cooled quartz flask with light from a high pressure mercury lamp 125 W (Phillips) for 10h with occasional bubbling of air through the solution. The volume of the solvent was reduced to a convenient amount by distillation. The concentrated solution so obtained was treated with ethereal solution of freshly prepared diazomethane for methylation. After completion of methylation (few drops of coloured solution taken into a test tube and a glass rod moistened with glacial acetic acid was introduced, immediate evolution of gas indicated completion of reaction), the solvent was distilled off and methylated photoproduct was again dissolved in methanol for identification of individual degraded products by Gas Chromatognaphy- Mass Spectroscopy (GC-MS).

3.4.5 Synthesis of Curcumin Photoproducts

3.4.5.1 4(3,4-Dimethoxyphenyl)-but-3-ene-2-one or (3,4-dimethoxy cinnamoyl methane)

Veratraldehyde (1.66 gm, 0.01 mol) and pure acetone (2.5 ml) were placed in a 250 ml conical flask equipped with magnetic stirrer. The reaction vessel was immersed in cold-water and 1 ml of 10% NaOH solution was added slowly during half an hour from a separatory funnel. The rate was so adjusted that the temperature was maintained between 25-30^{0}C. The reaction mixture was stirred for further two hours at room temperature. The reaction mixture was rendered just acidic to litmus paper adding dilute hydrochloric acid. The material was transferred to a separatory funnel and upper organic layer was taken out. The lower aqueous layer was extracted with 20 ml of toluene. The combined yellow organic layer was washed with 20 ml water and dried with little anhydrous magnesium sulfate. After removing toluene at atmospheric pressure, the product was crystallised from methanol as yellow crystals. Yield (1.95 gm), m.p 75-78^{0}C R_f 0.56 (benzene-ethanol, 95:5). MS (m/z, irl.int.) : 206 (47.27%, M^+), 191 (100%), 175 (23.63%), 163 (13.63%), 160 (5.45%), 148 (12.7%), 132 (10%), 119, 105, 91, 77. ^{1}H NMR: ($CDCl_3$) : δ 2.35 (s, 3H, -$COCH_3$), 3.95 (s, 6H, 2 x-OCH_3) 6.7 (d, 1H, J=16 Hz, Ar-C<u>H</u>=CH-), 7.2 (m, 3H, aromatic) 7.6 (d, 1H, J=16 Hz ar-CH=C<u>H</u>-C-).

3.4.5.2 3, 4-Dimethoxy cinnamic acid methyl ester

Veratraldehyde (1.66 gm) (0.01 mol) and malonic acid (1.75 g) were dissolved in a mixture of pyridine 4.5 ml and few drops of piperidine contained in a round bottom flask (100 ml) and

heated under reflux for 1h on water bath. Rapid evolution of CO_2 was observed. The reaction was completed by boiling the solution for 5 minutes. Reaction mixture was cooled and excess of water containing hydrochloric acid was added to combine with excess pyridine. Acid so obtained was filtered and recrystallised from hot water (m.p. 160°C).

Small quantity of the acid was dissolved in methanol and etheral solution of diazomethane was added. After completion of methylation, solvent was distilled off to obtain a brownish, sweet smelling semi-solid ester. MS (m/z, rel.int.) : 222 (100% M^+), 207 (20%), 191 (69.09%) 163/164 (13.63), 147, 137, 133, 132. ^1H NMR ($CDCl_3$) : δ 3.8 (s, 3H, -,$COOCH_3$), 3.9 (s 6H,- O CH_3, 6.35 (d, 1H, J=16.5 H_z, H_c), 6.9 (d, H, J= 8.5 H_z, H_a), 7.1 (m_1 2H, H_b and H_c), 7.7 (d, 1H, J= 16.H_z, H_b). IR (ν^{KBr})Cm-1 : 3019 - 2841 (α, β-unsaturated, and aryl C-H str), 1667, 1594 (C= O Str), 1514 (ring C ⎓ C str),1253,1137 (C-str).

3.4.5.3 3,4-Dimethoxybenzoic acid methyl ester

Vanillic acid (100 mg) taken in methanol was treated with sufficient quantity of the etheral solution of diazomethane. After completion of methylation, solvent was distilled off to obtain a brown coloured ester. MS (m/z, rel.int.) : 196 (78.16%, M^+), 181 (11.8%), 165 (100%), 137 (11.8%) 107 (10%) 125, 94, 79. ^1H NMR ($CDCl_3$) : δ 3.9 (s, 9H, 2 x - OCH_3, -$COOCH_3$), 6.9 (m, 1H, H_a), 7.6 (m, 2H, H_b and H_c). IR (ν^{KBr}) Cm^{-1} : 3019 (aryl C-H str), 1709, 1616 (ester C=O str), 1433 (ring C ⎓ C str), 1222 (C-O str).

3.5 Photodegradation of Azadirachtin

A 4000 ppm stock solution of 45% pure azadirachtin was prepared by dissolving 40 mg of the 45% pure azadirachtin in 10 ml of methanol. From this stock solution 2000, 1000, and 500 ppm sub-stocks were prepared by serial dilution for photodegradation studies.

3.5.1 UV-light, methanolic solution

Azadirachtin solution in methanol (20 ml, 500 ppm) was irradiated in three water cooled quartz tubes with UV-light emanating from a high pressure mercury lamp (125 W) for 5 h. The sample from each tube was collected at half an hour interval and were filtered before HPLC analysis.

3.5.2 UV-light, thin film on glass surface

Azadirachtin solution in methanol (1 ml, 1000ppm) was applied uniformly in sufficient number of 5 cm dia petri-plates with pipette. The solvent from petriplates was allowed to evaporate at room temperature to form a thin film of azadirachtin. Plates were irradiated upto 14h under germicidal lamp (254 nm). Samples were taken (3 at a time) at 1h intervals. The azadirachtin layer from each petriplate was extracted with methanol (2x1 ml) to obtain 2 ml of the extract for HPLC analysis.

3.5.3 Sunlight, methanolic solution:

Azadirachtin solution (20 ml, 500 ppm) in methanol was taken in three pyrex tubes and irradiated under sunlight (7-8h/ day) upto 7 days. 1 ml of the sample was withdrawn every day

from each tube and analysed on HPLC instrument.

3.5.4 Sunlight, thin film on glass surface

Azadirachtin solution (1 ml,1000 ppm) was spread uniformly in sufficient number of 5 cm dia. petriplates with the help of a pipette. The solvent from the plates was allowed to evaporate at room temperature to from an uniform thin layer of azadirachtin which was irradiated under sunlight for 7 days. Samples were taken everyday (3 replications) and thin layer of azadirchtin from each petriplate was extracted properly with methanol to obtain a final volume of 2 ml. The samples after filtration, were analysed by HPLC instrument.

3.5.5 Sunlight, thin film on leaf surface

Azadirachtin solution (0.5 ml, 2000 ppm) was applied uniformly on each leaf (Tobacco plant) on an area equivalent to that of a circle (5 cm dia) by a pipette. A total of 25 leaves were treated. Solvent was allowed to be evaporated off from the treated leaves under room temperature and kept under sunlight for a week. Samples (3 leaves) were taken daily and each leaf was extracted with methanol (2x1 ml) to obtain a final volume of 2 ml.

3.6. Photodegradation of Curcumins

3.6.1 UV-light, methanolic solution

In three different experiments, solution of curcumin-I, curcumin-II and curcumin-III (20 ml, 500 ppm) each in 3 replications contained in quartz tubes was irradiated in UV-light from 125 W high pressure mercury lamp for 7 h. Samples were

collected after half hourly intervals and analysed on HPLC.

3.6.2 UV-light, thin film on glass surface

Methanolic solution of each of the three curcuminoids (curcumin-I, curcumin-II and curcumin-III) (1 ml, 1000 ppm) was applied in 5 cm dia petriplates in equal and sufficient number. After evaporation of the solvent, plates were irradiated under UV-light from a germicidal lamp (254 nm) for 15 h and samples were collected at 1 hour intervals. Each plate was rainsed with methanol (2x1 ml) and samples filtered before HPLC analysis.

3.6.3 Sunlight, methanolic solution

Methanolic solution of circumin- I (20 ml, 500 ppm) in three different pyrex tubes were irradiated under sunlight for 7 days. Samples were taken from each tube everyday for analysis.

3.6.4 Sunlight, thin film on glass surface

1 ml of 1000 ppm methanolic solution of curcumin I was applied in petriplates (5 cm dia) taken in sufficient number. After evaporating off the solvent, plates were irradiated under sunlight upto 5 days and samples were collected (3 replications) everyday. Each plate was rinsed with methanol (2x1 ml) and samples analysed by HPLC.

3.6.5 Sunlight, thin film on leaf surface

0.5 ml methanolic solution (2000 ppm) was spread uniformly over each of the fifteen tobacco leaves. After evaporation of the solvent from the leaves, plants were kept under sunlight for 5 days. Everyday 3 leaves were clipped off and rainsed with

methanol (2x1 ml) separately. Filtered samples were analysed by HPLC.

3.7 Photostabilization of Azadirachtin

3.7.1 UV-light, methanolic solution

3.7.1.1 With curcumin-I, II and III

Methanolic solution of azadirachtin (1000 ppm, 5ml) and curcumin- I (1000 ppm, 5ml) was mixed to obtain a solution (10 ml) containing 500 ppm each of azadirachtin and curcumin-I in 1:1 ratio. This mixture was taken in three quartz tubes for irradiation under UV-light. Similarly, 5 ml each of the methanolic solution of azadirachtin (1000 ppm) and curcumin-I (500 ppm) were added in another three tubes to have a 2:1 azadirachtin : curcumin-I solution. Similarly solution of azadirachtin : curcumin-I (3:1) was obtained by mixing 5 ml each of azadirachtin (1000 ppm) and curcumin-I (333 ppm) in yet another three quartz tubes. All the tubes were irradiated simultaniously in UV-light emanating from a high pressure mercury lamp upto 7 h. Samples were withdrawn separately at half hourly intervals and all the samples were filtered before HPLC analysis.

Similar experiments were conducted by irradiating separately methanolic solution of azadirachtin-curcumin-II, azadirachtin-curcumin-III and azadirachtin-curcuminoid mixture (I, II, and III) in three different proportions (1:1, 2:1, 3:1), each with three replications. Samples collected after half hourly intervals were filtred and analysed by HPLC for azadirachtin content.

3.7.1.2 With trimethyl and di-butyl derivatives of curcumin-I

Methanolic solution of azadirachtin and trimethyl derivative of curcumin-I in three different ratios (1:1, 2:1, and 3:1), each with three replications kept in different quartz tubes were irradiated with UV-light upto 5 h as above. From each tube, samples were collected at half hourly intervals and after filtration were analysed on HPLC. In another set of experiment, methanolic solution of azadirachtin and dibutyl curcumin-I in three different proportions (1:1, 2:1 and 3:1) were irradiated in UV-light from high pressure mercury lamp as described above. The samples collected after half hourly intervals were filtered and analysed on HPLC for azadirachtin content.

3.7.1.3 With butylated hydroxy toluene (BHT)

Methanolic solution of azadirachtin and BHT in 1:1, 2:1, and 3:1 ratio, each with three replications was taken in quartz tubes and irradiated in UV-light upto 5h. Samples were collected at half hourly intervals and filtered before analysis by HPLC.

3.7.2 UV-light, thin film on glass surface

3.7.2.1 With curcumin I, II, and III, and mixture of curcumins

1 ml each of methanolic solution of azadirachtin (1000 ppm) and curcumin-I (1000 ppm) were spread uniformly in 5 cm dia. petriplates. Solvent from each plate was evaporated off in room temperature to from an uniform thin layer of azadirachtin-curcumin-I (1:1). In second experiment, a thin layer of 1 ml azadirachtin (1000 ppm) and curcumin-I (500 ppm) solution were

made containing azadirachtin-curcumin-I in 2:1 ratio. Similarly, when 1 ml each of aza (1000 ppm) and curcumin-I (333 ppm) were mixed and applied in petriplates after evaporation of the solvent, a thin layer of azadirachtin-curcumin-I (3:1) was obtained. All the three sets of plates were irradiated simultaniously under germicidal lamp (254 mm) for 16 h (2 x 1 ml) and the samples were collected in 3 replications in each case at 1h intervals. Each plate was washed properly with 2 ml of methanol.

Samples were filtered before HPLC analysis. Similar experiments were conducted separately with azadirachtin-curcumin-II, azadirachtin-curcumin-III, and azadirachtin-curcumin (I, II and III,) mixture.

3.7.2.2 With trimethyl and dibutyl drivatives of curcumin-I

An uniform thin film of methanolic solution of azadirachtin and trimethyl derivatives of curcumin-I in three different ratios (1:1, 2:1and 3:1) and with three replicates, was spread on glass surface (petriplate of five cm dia) by coating 1 ml of the test solution. It was subjected to UV-light (254nm) emanating from germicidal lamp. Similar experiment was planned with dibutyl curcumin-I. Samples in triplicate were collected as above and analysed by HPLC for azadirachtin content.

3.7.2.3 With butylated hydroxy toluene (BHT)

A thin film of 1 ml methanolic solution of azadirachtin-BHT mixture (1000 ppm each in 1:1, 2:1 and 3:1 ratio) was coated on sufficient number of petriplates (5 cm dia). Samples in

triplicates were collected as above and after filtration were analysed by HPLC for azadirachtin content.

3.7.3 Sunlight, methanolic solution

3.7.3.1 With curcumin-I

Three pyrex tubes containing 10 ml methanolic solution of azadirachtin and curcumin-I, (each 500 ppm) in 1:1 ratio were irradiated under sunlight for 7 days (7-8 h/day). Samples (0.5 ml) in triplicate were collected from each tube everyday and after filtration were analysed by HPLC.

3.7.3.2 With butylated hydroxy toluene (BHT)

Methanolic solution of azadirachtin and BHT (1:1, 500 ppm each, 10 ml) taken in three pyrex tubes was irradiated under sunlight outdoor and samples (in triplicate) were withdrawn everyday upto seven days and after filtration analysed by HPLC.

3.7.3.3 With turmeric oil (T.O)

10 ml methanolic solution containing azadirachtin and turmeric oil (500 ppm each) in 1:1 ratio was taken in three different pyrex tubes and irradiated under sunlight as described above. Samples were collected (in triplicate) from each tube everyday and after filtration were analysed by HPLC.

3.7.4 Sunlight, thin film on glass surface

3.7.4.1 With curcumin-I

1 ml methanolic solution containing azadirachtin (1000 ppm, 0.5 ml) and curcumin-I (1000 ppm, 0.5 ml) was applied in

sufficient number of petriplates (5 cm dia.) to make a thin film. After evaporation of the solvent in dark, the plates were irradiated under sunlight upto 7 days (7-8 h/day) and samples in three replications were collected everyday. Each plate was rinsed with methanol (2x1 ml) and samples filtered before HPLC analysis.

3.7.4.2 With turmeric oil and BHT

A thin film of azadirachtin-turmeric oil (1:1) and azadirachtin-BHT (1:1) was prepared in sufficient number of petri plates following above method, and irradiated separately under sunlight upto 7 days. The samples (3 replications) were collected everyday and rinsed with methanol (2x1 ml). All the samples were filtered before HPLC analysis.

3.7.5.1 Sunlight on leaf surface

A methanolic substock solution containing 2000 ppm each of azadirachtin and curcumin-I (1:1) was prepared by adding equal volume of each of azadirachtin (4000 ppm) and curcumin I (4000 ppm) solutions. 0.5 ml of the substock (2000 ppm), was uniformly spread on the upper surface of tobacco leaves in sufficient number, on an area equal to that of a circle of 5 cm dia. on each leaf. After evaporating off the solvent from the leaves in dark at room temperature, plants were irradiated under sunlight upto 7 days. Everyday three leaves were clipped from plants and rinsed with 2 ml of methanol (2x1 ml). Filtered samples were analysed by HPLC.

3.8 Photostabilization of Azadirachtin Rich Neem Oil

3.8.1 Preparation of azadirachtin rich neem oil solution with/without curcumin-I/turmeric oil

Neem oil (100 mg) was mixed with accurately weighed 100 mg of 45% pure azadirachtin to obtain 22.5% azadirachtin rich neem oil. 50 mg of this oil was taken into a 50 ml volumetric flask and volume made with methanol to produce azadirachtin rich neem oil solution containing 225 ppm azadirachtin (45%) equivalent to 112.5 ppm pure azadirachtin (100%). Similarly, a stock solution of azadirachtin enriched neem oil containing azadirachtin (225 ppm) was prepared for conducting experiments on leaf surface under sunlight conditions.

In another 50 ml volumetric flask 50 mg of azadirachtin rich neem oil and 11.25 mg of curcumin-I was taken. Volume was made up with methanol to produce azadirachtin rich neem oil solution containing 112.5 ppm each of azadirachtin and curcumin-I in 1:1 proportion. Similarly, a stock solution of azadirachtin (225 ppm), curcumin-I (225 ppm) in 1:1 ratio was prepared for conducting sunlight experiment on leaf surface.

In a 50 ml volumetric flask, 50 mg of azadirachtin rich neem oil was taken and 11.25 mg of turmeric oil was added to it. After making up the volume upto 50 ml with methanol, a solution of azadirachtin rich neem oil containing 112.5 ppm of azadirachtin was obtained in which azadirachtin : turmeric oil ratio was 1:1. In a similar way, a stock solution of azadirachtin rich neem oil containing azadirachtin (225 ppm) and turmeric oil (225 ppm) in 1:1 ratio was prepared for leaf surface experiment under sunlight.

3.8.1.1 Sunlight, methanolic solution

3.8.1.11Azadirachtin rich neem oil + curcumin-I

In each of the three pyrex tubes, 10 ml of the methanolic stock solution containing 5 ml of 225 ppm of azadirachtin and 5 ml of 225 ppm curcumin-I in 1:1 ratio was irradiated under sunlight upto 7 days. In equal number of tubes, methanolic solution (10 ml) from a stock solution containing only azadirach rich neem oil with 112.25 ppm azadirachtin was also irradiated simultaniously to assess the stabilizing ability of curcumin-I. Samples were collected from each tube everyday and after filtration samples were analysed by HPLC.

3.8.1.1.2 Azadirachtin rich neem oil + turmeric oil

Methanolic stock solution (10 ml) of azadirachtin rich neem oil containing 112.25 ppm each of azadirachtin and turmeric oil was taken in three different pyrex tubes and irradiated under sunlight for 7 days. In another set of experiment, three tubes of the methanolic solution (10 ml each) of azadirchtin enriched neem oil (112.25 ppm) was also irradiated for the same number of days. Samples (1 ml) were collected everyday and analysed by HPLC for their azadirechtin content.

3.8.1.2 Sunlight, thin film on glass surface

3.8.1.2.1 Azadirachtin rich neem oil + curcumin-I

A thin film of the methanolic solution of azadirachtin rich neem oil (1 ml) containing azadirechtin (225 ppm) and curcumin-I (225 ppm) in 1:1 ratio was coated uniformly in sufficient

number of petriplates (5 cm dia). After evaporating off the solvent, the plates were subjected to sunlight irradiation for seven days. Samples were collected after each day and each plate rinsed with 2 ml of methanol (2 x 1 ml) and after filtration were analysed on HPLC.

3.8.1.2.2 Azadirachtin rich neem oil with turmeric oil

A thin film of azadirachtin rich neem oil (1 ml) containing azadirachtin (225 ppm) and turmeric oil (225 ppm) in 1:1 ratio was spread uniformly in each of the petriplates (5 cm dia.). After evaporation of the solvent at room temperature, plates were irradiated under sunlight for 7 days and samples collected as above and analysed by HPLC.

3.8.2 Sunlight, on leaf surface

3.8.2.1 With curcumin-I

Methanolic stock solution (1 ml) of azadirachtin rich neem oil containing azadirachtin and curcumin-I (225 ppm each) in 1:1 ratio was applied on sufficient number of tobacco leaves on an area equal to that of a circle having diameter 5 cm, by a pipette slowly and cautiously.

Similarly, equal number of leaves were treated with 1 ml methanolic solution of azadirachtin rich neem oil containing azadirachtin (225 ppm). After solvent evaporation, the treated leaves were irradiated under sunlight upto 7 days. Three leaves were clipped off from the plant everyday and each leaf was rinsed with 2 ml of methanol (2x1 ml) and samples filtered before HPLC analysis.

3.8.2.2 With turmeric oil

Methanolic solution (1 ml) of azadirachtin rich neem oil containing azadirachtin and turmeric oil (225 ppm each) in 1:1 ratio was applied cautiously on sufficient number of tobacco leaves on an area equal to that of a circle having 5 cm dia by a pipette. Equal number of leaves were similarly treated with 1 ml solution of azadirachtin rich neem oil containing 225 ppm of azadirachtin. After evaporation of the solvent, the treated leaves in both set of experiments were irradiated under sunlight upto seven days. Three leaves were clipped off from the plant every day and extracted, filtered and analysed as above by HPLC.

Experiment conducted under non-illuminated condition (dark) in all the cases were taken as control.

Recovery experiments were conducted on zero day (1h) samples and in all the cases recovery was found to be in the range of 90-95% on glass surface and 85-90% on leaf surface. In samples collected as control after 0 day (1h), the concentration of azadirachtin/curcumin-I after recovery was taken as 100% for all calculations.

3.9 Bioassay

3.9.1 Test Insect

Bihar-hairy catterpiller (*Spilosoma obliqua*)

Third and fifth instar larvae of *Spilosoma obliqua* (Walker) (Arctiidae:Lepidoptera) were used in determining the insect growth inhibition and antifeedant activity of (45%)

azadirachtin and curcumin-I either alone or in various combinations.

3.9.2 Rearing of Bihar-hairy catterpiller

The larvae were collected from the fields and reared in the laboratory on castor leaves (*Ricinus communis* Linn.) in glass jars (20 cm x 15 cm) at 27±1°C and 60% relative humidity. Everyday fresh and clean leaves in sufficient amount were provided in each jar after removing the remnant leaves and excreta of the insects. Roughly about one hundred larvae were kept in each jar in order to avoid overcrowding and unsanitary conditions. In this way, a disease free culture was maintained. The larvae upto fourth instar were reared in these glass jars. The fully grown, about to pupate, larvae were then transferred to clean jars having thick layer of sterilized soil for pupation. The moths emerging after a week were collected and transferred to clean jars containing a suspended cotton swab soaked with honey solution and pieces of folded papers at the bottom for oviposition. The freshly hatched larvae of the same batch were removed and maintained separately on fresh tender castor leaves, in order to have larvae of third and fifth instars. These third and fifth instar larvae were exposed separately to the test compounds for studying their insect growth inhibition and antifeedant activity.

3.9.3 Stock solutions

333 mg of azadirachtin (45%) was weighed accurately in a 25 ml volumetric flask and dissolved into a little amount of distilled acetone with shaking. The volume was made upto 25 ml to obtain a stock solution containing 0.6% azadirachtin. Similarly,

315.8 mg of curcumin-I (95%) was weighed and dissolved into a 25 ml volumetric flask with distilled acetone to produce a 0.6% curcumin-I solution. From this 0.6% stock of curcumin-I, 0.3% and 0.2% sub-stocks were prepared.

Equal volume of each of azadirachtin (0.6%) and curcumin-I (0.6%) solutions were mixed to obtain azadirachtin solutions containing azadirachtin 0.3%) and cucrumin-I (0.3%) in 1:1 proportion. Equal volume of 0.6% azadirachtin and 0.3% curcumin-I solutions were mixed to obtain a azadirachtin : cucumin-I solution in 2:1 proportion. Similarly azadirachtin : curcumin-I (3:1) mixture was prepared by mixing equal volume of azadirachtin 0.6%) and curcumin-I (0.2%) solutions.

3.9.3.1 Test solutions

These stock solutions (0.3%) were serially diluted to optain 0.2%, 0.1%, 0.08%, 0.06% and 0.04% azadirachtin test solutions containing curcumin-I in 1:1, 2:1 and 3:1 ratio. Similarly test solutions of azadirachtin and curcumin-I (0.3%, 0.2%, 0.1%, 0.08% and 0.06%) were separately prepared by serial dilutions from respective stock solutions (0.6%).

3.9.4 Insect growth inhibition

Leaf disks of approximately 6 cm dia were cut from well grown castor leaves. After washing with water, the leaf disks were dried under shade and then treated with test solutions. Each leaf disk was treated with 1 ml of a particular concentration of a compound and dried under shade. The treated disks were then transferred to clean jars (15 cm x 10 cm) providing one leaf disk in each jar. Ten larvae of third and fifth instar were released

separately in each jar and each treatment (20 insects in two jars) was replicated thrice. Thus, for a particular treatment, six jars were required. Insects were exposed to test chemicals for 24 h after which non treated leaves were fed. Everyday, the left-over leaf disks and excreta of the insects were removed and fresh and clean untreated leaves were provided in each jar. Regular counting for the larval mortality, larval-pupal intermediates, pupal mortality, pupal-adult intermediates, abnormal adults, normal adults and normal dead adults were taken till the emergence of adults. Acetone treated leaf disks exposed to larvae, served as control. In this way, insect growth inhibition of various concentrations of azadirachtin and curcumin-I either alone or in different combinations were studied.

3.9.5 Antifeedant activity

In a similar experiment, jars containing treated leaf disks with azadirachtin, curcumin-I and azadirachtin-curcumin-I mixture in various proportions and each proportion with various concentrations (0.6 to 0.04%) were fed to third and fifth instar larvae of *S. obliqua* separately for a period of 24h. As a result of the forced feeding, the consumption of leaf was measured by taking observations on graph papers. Consumption data of acetone treated leaf disks was taken as control. Antifeedency (%) was calculated following the formula:

$$\% \text{ Antifeedency} = \frac{C - T}{C + T} \times 100$$

Where, C = Consumption of leaf in control; T = Consumption of leaf in treatement.

RESULTS AND DISCUSSIONS

Azadirachtin based neem products are quickly degradable and therefore, must be stabilized by stabilizers such as anti-oxidants, UV and sunlight screens etc. Since synthetic anti-oxidants are not likely to be environmentally safe, the use of natural anti-oxidants such as those derived from *Cucurma longa* (turmeric) may provide a solution to develop stable formulations with increased residual life of azadirachtin. The present investigation is aimed at exploring the potential of turmeric constituents in enhancing stability and efficacy of neem products.

4.1 Calibration of HPLC instrument

The developed chromatographic system is efficient enough to resolve the peak of azadirachtin (R_t 3.81) from other minor peaks. The linearity of PDA detector for azadirachtin was varified by injecting 20 µL solution of each of the eight standards (250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.95 ppm) in triplicates and integrating the peak area electronically. The regression analysis of the data indicated linearity [$F(1,6) = 5662.37$ and $R^2 = 0.999$, significant at 1% level] (Fig 1 and 2) with a lower limit of detection of 1 ppm of azadirachtin.

4.2 Isolation and Quantification of Azadirachtin

Azadirachtin was isolated from azadirachtin rich fraction separated from neem seed kernels following Schroeder and Nakanishi method (1987). It involved first defatting of neem seed kernels with hexane followed by extraction with ethanol. Further

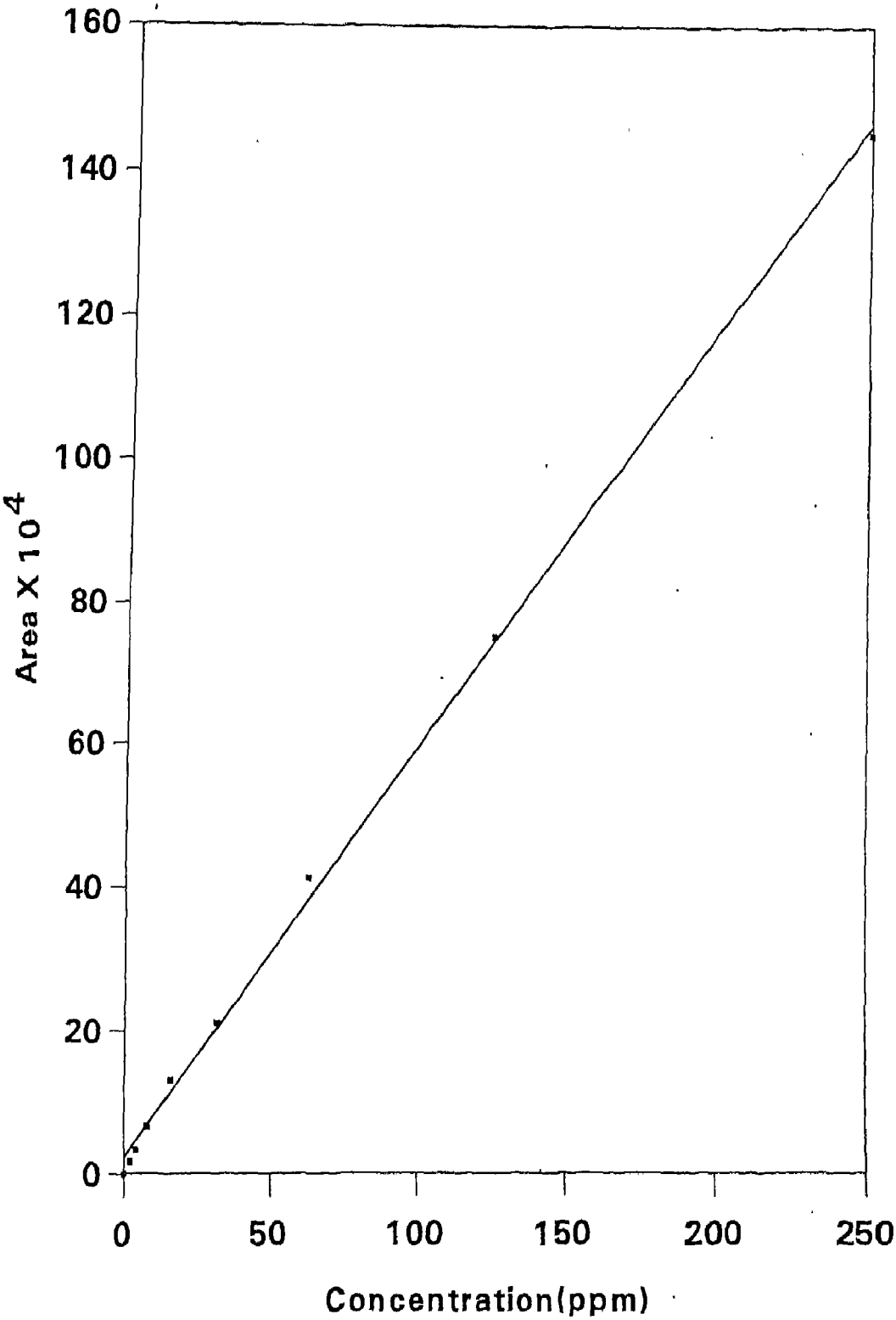


Fig.1. Standard curve of azadirachtin by HPLC

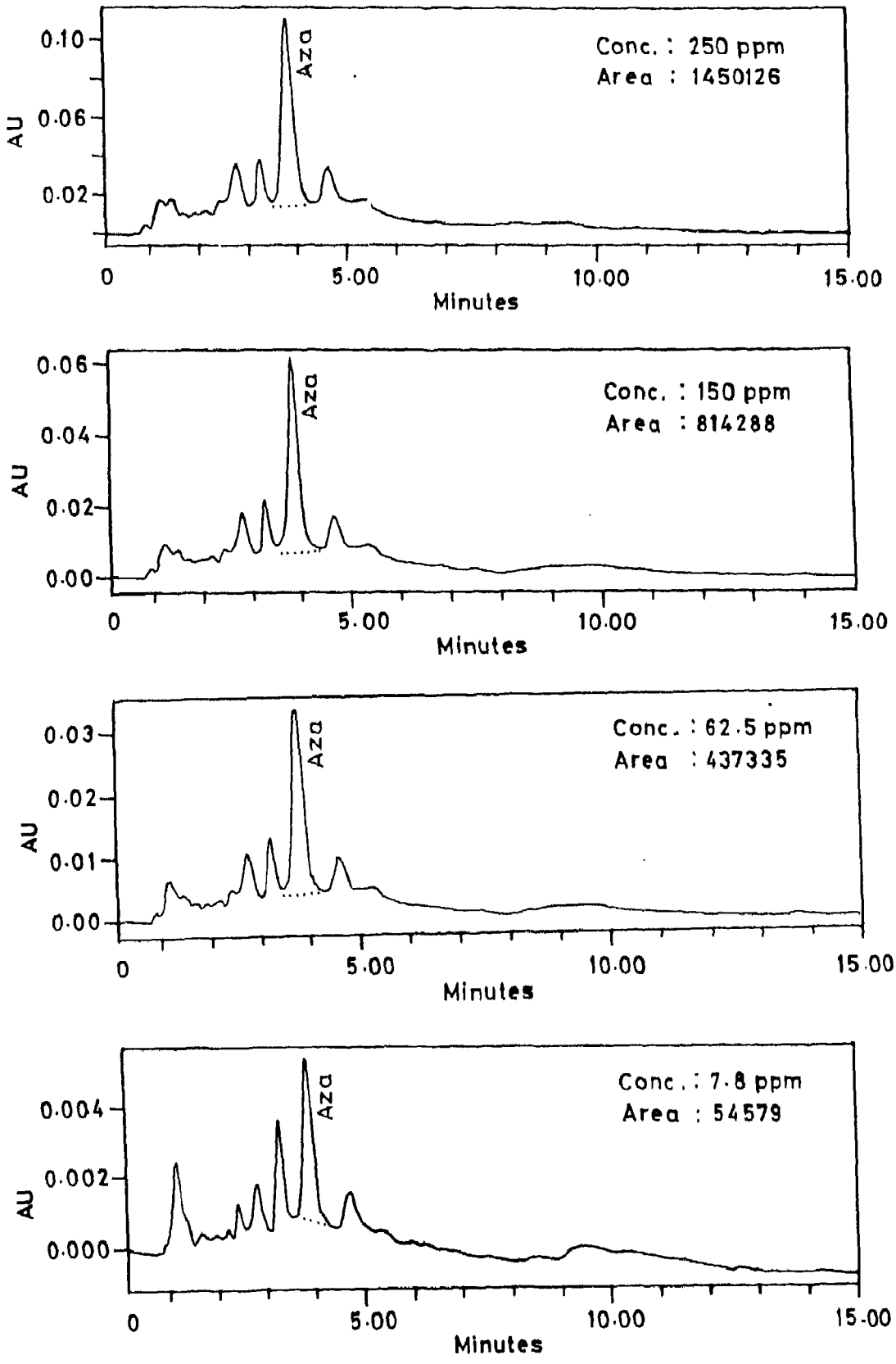


Fig. 2 : LC Chromatogram of (45%) azadirachtin at different concentrations.

partitioning with hexane and aqueous methanol followed by water and ethyl acetate, was necessary to remove remaining oil, non polar materials and, water soluble proteins and sugars. Ethyl acetate extract was then subjected to quick filtration through silica gel column to yield azadirachtin enriched fraction which was crystallised from CCl_4 twice to yield sufficiently pure azadirachtin sample. The yield of the azadirachtin from 2 kg of neem seed kernels was 3.5 gm. It was quantified by HPLC method following BIS specification and employing standard azadirachtin sample (50% pure) procured from Australia.

$$\text{Azadirachtin content} = [A_1/A_2] \times [m_2/m_1] \times P = 45\%$$

Where, A_1 (peak area of azadirachtin in sample) = 558339 μV-sec.

A_2 (peak area of azadirachtin in reference standard) = 618183 μV-sec.

m_1 (mass, in g, of the sample taken for test) = 2×10^{-6}g

m_2 (mass, in g, of the reference standard azadirachtin) = 2×10^{-6}g

P (purity of reference standard azadirachtin) = 50%

Thus, azadirachtin in the sample was calculated to be 45%. This material was used in various photodegradation and photostabilization experiments.

4.3 Isolation and Characterization of Turmeric Products

Turmeric powder was extracted with pet. ether (40-60°) in a Soxhlet apparatus to yield turmeric oil which has been reported to possess insect repellent activity (Jilani *et al.*, 1983). On further extraction with benzene, the residue yielded a dark brown oily mass which crystallised from benzene to furnish an orange coloured product comprising of three components (TLC). These three

components usually gave one peak at the same retention time as they could not be separated on phenyl and/or RP-18 column using methanol -water (60:35) and acetonitrile-water (60:40) as mobile phase. Acccording to literautre reports separation of curcumins is best achieved utillising fluorescence detctor (Tonnesen and Karlsen, 1983) and electrochemical detctor (Smith and Witowsk, 1984) using acetonitrile buffer as mobile phase. In the present study, curcumin mixture was conveniently separated with RP-8 column and acetonitrile as mobile phse in which distinct peaks of curcumin-I,II and III were detected at 5.892, 5.567 and 5.392 minutes respectively (Fig. 3). To obtain curcumins in sufficient quanitity these were separated by either repeated column chromatography or preparative thin layer chromatography over silica gel. IR spectra of all the three curcumins exhibited bands at 3200-3300, 1600-1626 and 1510-1545 cm^{-1} corresponding to 0-H str., C=0 and ring C $\doteqdot$ O stretching vibration respectively.

The first product, R_f 0.4 (benzene - ethanol, 9:1) was obtained as yellow powder. Its ^1H NMR spectrum showed two characteristic peaks at δ 3.897 and 9.25 for two methoxyl and two hydroxyl protons respectively. The latter peak was D_2O exchangeable. From the spectral data it appears that one-OH and one $-OCH_3$ substituent is located in each of the aromatic nucleus. Its structure was further confirmed by mass spectrum (Fig. 4) which showed molecular ion peak (M^+) at m/z 368 alongwith other characteristic ion peak at m/z 350 originated as a result of the loss of 18 mass units due to water molecule. Peaks located at m/z 217, 272 and 177, 150 and 137 originated as a result of a, b, and c

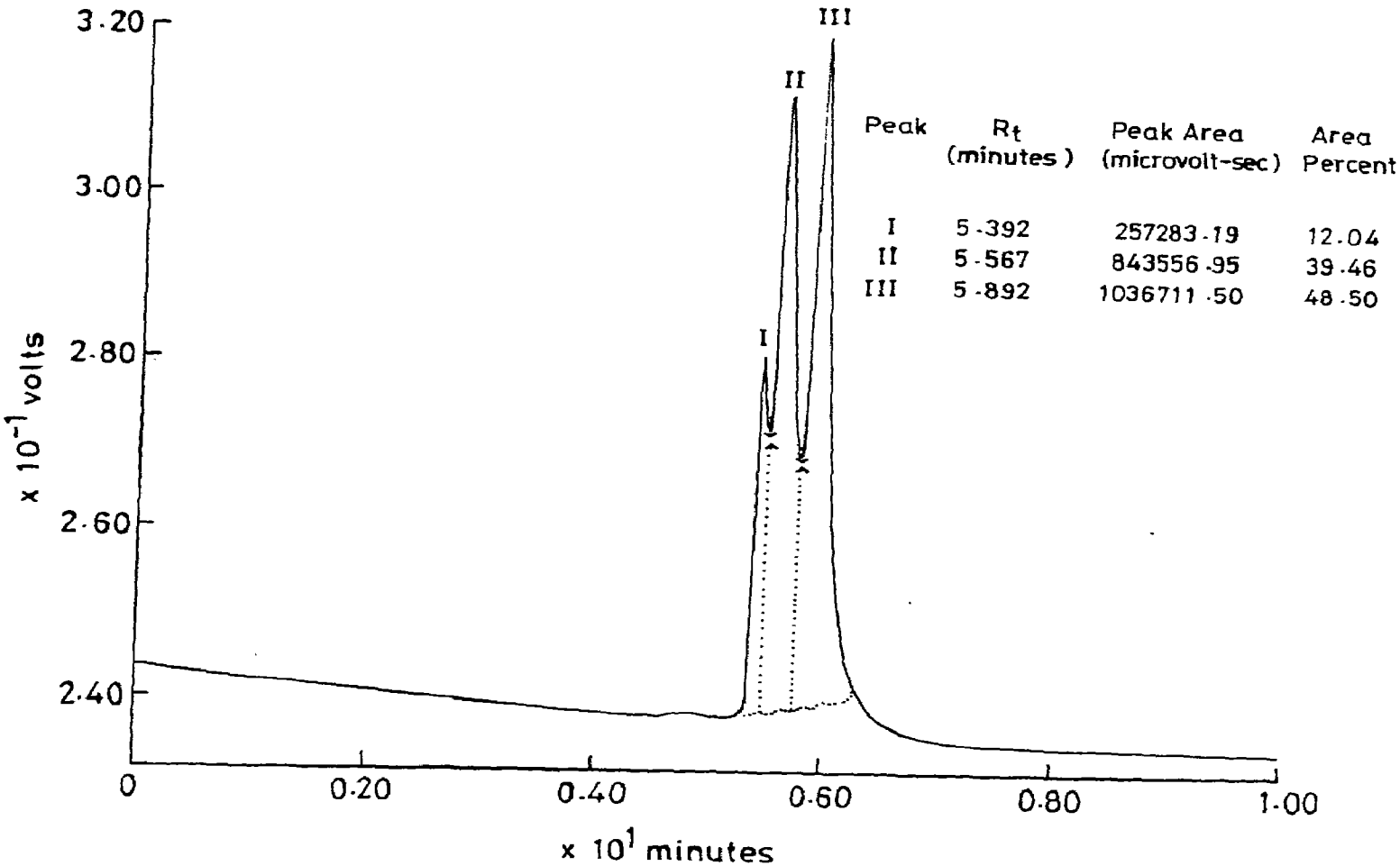


Fig.3:LC Chromtogram of curcumin mixture separating curcumin - I, II & III

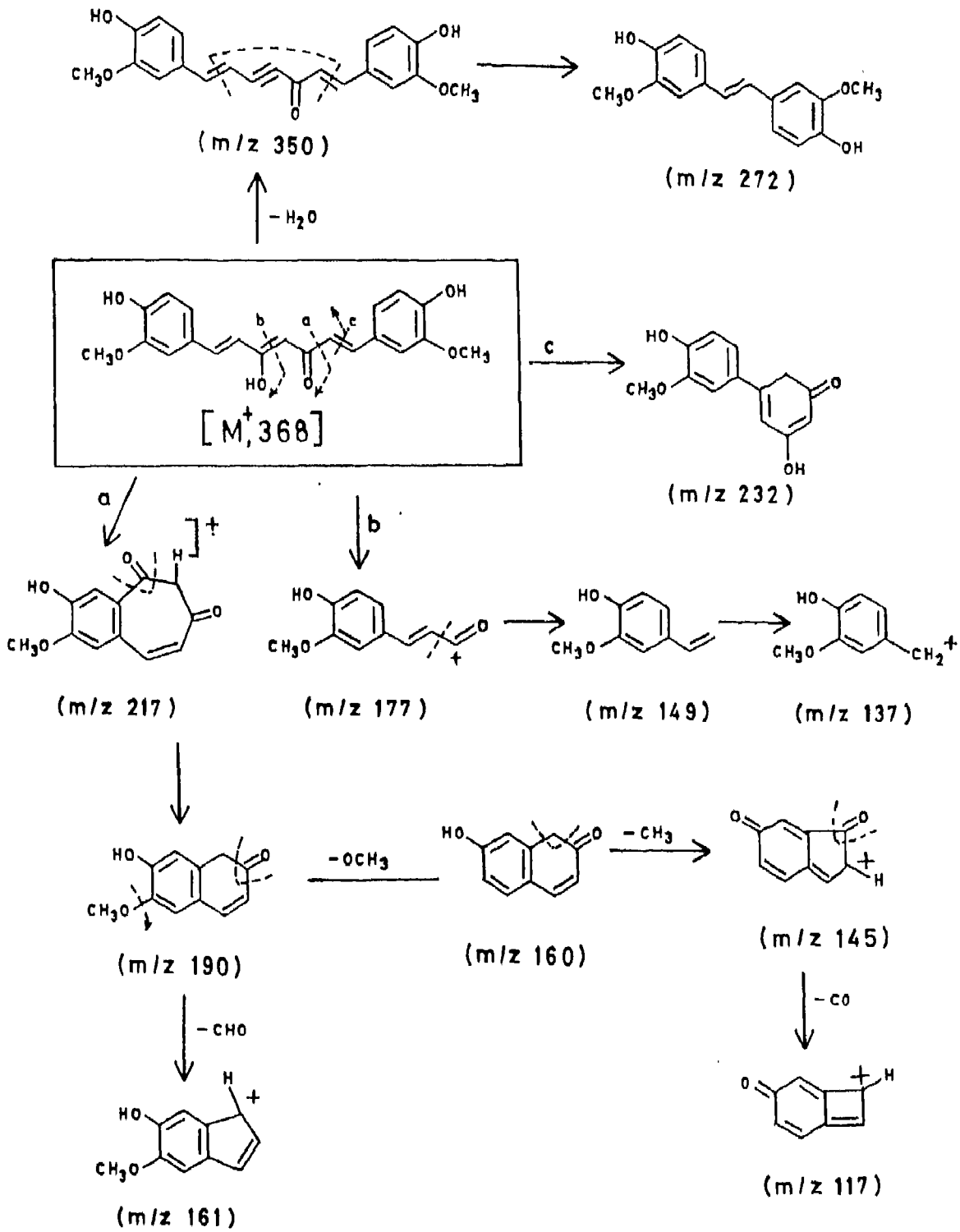


Fig. 4: Mass fragmentation pattern of curcumin - I

cleavages in the central alkene moiety of the compound were typical of a feruloyl moiety. The compound was therefore, identified as curcumin-I (XXXI) and was assigend the structure, 1,7-bis (4-hydroxy-3-methoxyphanyl)-1,6-heptadiene-3,5-dione.

The second product (R_f, 0.30) in benzene-ethanol (9:1) has a slightly lower R_f value than curcumin-I and gave in its ^1H NMR spectrum one peak typical of one methoxyl substituent (δ 3.897) and two peaks for hydroxyl substituents (δ 9.27 and 9.71) which were D_2O exchangeable. Other peaks characteristic of curcumin nucleus were also present. Its mass spectra gave a molecular ion peak (M^+) at m/z 338 alongwith another typical peak at 320 formed as a result of the loss of water molecule (Fig. 5). Two groups of peaks located at m/z 177, 150, 137, and 147, 119 and 107 were characteristic of two differently substituted aromatic moieties, namely feruloyl and p-hydroxy cinnamoyl moiety respectively. On the basis of ^1H NMR spectral data and mass fragmentation pattern the compound was identified as curcumin-II (XXXII) and was assigned the structure 4-hydroxy cinnamoly(feruloyl)methane.

The third orange coloured product with lowest R_f value (R_f 0.25, benzene-ethanol, 9:1) in its ^1H NMR spectrum did not exhibit any peak corresponding to methoxyl substituents, however, D_2O exchangeable peak located at δ 9.96 was typical of two aromatic hydroxyl groups. The structure of curcumin-III was further confirmed by its mass spectrum which showed molecular ion peak at m/z 308 alongwith a characteristic peak at m/z 290 due to the possible loss of H_2O from the parent molecule. Like in

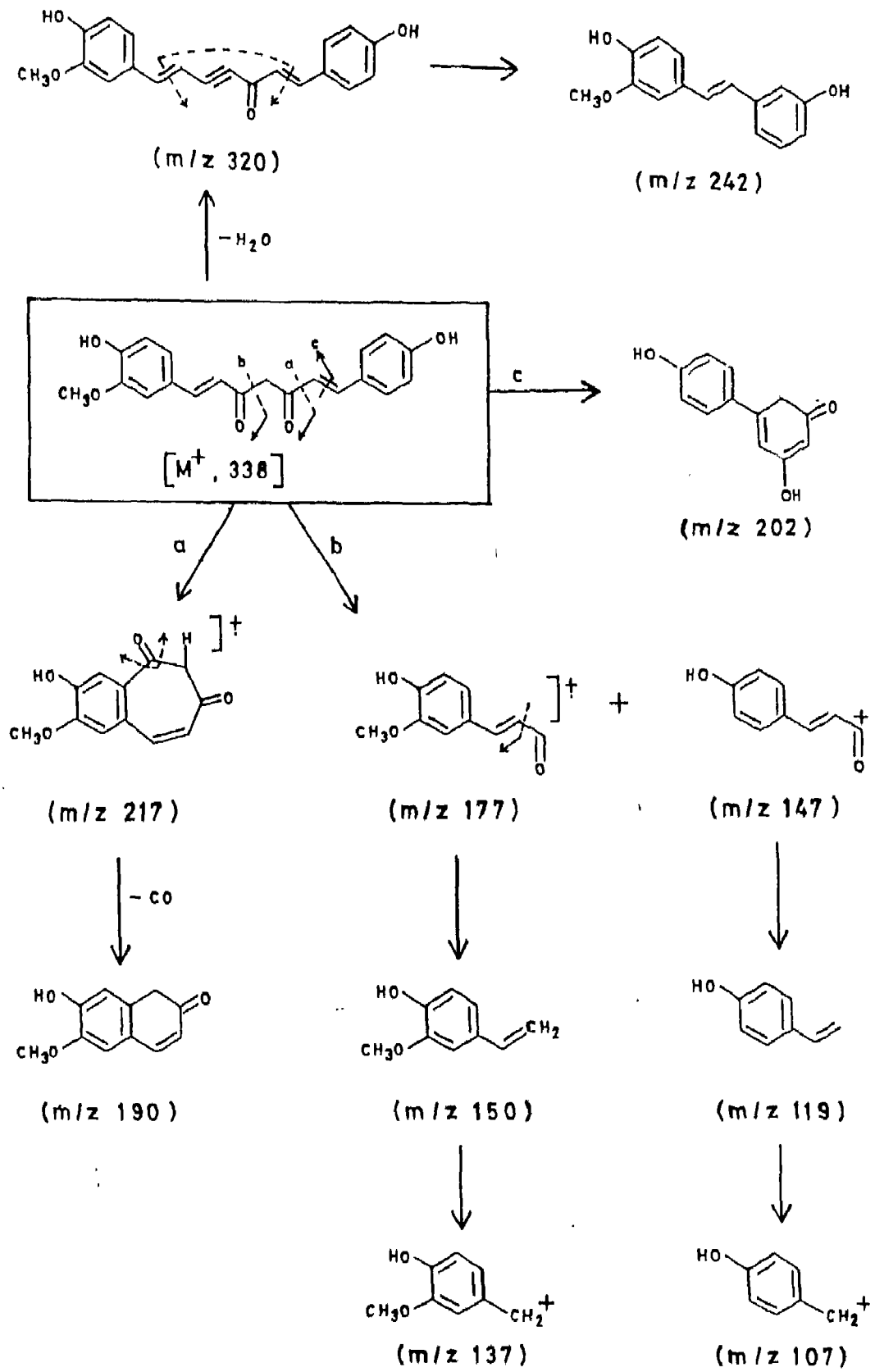


Fig. 5: Mass fragmentation pattern of curcumin - II

the mass spectrum of curcumin-I and II, curcumin-III, also exhibited fragment ion peaks at m/z 219, 202, 187, 160, 147 and 119, 107 characteristic of *p*-hydroxy cinnamoyl moiety (Fig. 6). Based on spectral evidences, the compound was assigned the structure bis (4-hydroxy cinnamoyl)methane (Curcumin-III) (XXXIII).

4.4 Synthesis of Curcumin-I Derivatives

Curcumin-I on treatment with methyl iodide yielded a methylated product which in its ^{1}H NMR spectrum exhibited peaks at δ 4.0 and 4.10 corresponding to four methoxyl substituents each alongwith a new singlet peak at δ 2.3 due to methyl group located in the central active methylene carbon in the aliphatic chain. Other peaks typical of dimethoxy cinnamoyl moiety were also present. On the basis of its ^{1}H NMR spectrum it was assigned the structure, 1,7-bis (3,4-dimethoxyphenyl)-4-methylhepta-1,6-diene-3,5-dione.

Curcumin-I was converted to its dibutyl analogue by refluxing acetone solution of curcumin-I with butyl bromide. Its structure was confirmed by its ^{1}H NMR spectum which exhibited peaks centred at δ 1.3 (t, 2 x-$OCH_2CH_2CH_2C\underline{H}_3$), 1.3 -1.8 (m x 2 x-$OCH_2(C\underline{H}_2)_2$-$CH_3$) and 4.25 (t, 2 x- $OC\underline{H}_2(CH_2)_2$-$CH_3$) corresponding to two butyl moieties. A peak located at δ 3.95 was assignable to two methoxyl substituents. Since both the hydroxyl substituents in the curcumin-I molecule have been butylated, the product was identified as dibutyl curcumin-I (1,7-bis (3-methoxy,4-*n*-butoxyphenyl)-hepta-1,6-diene-3,5-dione). The IR spectra of both methyl and butyl derivatives also did not show any band corresponding to free hydroxyl substituent.

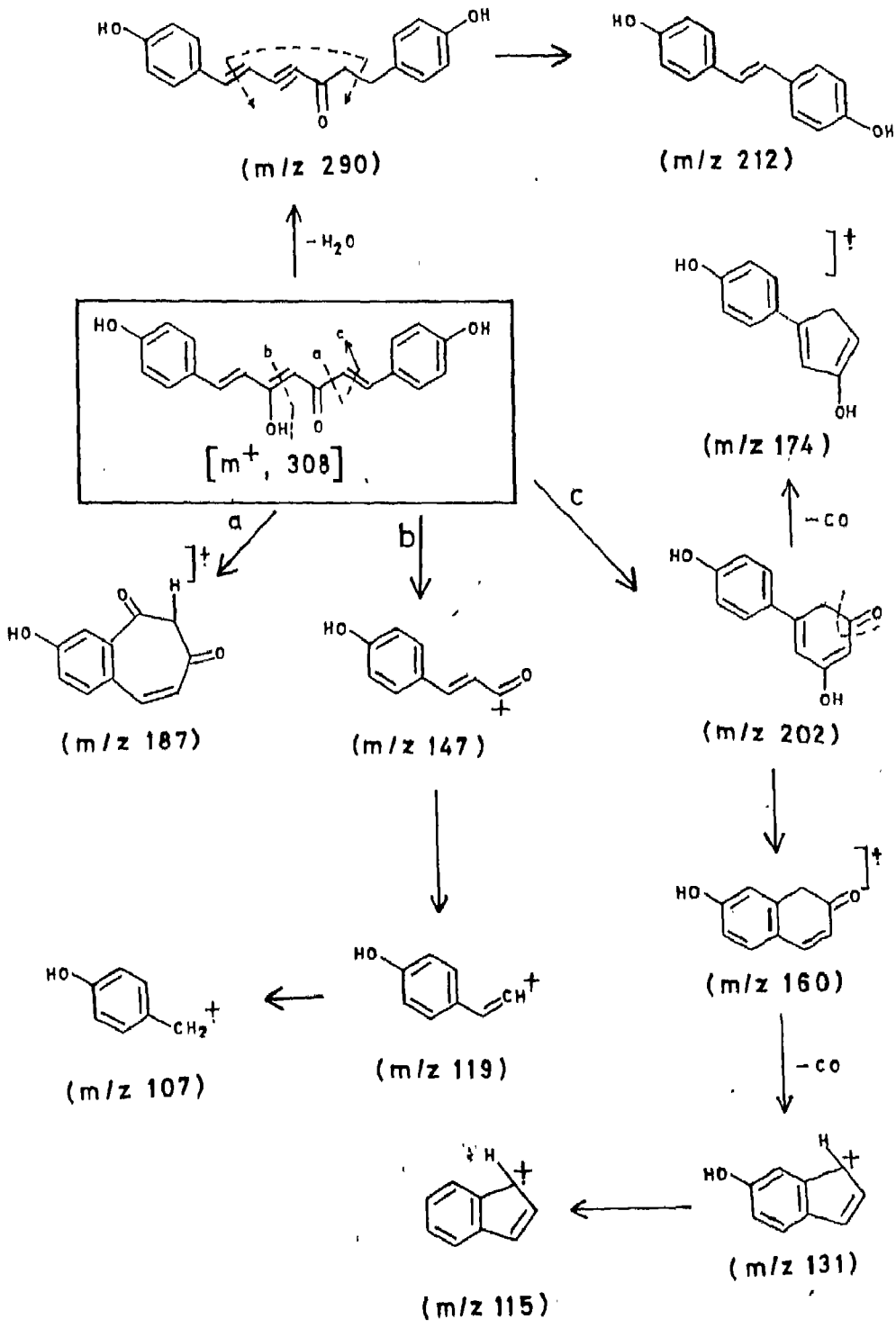


Fig. 6: Mass fragmentation pattern of curcumin - III

4.5 UV-Photolysis of Curcumin-I

Curcumin-I solution in methanol was irradiated with light from high pressure mercury lamp and occassional bubbling of air through the solution for 10h. Since some of the possible photo-products formed as a result of oxidative cleavage are likely to be acidic in nature, they are unlikely to be detected by GC/GCMS. The mixture was therefore, methylated following its treatment with diazomethane. The methylated mixture of the photoproducts was subjected to GC-MS analysis following which four photoproducts were identified (Fig. 7) on the basis of their mass fragmentation pattern. These compounds included i) 3,4-dimethoxy cinnamoyl methane (M^+, 206) , ii) methyl ester of 3,4-dimethoxy cinnamic acid [M^+, 222 (Fig. 8)], iii) methyl ester of 3,4-dimethoxy benzoic acid (M^+, 196) and iv) 3,4-dimethoxy benzaldehyde (M^+, 182) The methyl esters of the posible photoproducts were synthesised as follows to facilitate their identification by spectroscopic and chromatographic techniques.

The methyl analogue of one of the main products, 4(3,4-dimethoxyphenyl) but-3-ene-2-one or (3,4-dimethoxy cinnamoyl methane) was synthesised following Claisen reaction of veratraldehyde with acetone. The light yellow product was identified by its ^1H NMR spectra which showed peaks at δ 2.35 for $-COCH_3$ (s) and at δ 3.95 (s) for two methoxy substituents characteristic of the photoproducts. The structure was further confirmed by its mass spectrum which besides other fragment ion peaks, gave mass ion peak (M^+) at m/z 206.

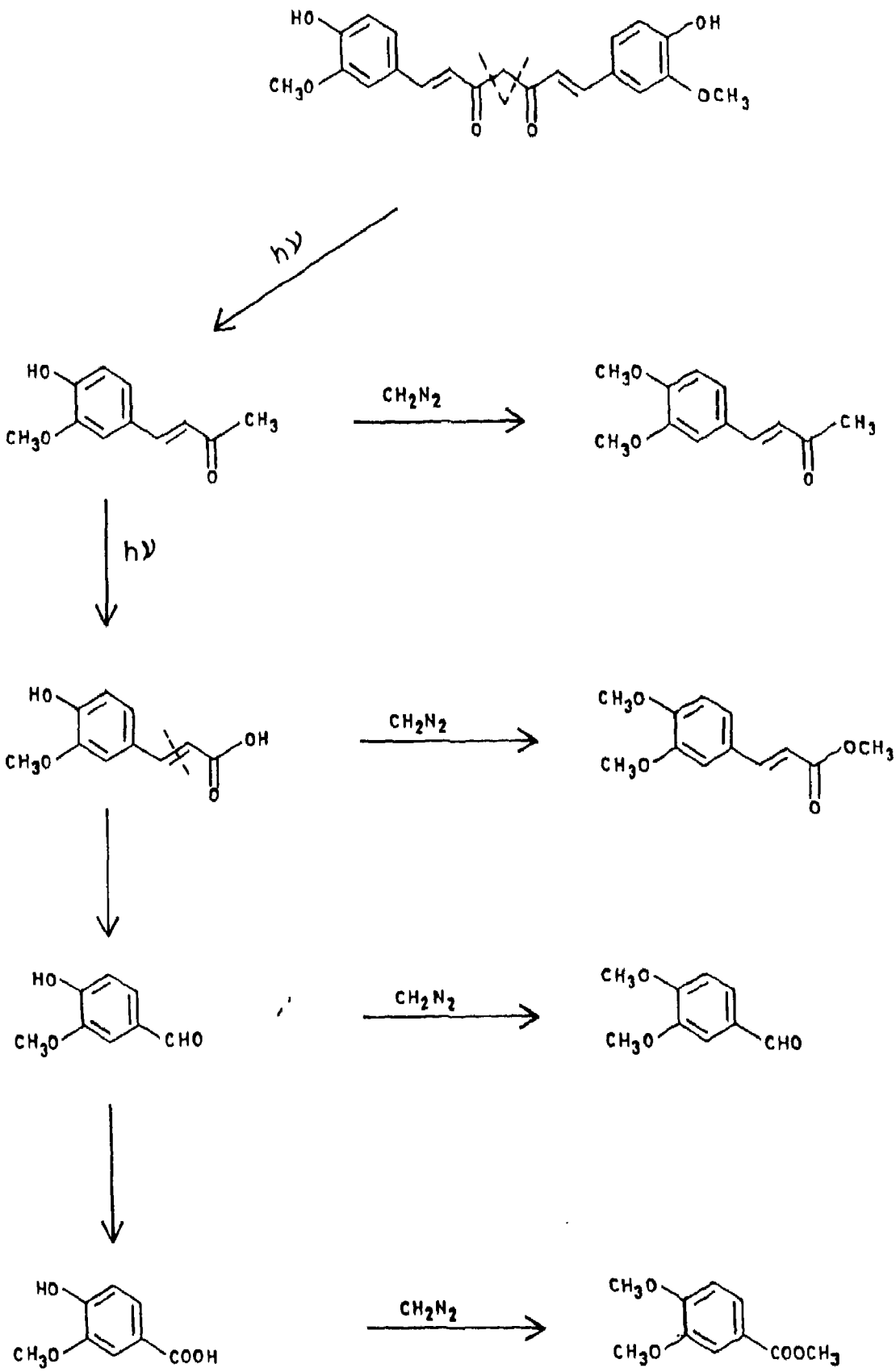


Fig. 7 : Photodegradation products of Curcumin - I.

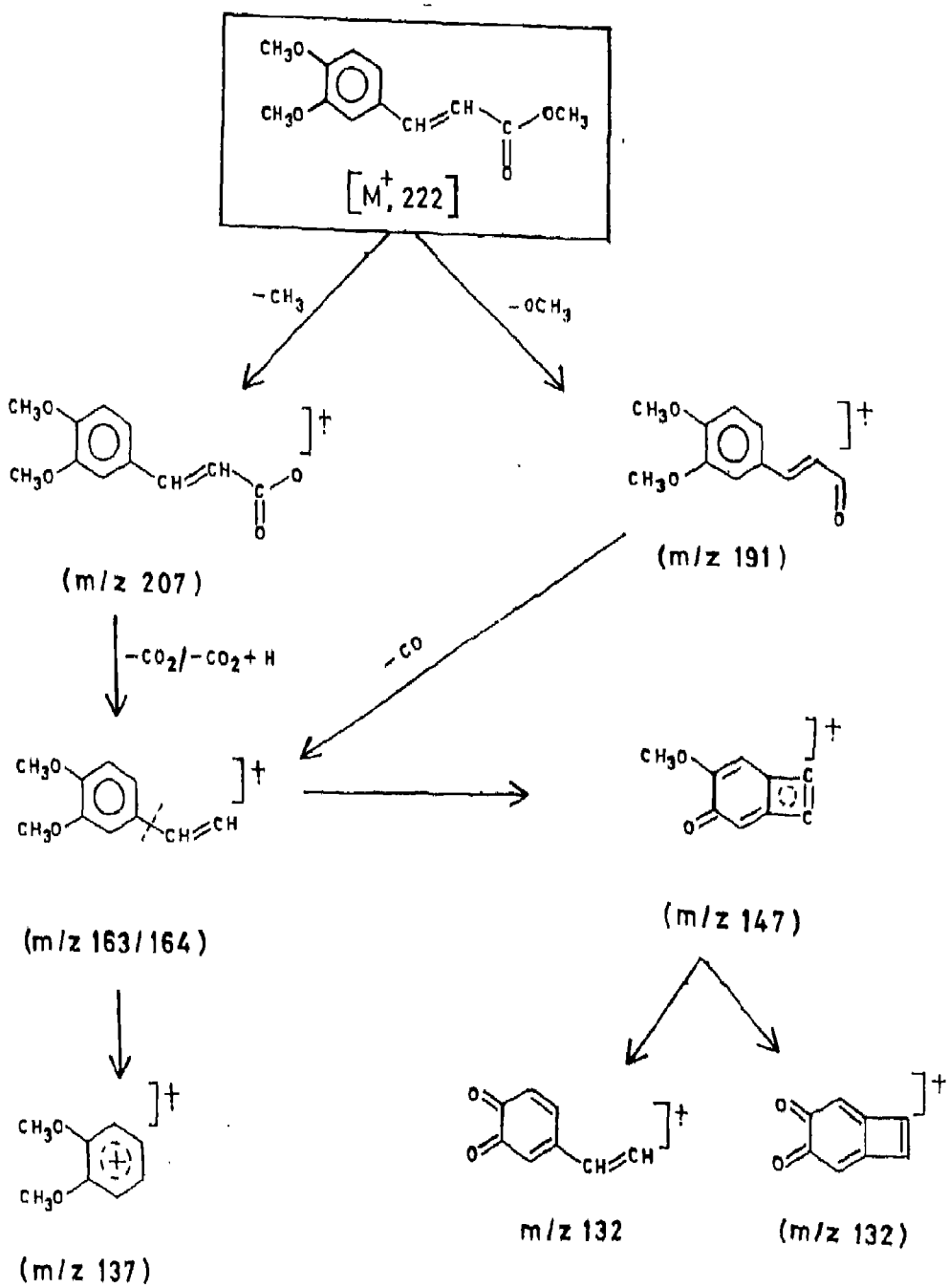


Fig. 8 : Mass fragmentation pattern of 3,4-dimethoxy cinnamic acid methyl ester.

The remaining two photoproducts were synthesised following methylation of 3,4 - dimethoxy cinnamic acid and 3, 4-dimethoxy benzoic acid with diazomethane. The former one (M^+ 222) in its ^1H NMR spectra showed two characteristic singlet peaks at δ 3.8 (-$COOCH_3$) and δ 3.9 (2X-OCH_3) whereas, the latter compound exhibted one characteristic singlet peak at δ 3.9 corresponding to 2 methoxyl and one -$COOCH_3$ substituents.

4.6 Effect of UV and Sunlight on Azadirachtin in Solution and Thin Film oln Glass and Leaf Surface

The use of azadirachtin based insecticies is likely to be limited because of their sensitivity to acids and bases (Szeto *et al.*, 1996) and susceptibility to photodegradation. Previous studies on photodegradation of azadirachtin have been conducted either in sunlight or UV-light (Barnby *et al.*, 1989; 1994 and Yakkundi *et al.*, 1996). UV-absorbing chromophores in azadirachtin like vinyl ether and α,β saturated ester [(*E*)-2-methyl but-2-enoate] groups are sensitive to light and as a result of various photochemical transformations produce a large proportions of polar products which remained undetected by HPLC.

Since azadirachtin is photolabile and degrades quickly, present studies were aimed at investigating the extent of photostabilization/degradation in the presence/absence of turmeric based bio-stabilizers under both UV and sunlight conditions. Photodegradation experiments were conducted in solution and on glass and leaf surface in the months of September and October when relative humidity was around 60-80%. In all the experiments, azadirachtin used was 45% pure and its purity was determined by

standard BIS procedure. HPLC analysis was used to monitor the degradative effects of ultraviolet and sunlight radiations on azadirachtin/curcumins at 217 nm.

4.6.1 UV-light, solution and as thin film on glass surface

Results indicated (Table 5, Fig. 9 & 10) that the methanolic solution of azadirachtin was highly sensitive to UV-light as 100% of it was degradable within 2.5 h. Within one hour, 72% of azadirachtin had decomposed. Its half life ($t_{1/2}$) was calculated to be 0.53 h. No degradation was however observed when samples were kept in dark.

When a thin film of azadirachtin coated on the glass surface was subjected to photodegradation, only 5% of the initial azadirachtin could be detected after 14h of UV-light exposure. Its half life was found to be 3.30 h. The studies indicated that under UV-light degradation of azadirachtin in methanolic solution was faster than as a thin film on glass surface. The significantly lower amount of recovered azadirachtin was due to the fact that azadirachtin was probably converted into a mixture of more polar materials from which no azadirachtin could be detected.

4.5.2 Sunlight, solution and as thin film on glass and leaf surface

Under sunlight irradiated conditions, methanolic solution of azadirachtin degraded comparatively slowly than in UV-light as 5.32% of azadirachtin still remained in solution after seven days. Similarly, as thin film on glass surface only 2.0% of azadirachtin was detected after 8 days (Table 6, Figs. 11 and 12). Its half life ($t_{1/2}$) in methanolic solution and as thin film on glass surface was

Table 5. Progressive degradation of azadirachtin under UV-light

Methanolic solution		Thin film on glass surface	
Time (hour)	% Azadirachtin remaining	Time (hour)	% Azadirachtin remaining
0.0	100.00	0	100.00
0.5	76.65	2	50.29
1.0	27.66	4	20.47
1.5	20.01	6	19.09
2.0	7.48	8	17.75
2.5	0	10	13.37
		12	5.03
		14	0

Table 6. Progressive degradation of azadirachtin under sun light

Methanolic solution		Thin film on glass surface	
Time (day)	% Azadirachtin remaining	Time (day)	% Azadirachtin remaining
0	100.00	0	100.00
1	43.02	2	22.05
2	35.00	4	12.72
3	27.33	6	4.62
4	18.07	8	2.91
5	11.81	10	0
6	9.49		
7	5.32		

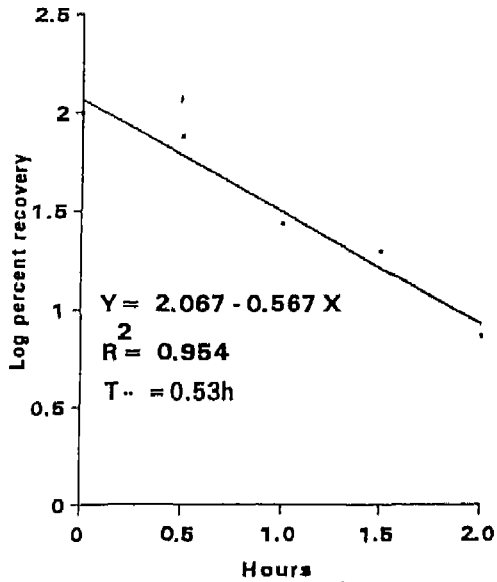


Fig.9. Linear plot for pseudo first order degradation of azadirachtin under UV-lihgt as methanolic solution

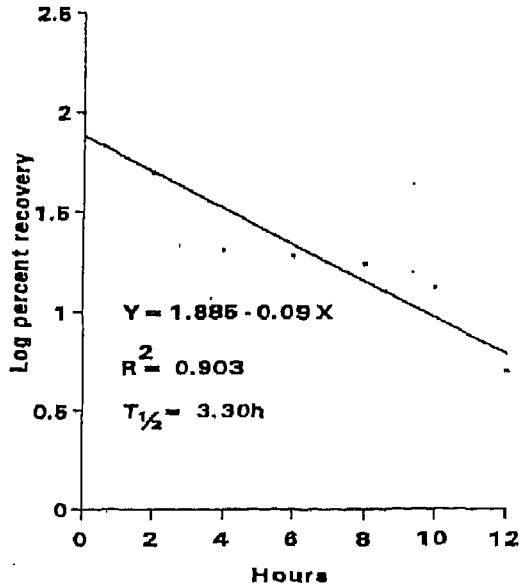


Fig.10. Linear plot for first order degradation of azadirachtin under UV-light as thin film on glass surface

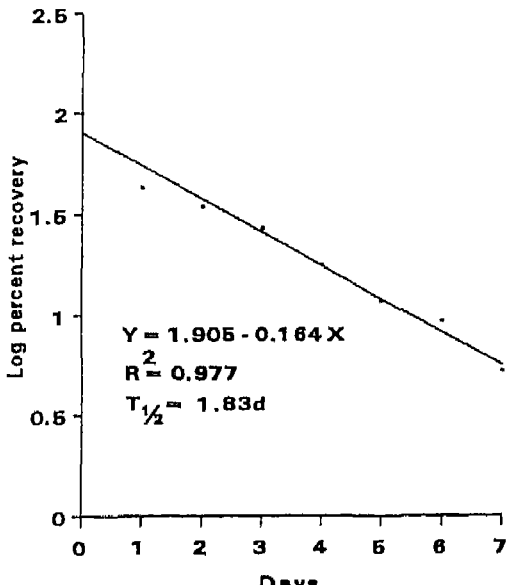


Fig.11. Linear plot for pseudo-first order degradation of azadirachtin under sunlight as methanolic solution

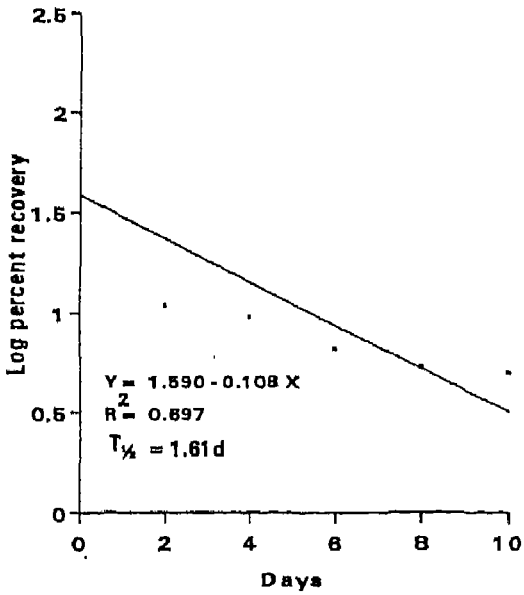


Fig.12. Linear plot for first order degradation of azadirachtin under sun light as thin film on glass surface

found to be 1.83 d and 1.61d respectively. When a thin film of azadirachtin spread uniformly on the leaf surface was subjected to sunlight degradation it was found to degrade quickly and within 3 days 95.62% of azadirachtin had dissipated (Table 7, Fig. 13). Even after one day, only 18% of azadirachtin could be detected. Its half life ($t_{1/2}$) was found to be 0.73 d. The faster degradation on leaf surface was attributed to i) possible moisture content on leaf surface ii) absorption of azadirachtin by leaves and iv) loss of azadirachtin content due to wind current.

In methanolic solution, degradation of azadirachtin in UV/ sunlight followed pseudo-first order kinetics [R^2=0.965, F(1,3),=83.348 and R^2=0.977, F(1,6)=255.826 respectively and both are significant at 5% level] whereas, as thin film on glass and leaf surface it followed first order kinetics [R^2=0.903, F(1,5),=46.551 for thin film on glass surface in UV light and R^2=0.697; F(1,4),=9.219 and R^2=0.922, F(1,2),=23.772 for thin film on glass surface and leaf surface under sunlight respectively, all are significant at 5% level]. In all the cases, initial degradation was faster which afterward became gradual. HPLC analysis of the non-irradiated compounds showed no change throughout the experiment as evident from the peak area/height of azadirachtin.

4.7 Photodegradation of Curcuminoids

Curcuma longa comprises of three main components namely curcumin-I, curcumin-II and curcumin-III. these curcumins have been reported to possess antioxidative activity and were therefore, considered for stabilizing azadirachtin in neem oil.

Table 7. Progressive degradation of azadirachtin under sunlight as thin film on leaf surface

Time (day)	% Azadirachtin remaining
0	100.00
1	18.12
2	9.52
3	5.38
4	0

Table 8. Progressive degradation of curcumin-I, II and III under UV-light

Time	% Curcumin remaining		
(hour)	Curcumin-I	Curcumin-II	Curcumin-III
Methanolic solution			
0	100.00	100.00	100.00
1	83.44	59.00	54.60
2	38.32	32.76	25.29
3	29.83	10.90	9.01
4	26.57	4.10	0
5	8.39	0	
Thin film on glass surface			
0	100.00	100.00	100.00
3	61.84	67.36	72.47
6	57.20	50.90	58.22
9	51.38	43.47	51.88
12	46.13	16.85	47.21
15	32.61	10.25	33.39

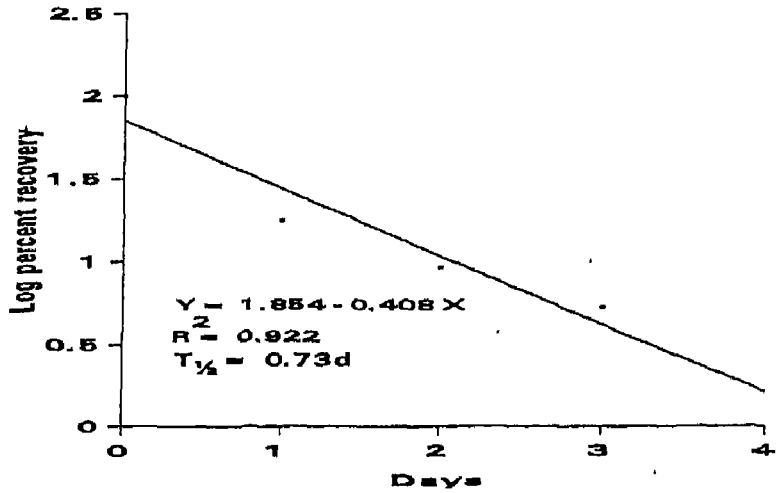


Fig.13. Linear plot for first order degradation of azadirachtin under sun light as thin film on leaf surface

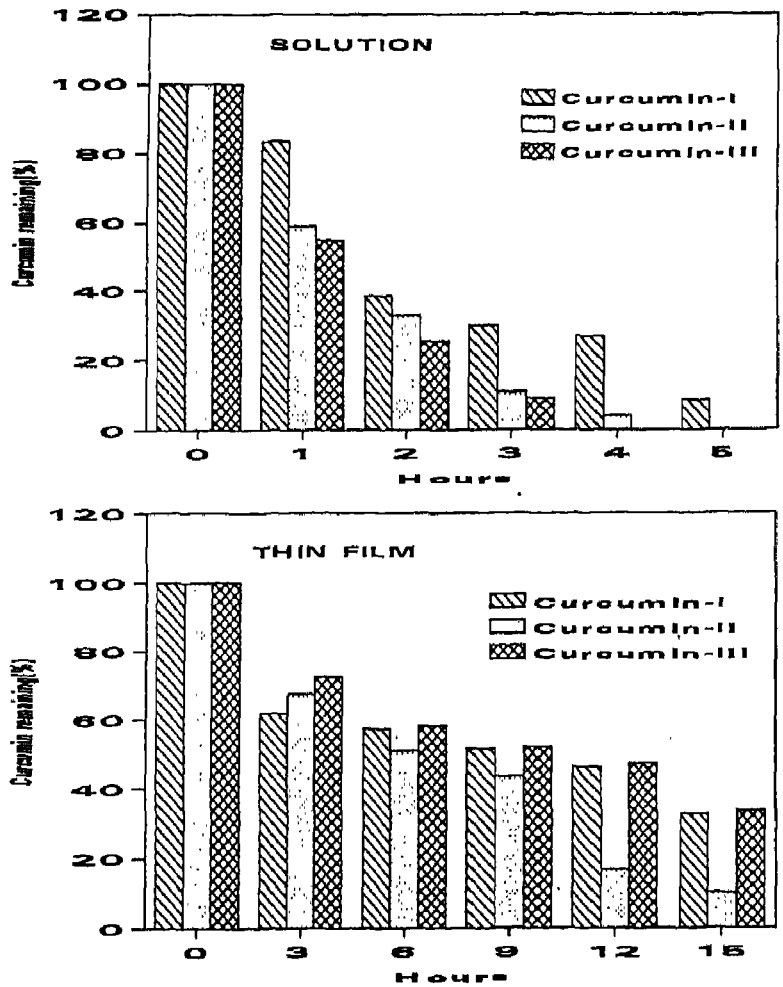


Fig.14. Progressive degradation of curcumin-I,II and III under UV-light

Before we undertake photostabilization of azadirachtin, it was necessary to study the effect of light on the degradation of curcumins.

4.7.1 Comparative photodegradation of curcumins (I, II and III) in solution and as thin film on glass surface

The three curcuminoids namely curcumin-I, curcumin-II and curcumin-III in methanolic solution and as thin film on glass surface were subjected to UV-radiation to assess their degradation behaviour. In solution phase curcumin-I was comparatively more stable to UV-light followed by curcumin-II and curcumin-III. After one hour of exposure, 83.44, 59.0 and 54.6% of curcumin-I, curcumin-II and curcumin-III, and after four hours 2.65, 4.1 and 0% of curcumin-I, curcumin-II and curcumin-III respectively were detected (Table 8, Fig. 14).

As thin film on glass surface the degradation pattern of curcumin-I and III was almost similar however, curcumin-II degraded comparatively rapidly. The study thus revealed that of the three curcumins, curcumin-I was most stable and its stability was attributed to the presence of methoxy substituent nearer to each -OH group in the curcumin nucleus. The presence of methoxy substituents have been earlier implicated in providing antioxidative activity to crucumins (Toda *et al.*, 1983) . Since curcumin-I was more stable, it was selected for further degradation and stabilization studies.

4.7.2 Effect of light on degradation of curcumins and its analogues in their combination with azadirachtin

Experiments were conducted to investigate the possibility of stabilizing azadirachtin with different curcumins alongwith

methyl and butyl analogues of curcumin-I. To begin with, studies were conducted to see the effect of light on the degradation of curcumins and derivatives of curcumin-I in combination with 45% azadirachtin in 1:1 ratio.

4.7.2.1 UV-degradation of curcumin I in curcumin I : azadirachtin (1:1) mixture

When methanolic solution of curcumin I : azadirachtin (1:1) mixture was irradiated with UV-light, 11.75 % of curcumin-I was found to remain in solution after 3h which after 6 h degraded completely. On the other hand, residues of curcumin I on glass surface persisted for longer duration and after 6 and 16h, they were detected to the extent of 58.78 and 6.87 % respectively (Table 9). The study indicated that photodegradation of curcumin-I: in curcumin I - azadirachtin (1:1) mixture was more in solution than as thin film on glass surface. When degradation pattern of curcumin-I in curcumin-I : azadirachtin mixture was compared with curcumin-I alone it was concluded that curcumin-I in mixture degraded rapidly.

4.7.2.2 UV-degradation of curcumin-II in curcumin-II : azadirachtin (1:1) mixture

Analysis of curcumin II content in curcumin II : azadirachtin (1:1) mixture following exposure to UV-light revealed that in methanolic solution curcumin-II degraded quickly as only 1.23 % of it could be detected in solution after 3 hours. On the other hand, as a thin film on glass surface, it degraded slowly as 21.55 % of it still remained after 16 hours (Table 10). Here also, degradation of curcumin II in curcumin II : azadirachtin (1:1) mixture was rapid as comapred to curcumin II alone.

Table 9. Progressive degradation of curcumin-I in curcumin-I : azadirachtin (1:1) mixture under UV-light

Methanolic solution		Thin film on glass surface	
Time (hour)	% Curcumin-I remaining	Time (hour)	% Curcumin-I remaining
0	100.00	0	100.00
1	82.21	2	85.39
2	30.40	4	65.56
3	11.75	6	58.78
4	6.84	8	52.34
5	1.13	10	37.59
6	0	12	31.83
		14	19.96
		16	6.87

Table 10. Progressive degradation of curcumin-II in curcumin-II : azadirachtin (1:1) mixture under UV-light

Methanolic solution		Thin film on glass surface	
Time (hour)	% Curcumin-II remaining	Time (hour)	% Curcumin-II remaining
0	100.00	0	100.00
1	24.39	2	75.84
2	14.39	4	67.60
3	1.23	6	60.68
4	0	8	53.24
		10	45.21
		12	32.47
		14	25.66
		16	21.75

4.7.2.3 UV-degradation of curcumin III in curcumin III : azadirachtin (1:1) mixture

Monitoring of curcumin III in various samples of curcumin-III : azadirachtin (1:1) mixture showed that degradation pattern of curcumin-III was almost similar to that of curcumin-I & II. In this case, curcumin-III residues in methanolic solution bacame non-detectable after 3 hours whereas, as thin film on glass surface curcumin-III was quite stable as after 16 hours of irradiation 46.72% of curcumin-III still remained on the glass surface (Table 11). As in curcumin-I and II, the degradation of curcumin-III in mixture was more than in alone.

4.7.2.4 UV-degradation of methyl and butyl derivatives of curcumin I in combination with azadirachtin

The methyl and butyl ether analogues of curcumin I were tested for their possible photolytic stability in combination with azadirachtin. The study revealed that these products were more susceptible to UV-light than their parent curcumin-I molecule (Table 12 and 13).

On the basis of these observations, only curcumins were selected for investigation for their potential for stabilizing azadirachtin or azadirachtin rich neem oil.

4.7.3 Photodegradation of curcumin-I

4.7.3.1 UV-light degradation in solution and as thin film on glass surface

Since curcumin-I absorbs in both UV and visible region, it quite susceptible to photodegradation. Under the impact of UV-light, methanolic solution of curcumin-I degraded faster than its thin film on glass surface. After six hours of exposure, 57% of

Table 11. Progressive degradation of curcumin-III in curcumin-III : azadirachtin (1:1) mixture under UV-light

Methanolic solution		Thin film on glass surface	
Time (hour)	% Curcumin-III remaining	Time (hour)	% Curcumin-III remaining
0	100.00	0	100.00
1	15.52	2	93.52
2	7.21	4	87.79
3	0	6	83.42
		8	69.81
		10	60.00
		12	58.04
		14	51.23
		16	46.72

Table 12. Progressive degradation of trimethyl curcumin-I in trimethyl curcumin-I : azadirachtin (1:1) mixture under UV-light

Methanolic solution		Thin film on glass surface	
Time (hour)	Trimethyl curcumin-I remaining (%)	Time (hour)	Trimethyl curcumin-I remaining(%)
0	100.00	0	100.00
1	52.96	2	73.45
2	3.30	4	60.69
3	0	6	59.91
		8	34.03
		10	26.10
		12	15.05
		14	9.33
		16	6.39

Table 13. Progressive degradation of dibutyl curcumin-I in dibutyl curcumin-I : azadirachtin (1:1) mixture under UV-light

Methanolic solution		Thin film on glass surface	
Time (hour)	% Dibutyl curcumin-I remaining	Time (hour)	% Dibutyl curcumin-I remaining
0	100.00	0	100.00
1	50.23	2	81.02
2	3.77	4	63.48
3	0	6	57.02
		8	32.69
		10	23.62
		12	16.22
		14	8.11
		16	4.53

Table 14. Progressive degradation of curcumin-I under sunlight

Methanolic solution		Thin film on glass surface	
Time (hour)	% Curcumin remaining	Time (day)	% Curcumin remaining
0	100.00	0	100.00
2	70.10	1	17.25
4	55.30	2	8.32
6	48.30	3	8.32
8	30.80	4	1.42
10	25.10	5	0
12	20.90		
14	14.60		
16	9.36		
18	6.32		

curcumin-I remained on the glass surface whereas, in methanolic solution it degraded completely by this time (Table 8). Its half life ($t_{1/2}$) in methanolic solution and as thin film on glass surface was found to be 1.5 h and 11.04 h respectively (Figs. 15 & 16). The study revealed that degradation of curcumin-I followed pseudo-first order kinetics [(R^2=0.922, F(1,4) = 47.314) significant at 1% level] in solution and first order kinetics [R^2=0.910, F(1,4) = 40.563) significant at 1% level] in thin film on glass surface respectively.

4.7.3.2 Sunlight degradation in solution and as thin film on glass surface

When methanolic solution of curcumin-I was exposed to sunlight for 8h, at the end of exposure only 6.32% of its initial concentration was detected in solution, within 6 hours, 52% of curcumin-I had already degraded. As thin film on glass surface, curcumin-I residue disappeared slowly. Half life ($t_{1/2}$) of curcumin-I in methanolic solution and as thin film on glass surface was found to be 4.73h and 4.08h respectively (Table 14, Figs. 17 & 18). The study revealed that degradation of curcumin-I followed pseudo-first order kinetics [R^2 = 0.987, F(1,8) = 615.075, significant 1% level] in solution and first order kinetics [R^2 = 0.988, F (1,2) = 85.225 significant at 5 % level] when applied as thin film on galss surface. In this experiment, the rate of degradation was faster in film on glass surface than in solution phase. In another experiment, a thin film on glass surface under UV-light degraded slowly (50%, 15 h) than thin film under sunlight (15%, 15 h). It was probaly due to the fact that volatilization loss were more in sunlight than in UV-light as sunlight experimments were conducted

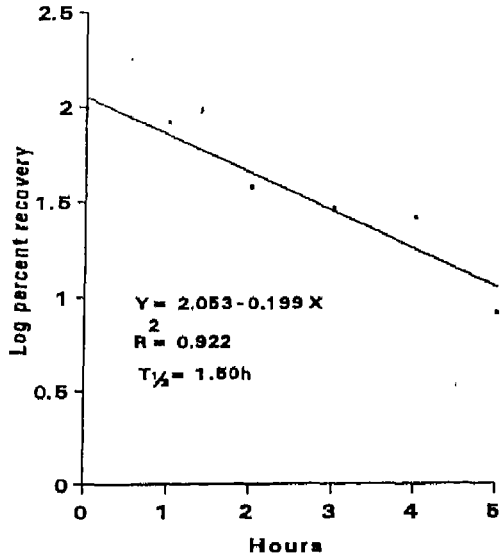


Fig.15. Linear plot for pseudo-first order degradation of curcumin1 under UV-light as methanolic solution

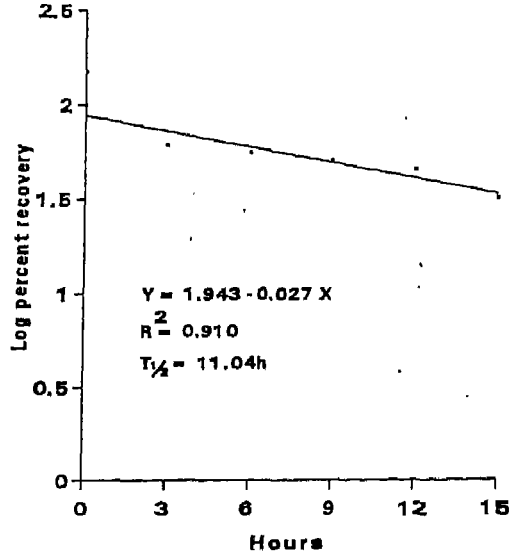


Fig.16. Linear plot for first order degradation of curcumin-I under UV-light as thin film on glass surface

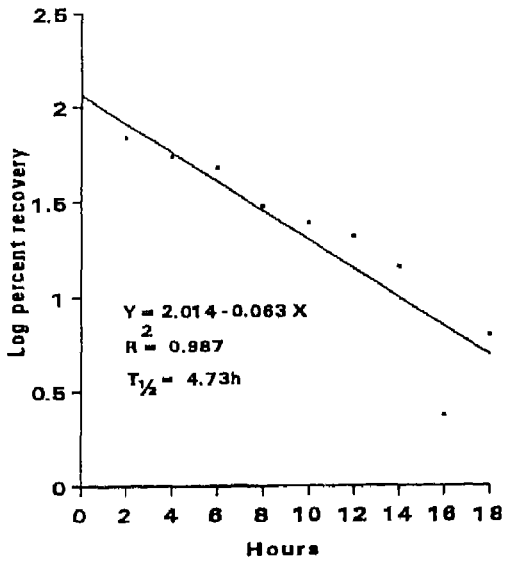


Fig.17. Linear plot for pseudo-first order degration of curcumin-I under sun light as methanolic solution

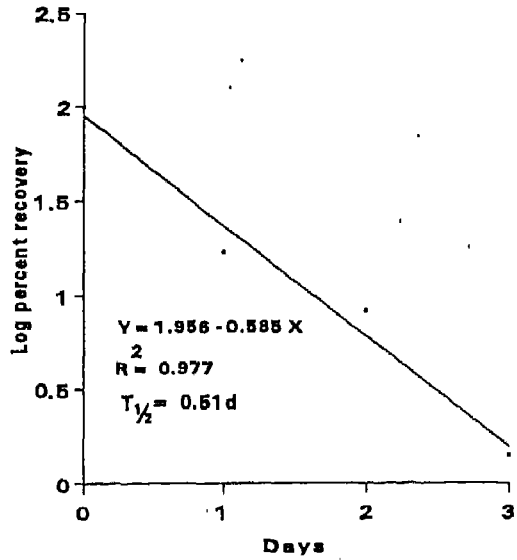


Fig.18. Linear plot for first order degradation of curcumin-I under sun light as thin film on glass surface

outdoor whereas, UV degradation studies were carried out in the laboratory at 254 nm.

4.7.3.3 Sunlight degradation on leaf surface

A thin film of curcumin-I coated on tobacco leaves when subjected to sunlight radiation, degraded rapidly as after 3 days only 4.82% of curcumin-I remained on the leaf surface (Table 15, Fig. 19). Most of the compound (95.28%) had dissipated mainly due to volatilization and degradative processes. Its half life (t1/2) was found to be 0.73 day. As compared to leaf surface, degradation of curcumin-I on glass surface was comparatively slower. The degradation of curcumin-I as thin film on leaf surface followed first order kinetics [R^2=0.756, F(1,2)=6.209 significant at 1% level].

Table 15. Progressive degradation of curcumin-I under sunlight as thin film on leaf surface

Time (day)	% Curcumin-I remaining
0	100.00
1	8.54
2	6.44
3	4.82
4	0

4.8.1 Effect of curcumin-I on stability of azadirachtin under UV-light

Under UV-photolylic conditions, azadirachtin in methanolic solution degraded rapidly and disappeard within 3 h.

However, when azadirachtin: curcumin-I mixture was subjected to photodegradation, it was stabilized to considerable extent. In three different ratios of azadirachtin and curcumin-I (1:1, 2:1, 3:1), azadirachtin content was found to be highest in 1:1 followed in by 2:1 and 3:1 ratio. After 8h of UV-exposure of the methanolic solution, curcumin-I was able to maintain azadirachtin content upto 36%, in 1:1, 26% in 2:1 and 9 % in 3:1 mixtures.

In a similar study as thin film on glass surface, azadirachtin was found to degrade completely within 14h. When a thin film of azadirachtin : curcumin I mixture (1:1, 2:1, 3:1) on glass surface was subjected to UV-exposure, photodecomposition was arrested to considerable extent as 45.36, 31.06 and 14.56 % of azadirachtin was still detectable after 14h. Results indicated (Table 16, Fig. 20) that of the three ratios, azadirachtin content in solution and as thin film on glass surface was again found maximum in 1:1 followed by 2:1 and 3:1 proportions. Thus azadiractin solution in methanol and as thin film, which degraded completely within 3h and 14h could be stabilized with curcumin-I upto 8h and 14h with azadiracthin content of 36 and 45.36 % in solution and thin film respectively.

4.8.2 Effect of curcumin-II on stability of azadirachtin under UV-light

Under UV-photolytic conditions, azadirachtin in azadirachtin : curcumin II solution was less susceptible to UV-light than azadirachtin alone. Whereas, in methanolic solution azadirachtin degraded completely within 3 h but in combination with cucurmin-II, 23, 11, and 2.5 % of it

Table 16. Effect of UV-light on the degradation of azadirachtin in azadirachtin : curcumin-I mixture

Time (hour)	% Azadirachtin remaining		
	Aza+Cur-I (1:1)	Aza+Cur-I (2:1)	Aza+Cur-I (3:1)
Methanolic solution			
0	100.00	100.00	100.00
1	77.85	62.63	56.90
2	60.15	53.70	48.37
3	54.75	43.52	44.77
4	49.23	41.99	28.60
5	47.12	32.96	18.72
6	39.54	31.46	15.17
7	37.60	28.71	12.18
8	35.98	25.66	9.96
Thin film on glass surface			
0	100.00	100.00	100.00
2	97.32	63.54	50.32
4	90.39	55.96	38.65
6	82.28	37.78	18.69
8	79.56	35.45	16.33
10	52.39	33.99	15.28
12	48.60	32.79	14.76
14	45.36	31.06	14.56
16	37.41	30.01	12.56

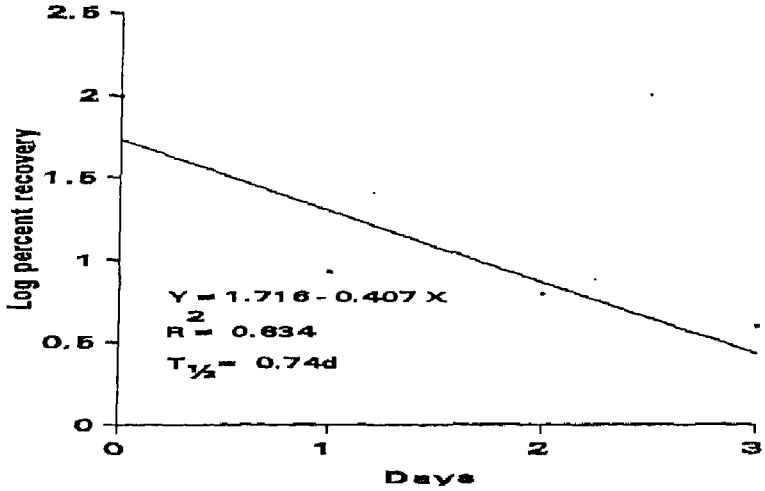


Fig.19. Linear plot for first order degradation of curcumin-I under sun light as thin film on leaf surface

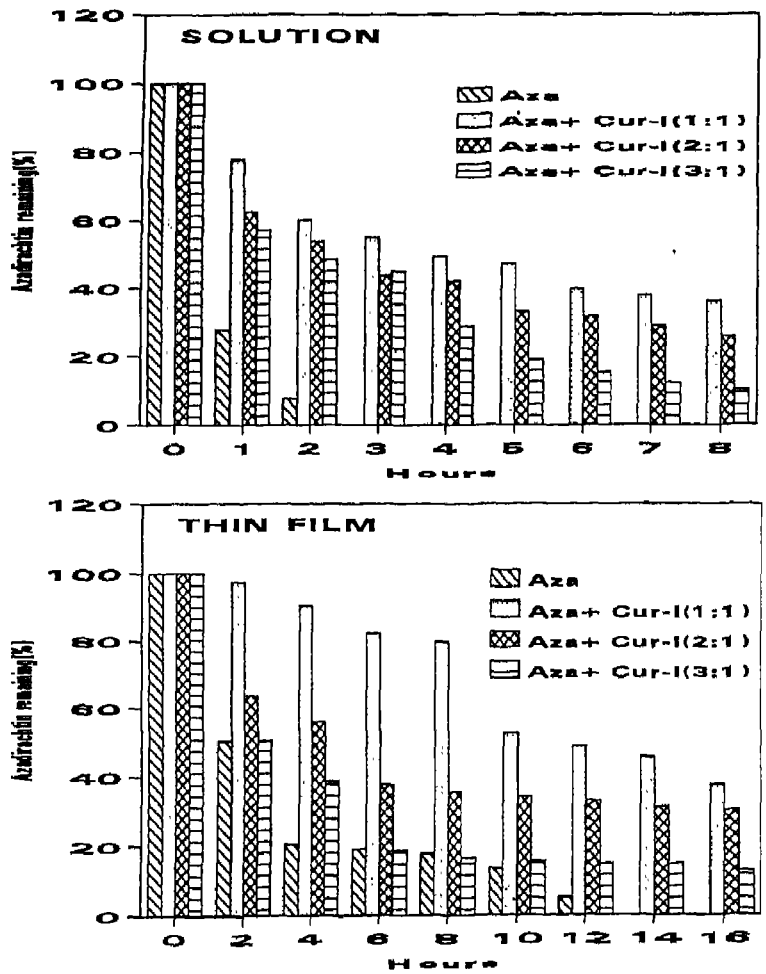


Fig.20.Effect of UV-light on the degradation of azadirachtin in azadirectin:curcumin-I mixture

could be detected in 1:1, 2:1 and 3:1 ratios after 5 h. respectively. Similarly as thin film on glass surface azadirachtin content after 16h were found to be 31.98, 20.39 and 11.04 % in 1:1, 2:1 and 3:1 proportions respectively (Table 17). Like in curcumin I, degradation of azadirachtin in curcumin-II : azadirachtin mixture on glass surface was slower than in solution. In this experiment also the azadirachtin content after 18h remained highest in 1:1 proportions.

4.8.3 Effect of curcumin-III on stability of azadirachtin under UV-light

Photodecomposition of azadirachtin in methanolic solution of azadirachtin : curcumin-III mixture was faster than azadirachtin alone indicating that curcumin III has no impact in stabilizing azadirachtin content in solution. On the other hand, as thin film on glass surface, curcumin II has maximum effect in imparting stability to azadirachtin thin layer. Unlike azadirachtin which became non-detectable after 14h, azadirachtin in azadirachtin: curcumin III mixture remained upto 48.6, 35.01 and 39.9 % after 16h in 1:1, 2:1 and 3:1 mixture respectively (Table 18). The maximum stability was again achieved when azadirachtin: curcumin-III ratio was 1:1. Thus unlike curcumin-I and II, curcumin-III in solution phase behaved differently on exposure to UV-light.

The quick degradation in solution is perhaps due to the fact that methanolic solution of curcumin-III having hydroxy substituent at para position is more susceptibile to UV-light. Similarly, higher stabilization of azadirachtin in thin film by

Table 17. Effect of UV-light on the degradation of azadirachtin in azadirachtin : curcumin-II mixture

Time (hour)	% Azadirachtin remaining		
	Aza+Cur-II (1:1)	Aza+Cur-II (2:1)	Aza+Cur-II (3:1)
Methanolic solution			
0	100.00	100.00	100.00
1	55.31	47.87	45.29
2	47.21	32.65	25.96
3	31.22	19.84	13.71
4	28.39	16.59	7.44
5	22.81	11.19	2.53
Thin film on glass surface			
0	100.00	100.00	100.00
2	94.04	84.07	67.49
4	91.48	58.46	48.41
6	64.42	43.91	28.06
8	61.63	35.25	23.69
10	57.27	31.39	23.35
12	39.55	25.72	19.93
14	34.33	22.23	15.03
16	31.98	20.39	11.54

Table 18. Effect of UV-light on the degradation of azadirachtin in azadirachtin : curcumin-III mixture

Time (hour)	% Azadirachtin remaining		
	Aza+Cur-III (1:1)	Aza+Cur-III (2:1)	Aza+Cur-III (3:1)
Methanolic solution			
0	100.00	100.00	100.00
0.5	64.92	59.00	59.55
1.0	3.93	3.40	3.20
1.5	2.42	2.13	2.51
2.0	0	0	0
Thin film on glass surface			
0	100.00	100.00	100.00
2	92.92	77.36	73.28
4	79.34	63.81	60.62
6	75.82	54.47	58.37
8	71.15	44.36	55.55
10	60.82	42.46	42.30
12	60.35	39.48	42.15
14	52.36	37.21	40.91
16	48.59	35.01	39.86

curcumin-III is likely to be attributed to the fact that curcumin-III is more stable as thin film either alone or in mixture with azadirachtin.

4.8.4 Effect of curcuminoids (mixture of curcumin-I, II, and III) on stability of azadirachtin under UV-light

Persual of the data (Table 19) revealed that in methanolic solution of azadirachtin - curcuminoid mixture, azadirachtin degraded comparatively rapidly. It was in contrast to our earlier observation that individual curcumins were able to considerably stabilize azadirachtin. In all the samples, azadirachtin degraded completely within 2.5h indicating no stabilization of azadirachtin with curcumin mixture. However, as thin film on glass surface some stabilization was achieved. Unlike azadirachtin which degraded completely within 14 h, in combination with curcuminoid mixture, azadirachtin content in three different conbinations (1:1, 2:1, 3:1) was found to be 37.52, 18.14 and 20.40% respectively. The extent of stabilization was however, less than using curcumin-I, II or III alone individually. As observed earlier, out of the three proportions, highest degree of stabilization was achieved when azadirachtin and curcumin mixture was taken in 1:1 proportion.

4.8.5 Effect of trimethyl curcumin I on azadirachtin stability under UV-light

Upon methylation, curcumin-I was converted to its trimethyl analogue in which two free - OH groups were converted to methoxyl substituents. It was then evaluated for stabilization effect to assess the contribution of -OH/-OCH_3 groups in the curcumin molecule. The study revealed that in methanolic solution' azadirachtin content after exposure to UV-light for 3.5h in three

Table 19. Effect of UV-light on the degradation of azadirachtin in azadirachtin : curcumin oil mixture (I, II and III)

Time (hour)	% Azadirachtin remaining		
	Aza+Cur-mixture (1:1)	Aza+Cur-mixture (2:1)	Aza+Cur-mixture (3:1)
Methanolic solution			
0	100.00	100.00	100.00
0.5	82.94	68.51	63.15
1.0	52.09	51.25	27.76
1.5	16.56	16.03	17.56
2.0	10.22	9.35	8.56
Thin film on glass surface			
0	100.00	100.00	100.00
2	71.16	74.57	79.80
4	65.21	63.20	71.15
6	59.72	37.54	43.87
8	48.71	31.02	39.95
10	41.18	23.94	33.46
12	39.23	19.85	23.87
14	37.52	18.14	20.40
16	29.25	13.35	12.52

proportions (1:1, 2:1, 3:1) was found to be 35, 5.3 and 0% respectivley. As thin film on glass surface, methyl curcumin-I was able to stabilize azadirachtin considerably as after 14h, 40.23, 30.32 and 32.2 % of azadirachtin was still detectable (Table 20). The extent of stabilization with methyl curcumin-I was slightly less than curcumin-I and II but slightly more than curcumin-III. Similarly, as thin film on glass surface, stabilization of azadirachtin by methyl curcumin-I was lower than curcumin-II. It was thus inferred that presence of free OH group adjacent to - OCH_3 moiety in curcumin nucleus is important for imparting stability to azadirachtin molecule.

4.8.6 Effect of di-O-butyl curcumin-I on azadirachtin stability under UV-light

Curcumin-I was converted to its butyl analogue by its reaction with *n*-butyl bromide and the butylated product tested for its possible stabilizing effect on azadirachtin. The study revealed that azadirachtin content in the methanolic solution of azadirachtin: di- butyl curcumin-I mixture after 4h of UV exposure was 15.68, 10.51 and 0 %. Whereas, as thin film on glass surface azadirachtin residues after 14h of exposusre were found to be 38.32, 31.21 and 32.85 in 1:1, 2:1 and 3:1 proportions respectively (Table 21). In both the cases, 1:1 proportion provided maximum stabilization of azadirachtin.

4.8.7 Effect of butylated hydroxy toluene (BHT) on azadirachtin stability under UV-light

In the presence of butylated hydroxy toluence (BHT), azadirachtin could be stabilized to some extent in the presence of UV-light but its stabilization was not to the extent of curcumins

Table 20. Effect of UV-light on degradation of azadirachtin in azadirachtin : trimethyl curcumin-I mixture

Time (hour)	% Azadirachtin remaining		
	Aza+Meth-Cur-I (1:1)	Aza+Meth-Cur-I (2:1)	Aza+Meth-Cur-I (3:1)
Methanolic solution			
0	100.00	100.00	100.00
0.5	82.61	67.24	60.40
1.0	79.09	55.09	33.08
1.5	75.97	49.85	20.78
2.0	67.61	25.55	8.26
2.5	60.06	19.63	3.22
3.0	43.00	10.60	1.12
3.5	35.00	5.30	0
Thin film on glass surface			
0	100.00	100.00	100.00
2	91.87	65.11	88.22
4	82.32	55.23	76.31
6	70.11	42.70	63.03
8	60.78	40.79	57.37
10	57.47	38.18	51.71
12	46.91	36.68	39.48
14	40.23	30.32	32.21

Table 21. Effect of UV-light on degradation of azadirachtin in azadirachtin : butylated curcumin-I mixture.

Time (hour)	% Azadirachtin remaining		
	Aza+But-Cur-I (1:1)	Aza+But-Cur-I (2:1)	Aza+But-Cur-I (3:1)
Methanolic solution			
0	100.00	100.00	100.00
0.5	86.02	70.36	40.16
1.0	83.43	64.13	30.52
1.5	81.38	59.67	20.92
2.0	71.31	39.98	10.94
2.5	59.86	32.59	5.32
3.0	42.27	25.59	0
3.5	31.85	18.21	
4.0	15.68	10.51	
Thin film on glass surface			
0	100.00	100.00	100.00
2	90.16	63.64	62.67
4	80.86	54.95	54.87
6	70.46	42.06	52.39
8	63.06	39.58	41.22
10	55.85	37.77	40.48
12	45.26	36.21	35.21
14	38.32	31.21	32.35

used under similar conditions. Whereas, azadirachtin residues in methanolic solution after 2h were found to be 7.48%, in presence of BHT, azadirachtin could be detected to the extent of 36.56, 25.69 and 20.59% after 2 h and 5.39, 2.39 and 0% after 6 h in 1:1, 2:1 and 3:1 proportions respectively (Table 22, Fig. 21).

Since in all three experiments, stabilization was best achieved when azdirachtin and stabilizers were taken in 1:1 followed by 2:1 and 3:1 proportions respectively, subsequent experiments under sunlight conditions were conducted by using 1:1 ratio of azadirachtin and stabilizers.

4.9 Sunlight degradation of azadirachtin in presence of curcumin-I and BHT

4.9.1 In methanolic solution

When exposed to sunlight, azadirachtin solution in methanol degraded rapidly and by the end of first day, 57% of azadirachtin had already degraded. Subsequent degradation was comparatively slower as by the end of sixth day 9.5% of the initial azadirachtin still remained in the solution. In the presence of curcumin-I, azadirachtin was considerably stabilized as 49.23% was still detected after 7 days of exposure (Table 23, Fig. 22). When stabilization of azadirachtin was attempted with BHT, a standard reference stabilizer, azadirachtin could not be stabilized rather it degraded a bit faster than azadirachtin alone.

Thus under sunlight irradiated conditions, unlike BHT, curcumin I was able to significantly stabilize azadirachtin upto seven days.

Table 22. Effect of UV-light on degradation of azadirachtin in azadirachtin : butylated hydroxy toluene (BHT) mixture

Time (hour)	% Azadirachtin remaining		
	Aza+BHT (1:1)	Aza+BHT (2:1)	Aza+BHT (3:1)
Methanolic solution			
0	100.00	100.00	100.00
1	45.97	35.84	26.65
2	36.56	25.64	20.59
3	26.25	20.39	14.68
4	19.31	13.64	4.29
5	10.25	7.26	0
6	5.39	2.39	
7	0	0	
Thin film on glass surface			
0	100.00	100.00	100.00
2	63.76	62.91	58.16
4	50.64	48.16	47.52
6	35.08	34.92	34.92
8	21.33	21.69	15.95
10	14.07	13.75	9.32
12	4.84	4.69	3.78
14	3.42	2.37	1.54

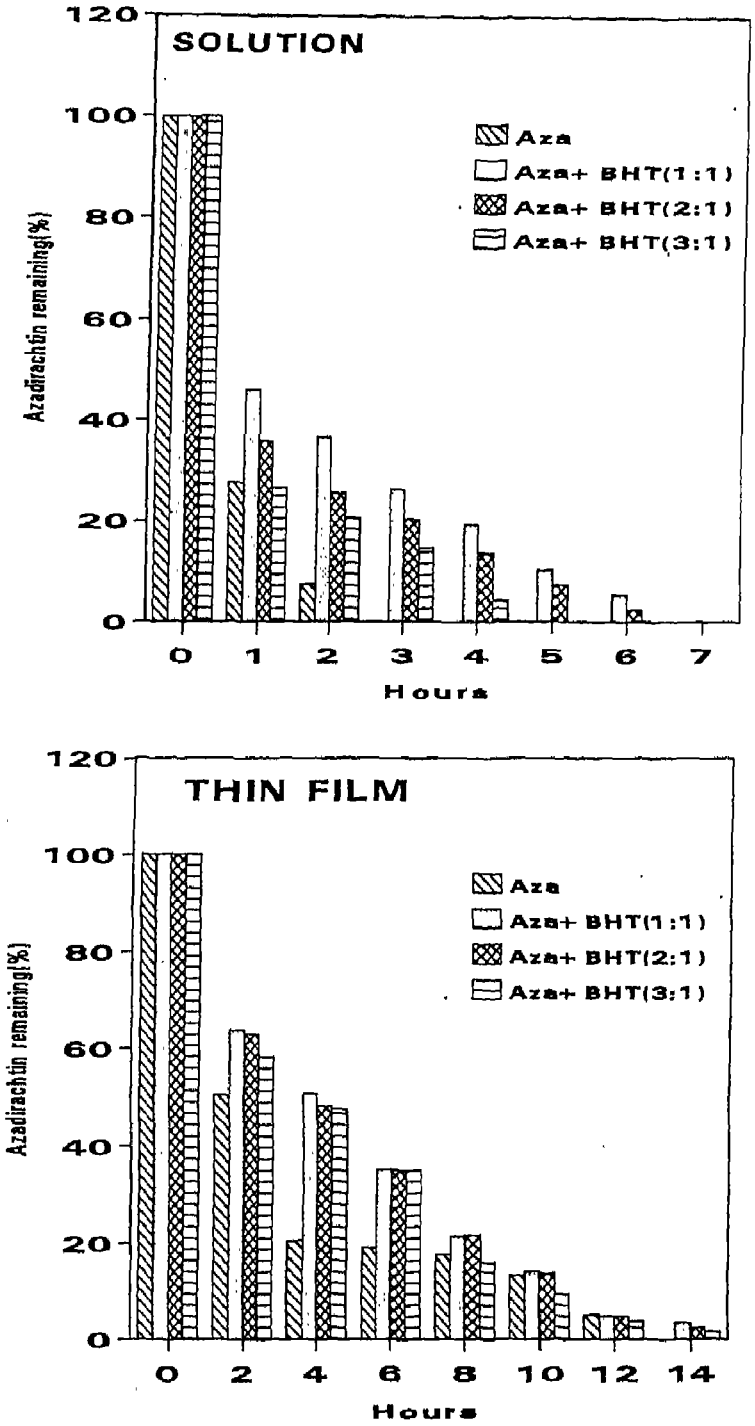


Fig.21. Effect of UV-light on degradation of azadirachtin in azadirachtin: butylated hydroxy toluene(BHT) mixture

Table 23. Effect of sunlight on degradation of azadirachtin in azadirachtin and curcumin-I/BHT/neem oil (NO) mixture

Time (day)	% Azadirachtin remaining		
	Aza+Cur-I (1:1)	Aza+BHT (1:1)	Aza+NO
Methanolic solution			
0	100.00	100.00	100.00
1	85.33	92.66	-
2	79.42	54.06	66.87
3	73.41	8.03	-
4	69.53		58.60
5	67.69		-
6	55.96		52.47
Thin film on glass surface			
0	100.00	100.00	100.00
1	95.21	54.89	-
2	54.90	36.06	32.17
3	41.28	11.91	-
4	30.21	0	22.00
5	15.35		-
6	10.25		6.00

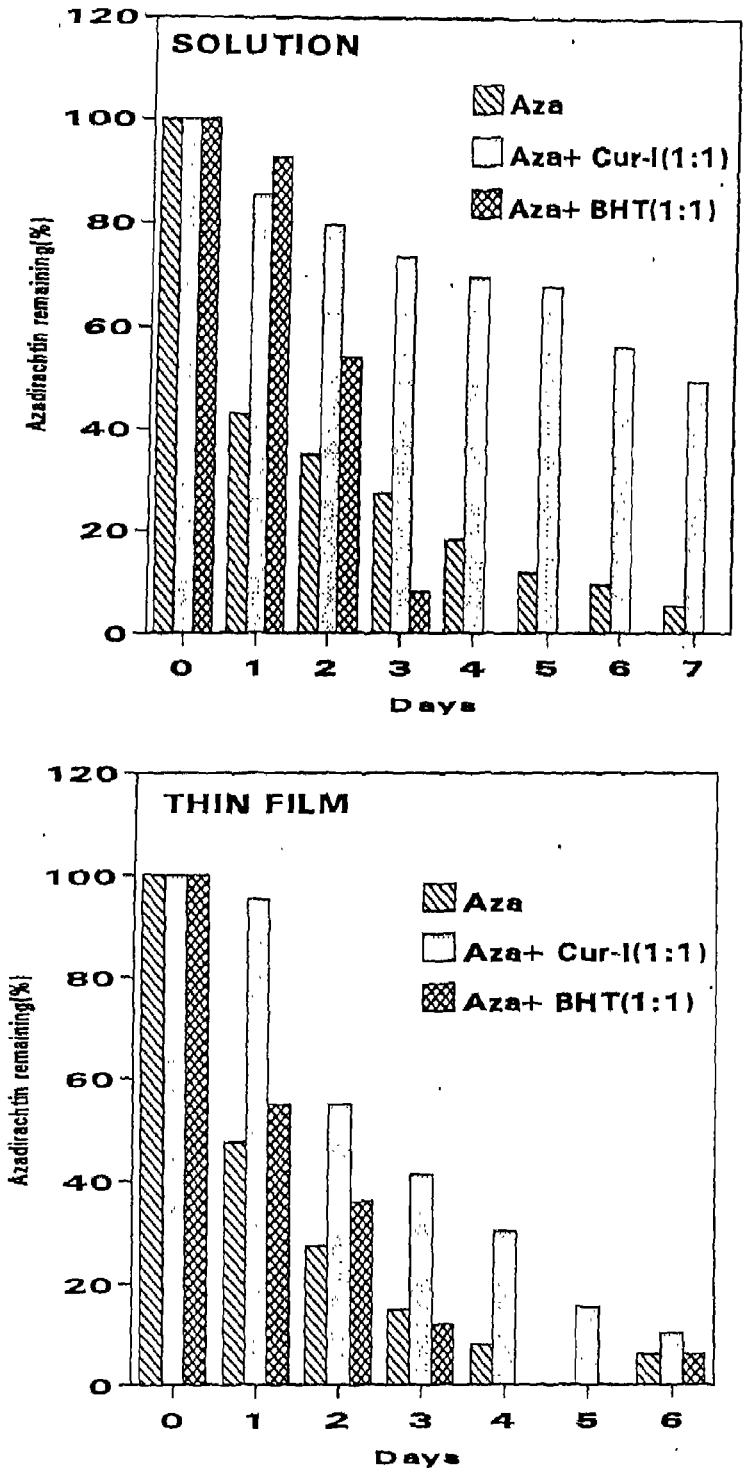


Fig.22. Effect of sunlight on degradation of azadirachtin in azadirachtin and curcumin-I/BHT mixture

4.9.2 Thin film on glass surface

When a thin film of azadirachtin on glass surface was subjected to solar radiations, as much as 92% of its initial deposit was found to degrade within 4 days. However, in the presence of curcumin-I (azadirachtin:curcumin-I, 1:1), azadirachtin degraded comparatively slowly as after 2, 4 and 6 days, 55, 30 and 10% of azadirachtin was still detectable. When similar experiment was conducted using BHT, azadirachtin could not be stabilized as after 3 days only 11.9% of azadirachtin was detected on glass surface.

4.9.3 Sunlight degradation of azadirachtin in solution and as thin film on glass surface in the presence of neem and turmeric oil

4.9.3.1 Azadirachtin + neem oil (NO)

Unlike azadirachtin which after six days of sunlight exposure was found to be 9.5%, in the presence of neem oil as stabilizer, azadirachtin content after similar time interval was 52.47% (Tables 23, Fig. 23). Thus azadirachtin rich neem oil is more stable to sunlight than azadirachtin alone in solution phase. These results are in agreement with earlier observations stating that neem oil is capable of stabilizing azadirachtin.

4.9.3.2 Azadirachtin + turmeric oil (TO)

In the presence of turmeric oil, azadirachtin in azadirachtin : T.O (1:1) mixture was stabilized considerably and the extent of stabilization was more or less same as observed with neem oil, as after 6 days, 45.34% of azadirachtin was detectable in methanolic solution. It was thus inferred that in the presence of neem oil or turmeric oil, azadirachtin was considerably stabilized

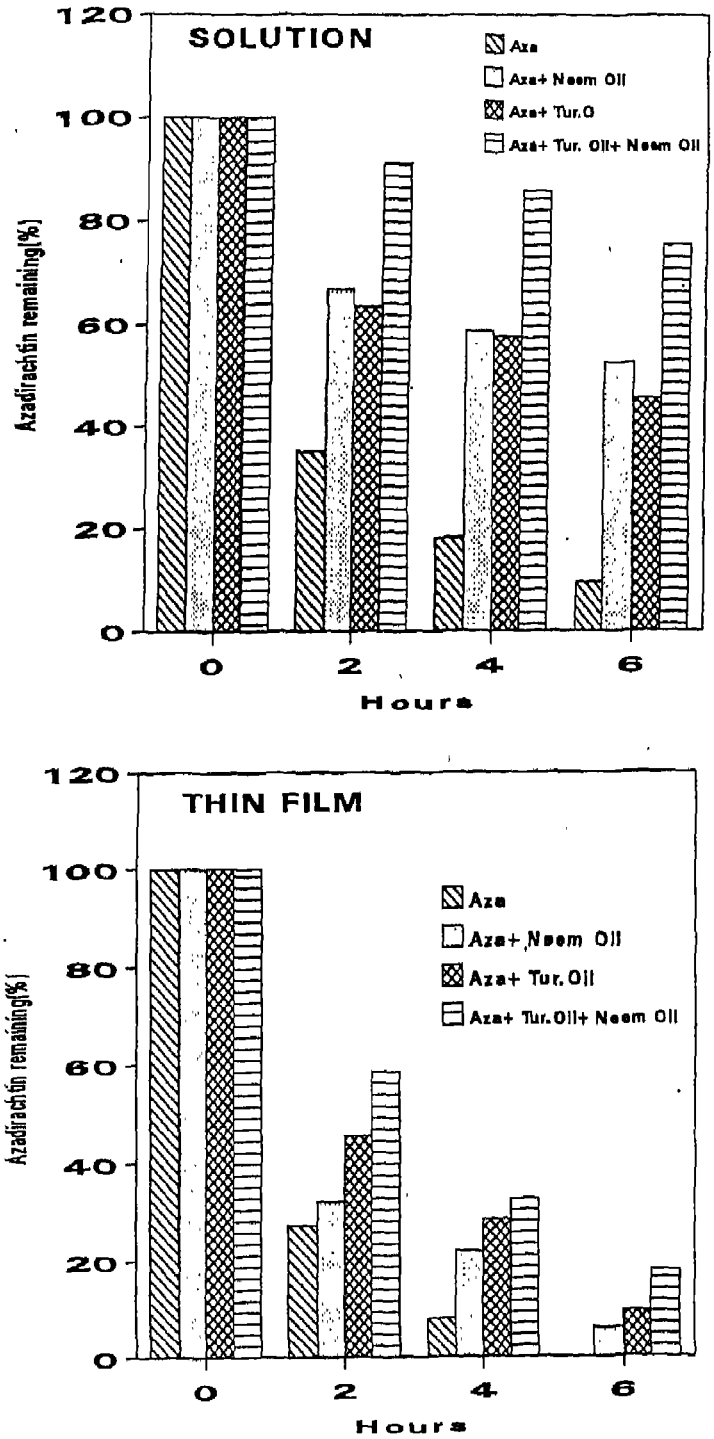


Fig. 23. Effect of sunlight on degradation of azadirachtin in combination with turmeric oil(TO) and neem oil(NO)

and did not degrade (Table 24, Fig. 23) as rapidly as it would have degraded in their absence. However, as thin film on glass surface, azadirachtin degradation was fairly more as only 25% of azadirachtin was detectable after 4 days. Lesser stabilization may be attributed to the possible volatilization loss of turmerones which are the major constituent of turmeric oil.

4.9.3.3 Azadirachtin + neem oil + turmeric oil

Because of the encouraging response obtained independently with turmeric oil and neem oil, next experiment was conducted to stabilize azadirachtin with mixture of both neem oil and turmeric oil. The study revealed that unlike azadirachtin which degraded within six days to 9.49% of its initial concentration in solution, turmeric oil -neem oil mixture could stabilize azadirachtin considerably as after 6 days 75.42% of azadirachtin was still detected (Fig. 24). The extent of stabilization was more than with either neem oil or turmeric oil which could retain 52.47 and 45.34% of azadirachtin in neem oil : azadirachtin or turmeric oil : azadirachtin mixtures. Thus, of the various combinations turmeric oil-neem oil mixture was considered the best for stabilizing azadirachtin in solution phase (Table 24, Fig. 23). As thin film on glass surface, disappearance of azadirachtin was faster as only 20% of it could be detected after 4 days as compared to 85.86% in methanolic solution. The rapid decrease in azadirachtin content on thin film was attributed to several environmental factors such as humidity temperature, wind velocity etc. contributing to volatilization losses.

Table 24. Effect of sunlight on degradation of azadirachtin in combination with turmeric oil (TO) neem oil (NO) and curcumin-I

Time (day)	% Azadirachtin remaining		
	Aza+TO (1:1)	Aza+TO+NO	Aza+NO+Cur-I
Methanolic solution			
0	100.00	100.00	100.00
2	63.51	91.21	86.64
4	57.52	85.86	78.34
6	45.34	75.42	62.26
Thin film on glass surface			
0	100.00	100.00	100.00
2	45.60	58.50	56.00
4	28.50	32.70	26.23
6	9.50	17.90	14.95

Table 25. Effect of sunlight on degradation of azadirachtin in combination with neem oil (NO), curcumin-I and turmeric oil (TO) on leaf surface

Time (day)	% Azadirachtin remaining				
	Aza	Aza+NO	Aza+Cur-I (1:1)	Aza+NO+ Cur-I	Aza+NO+ TO
0	100.00	100.00	100.00	100.00	100.00
2	9.52	28.25	40.20	49.98	66.20
4	0	19.50	19.90	20.65	32.80
6		3.90	8.90	9.78	13.80

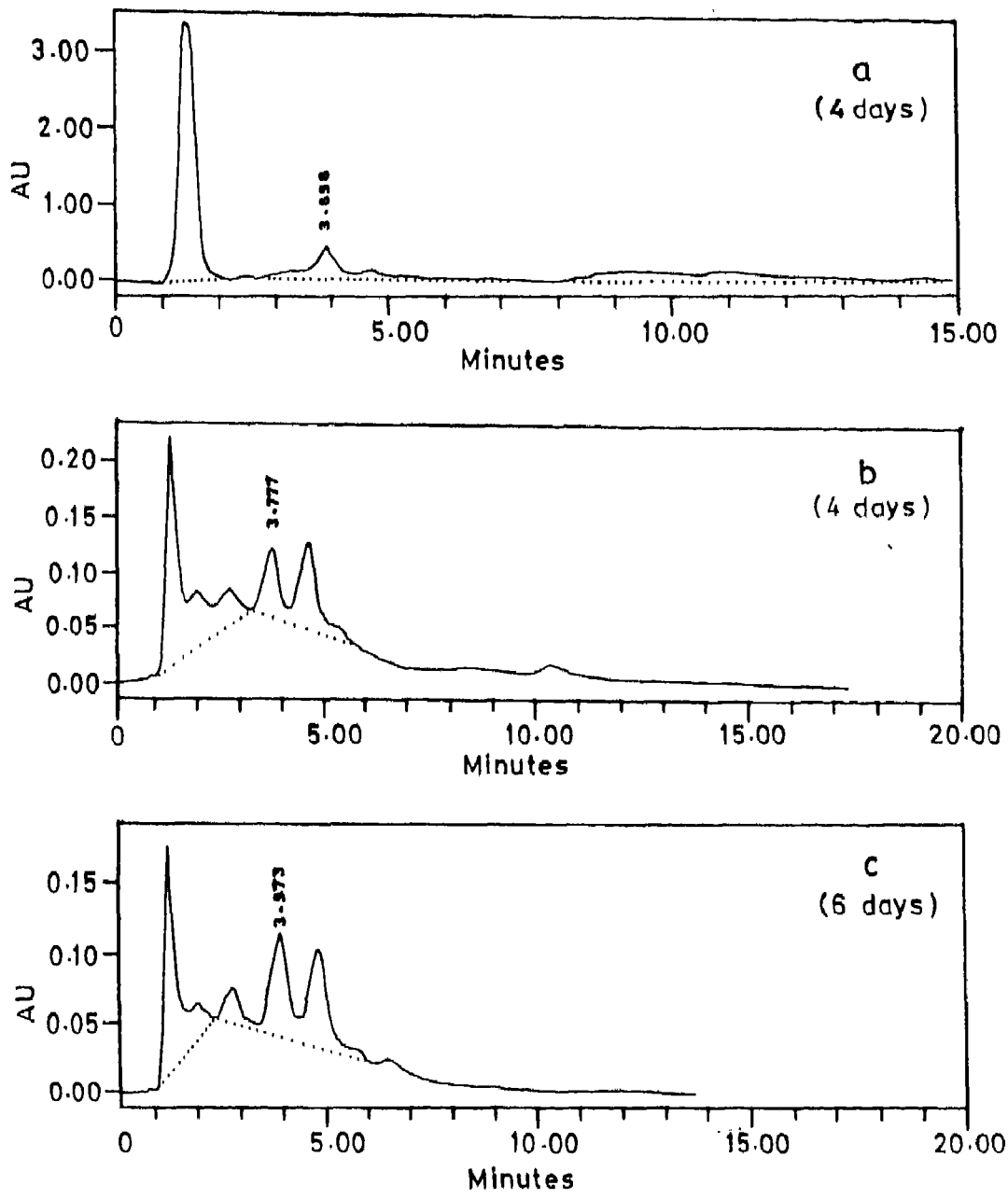


Fig. 24: HPLC Chromatogram showing azadirachtin peak in (a) methanotic solution of af azadirachtin (b) aza: neem oil mixture (c) aza + neem oil + turmeric oil mixture at different time intervals.

4.9.3.4 Azadirachtin + neem oil + curcumin I

As observed with turmeric oil, curcumin-I was also able to stabilize azadirachtin considerably. Thus, in presence of neem oil-curcumin-I mixture, azadirachtin content in the mixture in solution phase after 6 days of sunlight exposure was found to be 62.26% which was slightly higher than when curcumin-I (55.96%) and neem oil (52.57%) alone were used. Like in previous experiment with turmeric oil and neem oil mixture, degradation of azadirachtin as thin film on glass surface was higher because of the various environmental factors (Table 24, Fig. 25).

4.9.4 Sunlight degradation of azadirachtin in presence of curcumin-I, turmeric oil and neem oil on leaf surface

4.9.4.1 Azadirachtin

Dissipation of azadirachtin as thin film on leaf surface was comparatively higher as compared to on glass surface. Unlike on glass surface, in which azadirachtin deposit was found to be 8%, on leaf the residues became non detectable after four days of exposure (Table 25, Fig. 26). The rapid loss on leaf surface was possibly due to wind velocity, higher moisture content on leaf surface as well as possible photosensitization of azadirachtin with leaf green pigment chlorophyll.

4.9.4.2 Azadirachtin rich neem oil

In order to see the effect of neem oil on the degradation of azadirachtin, a thin film of azadirachtin -neem oil mixture (1:1) on leaf surface was exposed to sunlight. Unlike azadirachtin alone which disappeared completely within four days, 19.25 of

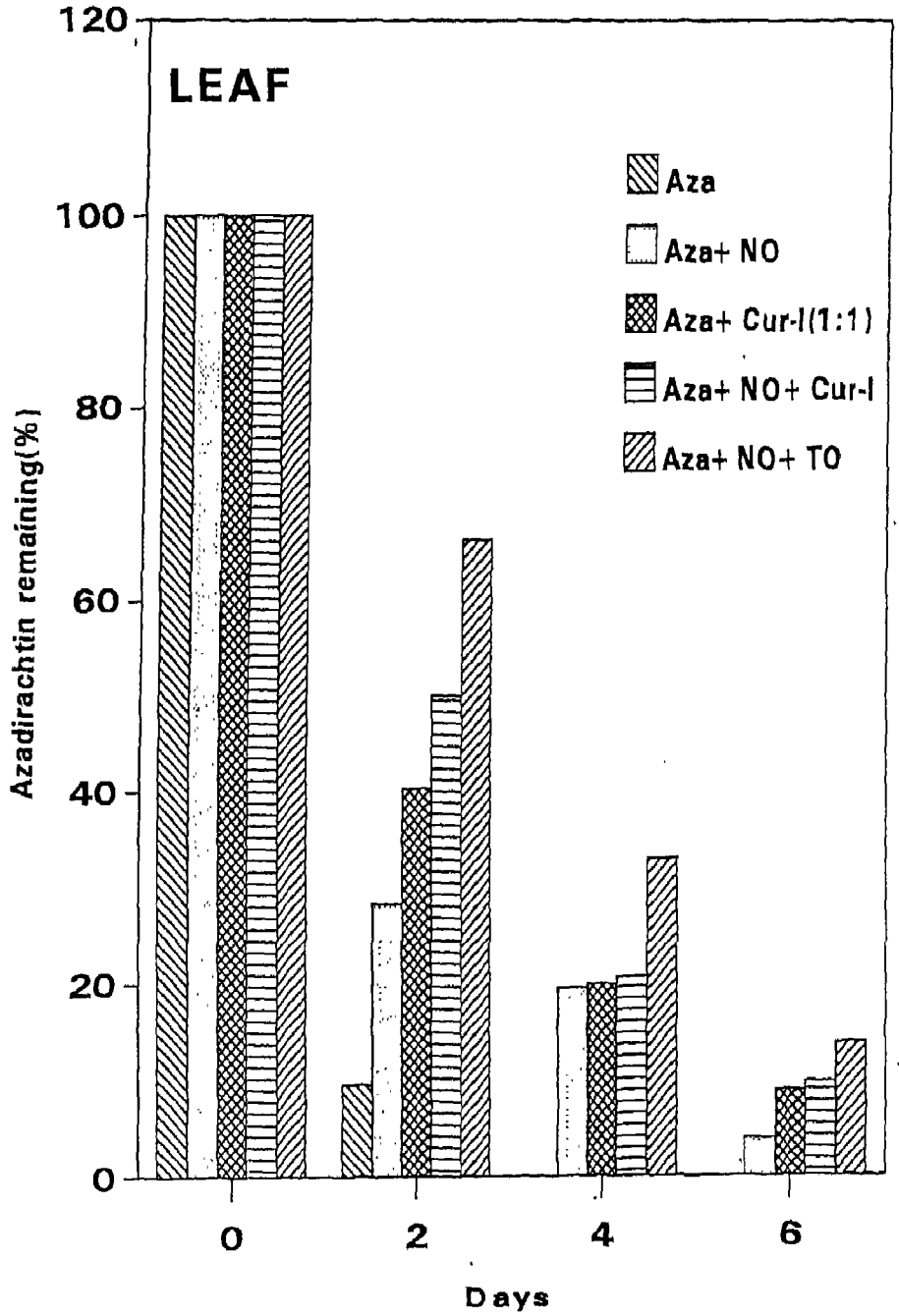


Fig.25. Effect of sunlight on degradation of azadirachtin in combination with neem oil(NO), curcumin-I and turmeric oil(TO)

azadirrachtin was detected when azadirachtin : neem oil, (1:1) mixture was exposed to sunlight (Table 25, Fig. 25).

4.9.4.3 Azadirachtin with curcumin-I

In the presence of curcumin-I, decomposition of azadirachtin in sunlight on leaf surface was significantly arrested. Azadirachtin content after 2 and 6 days of exposure was found to be 40.2 and 8.9% respectively. It was noticed that as compared to neem oil, curcumin-I has more stabilizing ability (Table 25, Fig. 25).

4.9.4.4 Azadirachtin rich neem oil + curcumin-I/turmeric oil

Unlike azadirachtin on leaf surface which degraded completly within four days this combination of azadirachtin rich neem oil : cucurmin-I provided considerable stability to azadirachtin as 50 and 9.78% of azadirachtin was detected after 2 and 6 days of exposure respectively. When a thin film of azadirachtin rich neem oil containining turmeric oil coated on leaf surface was exposed to sunlight, azadirachtin degradation was significantly reduced as after 2 and 6 days of exposure 66.2 and 13.8% of azadirachtin was detected which was significantly higher than when similar study was conducted with neem oil, curcumin I and/or other combinations (Table 25, Fig. 25).

From these studies it was concluded that to major constituents of *Curcuma longa* namely curcumin-I and turmeric oil provided maximum stability to azadirachtin or azadirachtin rich neem oil. The stability was more pronounced in solution phase than on glass or leaf surface where environmental factors also

influence the degradation of azadirachtin. Neem oil was also found to stabilize azadirachtin in both solution and as thin film on glass surface. Maximum photostablity of azadirachtin was achieved in combination with turmeric oil - neem oil mixture.

4.10 Bio activity of Azadirachtin and Azadirachtin-Curcumin-I Mixture against Third/Fifth Instar Larvae of *Spilosoma obliqua*

Whereas, azadirachtin is well known for insect growth inhibition and antifeeding activity, curcumin-I, one of the chief constituent of *Curcuma longa* possesses anti-oxidant activity. The present bioassay studies were conducted to see the effect of azadirachtin, curcumin-I and azadirachtin-curcumin-I mixture on larval mortality, insect growth inhibition and anti-feedant activity against 3rd/5th instar larvae of *Spilosoma obliqua*.

4.10.1 Insect growth regulatory (IGR) activity against third instar larvae

When leaves coated with known concentration of azadirachtin (0.3 to 0.04 %, 1ml) were fed to third instar larvae of *S. obliqua*, azadirachtin was found to elicit significant disturbance in insect growth. It was found to induce larval mortality in a dose dependent manner. With decrease in dose, mortality decreases, whereas, insect growth inhibition increases. Thus at 0.3 and 0.04 % concentration levels, larval mortality and moulting inhibition activity was 55, 45 and 40, 35 % respectively. Abnormal adults eventually died as they could not survive for longer duration.

When known concentration of curcumin-I : azadirachtin (1:1) mixture was fed (leaf disc method) to third instar larvae, there was no significant increase in larval/pupal mortality or moulting

inhibition and the activity remained at par with that of azadirachtin alone. However, when azadirachtin concentration was increased in the mixture (2:1 and 3:1 ratio) both the proportions imparted 100 % mortality upto 0.08 % concentration. At lowest dose of 0.04 % larval mortality was upto 80-90 % and the remaining (10-20 %) were abnormalities recorded during pupal and adult emergence. The delayed lethal effect or moulting abnormalities of azadirachtin with or without curcumin-I were attributed to their interference with insect endocrine system. Based on the bioassay experiment on third instar larvae it was inferred that bioefficacy of 45% azadirachtin in combination with curcumin-I (1:1, 2:1 and 3:1) was better than azadirachtin alone. The highest emergence of adults was witnessed either with azadirachtin alone (15-25 %) or azadirachtin : curcumin-I (1:1) mixture (15-35 %), whereas, no adult emergence was recorded in azadirachtin: curcumin-I 2:1 and 3:1 proportions (Table 26 and 27).

4.10.2 Insect growth regulatory (IGR) activity against fifth instar larvae

When fifth instar larvae of *S. Obliqua* were exposed to different concentrations of azadirachtin (0.3 to 0.04 %), the IGR activity was found to be dose dependent. Thus at 0.3 and 0.06 % dose level, 30 and 25 % of larval mortality and 70 and 45 % of insect growth inhibition activity was respectively recorded. This dose dependent action was further increased following addition of curcumin-I. Of the three proportions, IGR activity of azadirachtin : curcumin-I (3:1 combination) was a shade better than azadirachtin alone or azadirachtin curcumin-I 2:1 and 1:1 ratio. The adult

Table 26. Effect of feeding of azadirachtin and azadirachtin : curcumin-I mixture on development of third instar larvae of *S. obliqua* (N=20, R=3)

Conc. (%)	Larval mortality (%)	Larval-pupal /pupal-adult intermediates (%)	Pupal mortality (%)	Abnormal adults/adults dead (%)	Normal adults (%)	Moult inhibition (%)
Azadirachtin						
0.30	55	05	40	-	-	45
0.20	50	10	35	05	-	50
0.10	45	15	30	10	-	55
0.08	45	05	25	10	15	40
0.06	45	10	25	05	15	40
0.04	40	10	20	05	25	35
Azadirachtin : curcumin-I (1:1)						
0.30	60	-	40	-	-	40
0.20	55	-	40	05	-	45
0.10	50	15	20	-	-	50
0.08	45	10	20	10	15	40
0.06	45	15	20	05	15	40
0.04	45	05	10	05	35	20
Control	05	-	-	-	95	-

Table 27. Effect of feeding of azadirachtin and azadirachtin : curcumin-I mixture on development of third instar larvae of *S. obliqua* (N=20, R=3)

Conc. (%)	Larval mortality (%)	Larval-pupal /pupal-adult intermediates (%)	Pupal mortality (%)	Abnormal adults/adults dead (%)	Normal adults (%)	Moult inhibition (%)
Azadirachtin : curcumin-I (2:1)						
0.30	90	-	10	-	-	10
0.20	95	-	05	-	-	5
0.10	100	-	-	-	-	-
0.08	100	-	-	-	-	-
0.06	90	-	10	-	-	10
0.04	80	-	20	-	-	20
Azadirachtin : curcumin-I (3:1)						
0.30	100	-	-	-	-	-
0.20	100	-	-	-	-	-
0.1	100	-	-	-	-	-
0.08	100	-	-	-	-	-
0.06	95	-	05	-	-	05
0.04	90	-	10	-	-	10
Control	05	-	-	-	95	-

emergence was least in 3:1 proportion followed by 2:1 and 1:1 proportion (Table 28 and 29).

4.10.2 Antifeedant activity

Like insect growth inhibition, feeding inhibition activity of azadirachtin and azadirachtin curcumin-I mixture was also found to be dose dependent. As evident from the data (Tables 30 and 31) antifeeding activity was more pronounced in fifth instar than in third instar larvae. Thus at lower dose level of 0.04 %, antifeedant activity against third and fifth instar larvae was found to be 46 and 88.9 % respectively. In combination with curcumin-I, antifeeding activity of azadirachtin was further increased. Thus, of the three proportions, azadirachtin curcumin-I combination in 3:1 ratio provided the best antifeedant activity followed by 2:1 and 1:1 ratio. In all the dose levels, the corrected feeding inhibition ranged mostly between 90 to 100 %. Almost similar feeding inhibition behaviour of azadirachtin and azadirachtin curcumin-I mixture was attributed to the fact that curcumin-I itself had antifeeding activity of more than 80 % at 0.06 % dose level (Table 30 and 31).

It was concluded that unlike in third instar, larval mortality and moulting inhibition in fifth instar larvae was less due to the fact that older fifth instar larvae being more tolerant are able to resist the effect of toxicants. Further prolongation of instar duration after treatment led to moult disruption and mortality. The increased larval and pupal mortality in both third and fifth instar larvae was attributed to the fact that treated larvae

Table 28. Effect of feeding of azadirachtin and azadirachtin : curcumin-I mixture on development of fifth instar larvae of S. *obliqua* (N=20, R=3)

Conc. (%)	Larval mortality (%)	Larval-pupal /pupal-adult intermediates (%)	Pupal mortality (%)	Abnormal adults/adults dead (%)	Normal adults (%)	Moult inhibition (%)
Azadirachtin						
0.30	30	20	50	-	-	70
0.20	25	35	30	-	10	65
0.10	30	10	30	10	20	50
0.08	25	10	20	20	25	50
0.06	25	05	25	10	35	40
Azadirachtin : curcumin-I (1:1)						
0.30	35	10	30	15	10	55
0.20	30	10	30	15	15	55
0.10	30	10	20	15	25	45
0.08	25	05	15	10	45	30
0.06	25	05	10	10	55	25
Control	05	-	-	-	95	-

Table 29. Effect of feeding of azadirachtin and azadirachtin : curcumin-I mixture on development of fifth instar larvae of *S. obliqua* (N=20, R=3)

Conc. (%)	Larval mortality (%)	Larval-pupal /pupal-adult intermediates (%)	Pupal mortality (%)	Abnormal adults/adults dead (%)	Normal adults (%)	Moult inhibition (%)
Azadirachtin : curcumin-I (2:1)						
0.30	40	30	10	15	05	55
0.20	40	15	15	15	15	45
0.1	30	10	15	15	35	40
0.08	25	10	10	10	45	30
0.06	25	5	5	10	55	20
Azadirachtin : curcumin-I (3:1)						
0.30	40	20	30	10	0	60
0.20	40	15	20	15	10	50
0.10	35	20	15	10	20	45
0.08	30	20	05	05	40	30
0.06	30	15	05	05	45	25
Control	05	-	-	-	95	-

Table 30. Antifeedent activity of azadirachtin against third and fifth instar larvae of *S. obliqua* (N=20, R=3)

Conc. (%)	Third instar larvae		Fifth instar larvae	
	Feeding inhibition (%)	Corrected feeding inhibition (%)	Feeding inhibition (%)	Corrected feeding inhibition (%)
Azadirachtin				
0.30	90.0	81	100	100.0
0.20	82.5	69	100	100.0
0.10	70.0	52	98	83.0
0.08	70.0	52	100	100.0
0.06	70.0	52	95	88.9
0.04	65.0	46	95	88.9
Curcumin-I				
0.30	97	93.5	98.5	96.7
0.20	97	93.5	98.0	95.6
0.10	95	89.5	97.0	93.5
0.08	95	89.5	95.5	90.5
0.06	92	83.7	90.0	80.0
Control	10	-	15.0	-

Table 31. Antifeedant activity of azadirachtin : curcumin-I mixture against third and fifth instar larvae of *S. obliqua*

Conc. (%)	Third instar larvae		Fifth instar larvae	
	Feeding inhibition (%)	Corrected feeding inhibition (%)	Feeding inhibition (%)	Corrected feeding inhibition (%)
Azadirachtin : curcumin-I (%)				
o.30	85	71.42	95	88.9
0.20	80	63.36	95	88.9
0.10	77	59.29	95	88.9
0.08	76	57.89	95	88.9
0.06	76	57.89	90	78.9
0.04	75	56.52	92	83.8
Azadirachtin : curcumin-I (2:1)				
0.30	100	100.0	100	100.0
0.20	100	100.0	100	100.0
0.10	100	100.0	100	100.0
0.08	98	95.6	95	88.9
0.06	98	95.6	95	88.9
0.04	96	91.6	92	83.8
Azadirachtin : curcumin-I (3:1)				
0.30	100	100.0	100	100.0
0.20	100	100.0	100	100.0
0.10	100	100.0	100	100.0
0.08	100	100.0	100	100.0
0.06	98	95.6	98	95.4
0.04	95	89.5	96	91.0
Control	10	-	15	-

or survived pupae were unable to shed their cuticle during moulting and died subsequently. The apparent enhancement of azadirachtin activity in the presence of curcumin-I could be related to the fact that curcumin-I might exert additive or stablizing effect leading to increased bioavailability of azadirachtin to the target insect.

SUMMARY AND CONCLUSIONS

Being environmentally benign, secondary metabolites of neem tree having high degree of structural complexity like azadirachtin, are the focus of attention because of their increasing use in pest control. Because of the negative influence of various environmental factors such as UV and sunlight, residual life of azadirachtin based neem products is limited to few hours/days. It is therefore, necessary to photostabilize azadirachtin based products to increase their residual life and efficacy. Since synthetic stabilizers are likely to be unsafe, studies have been conducted to explore the possibility of stabilizing azadirachtin and azadirachtin rich neem oil with naturally occurring stabilizers such as curcumins and turmeric oil isolated from *Curcuma longa* rhizomes. The study incorporates i) isolation of azadirachtin, ii) isolation of curcumins in pure form, iii) synthesis of methyl and butyl ether analogues of curcumin-I, iv) isolation and identification of possible photoproducts of curcumin-I and v) assessing the impact of various turmeric based products on stability and efficacy of azadirachtin. The salient features of the research findings are summerised below.

1. From neem seed kernel extract, light cream coloured azadirachtin has been isolated following Schroeder and Nakanishi (1987) method. It was found to be 45% pure by its comparison with the authentic sample of 50 % purity.

2. Turmeric rhizome powder was extracted with petroleum ether (40-60°) in Soxhlet apparatus to obtain turmeric oil. The

deoiled powder on further extraction with benzene yielded a dark brown oily mass which was separated by column chromatography/ prep TLC into three pure components. On the basis of spectral evidences (^{1}H NMR, FTIR and MS) three components were indentified as curcumin-I, curcumin-II and curcumin-III.

3. Curcumin-I, the major yellow pigment was further converted to its methyl and butyl analogues by treatment with methyl iodide/butyl bromide.Their structures were confirmed with IR, ^{1}H NMR and elemental analysis.

4. When subjected to UV-light radiation, methanolic solution of curcumin-I gave number of photoproducts which after methylation with CH_2N_2 were identified as i) 4(3,4-dimethoxyphenyl) but-3-ene-2-one, ii) 3,4-dimethoxy cinnamic acid methyl ester, iii) methyl (3,4-dimethoxy) benzoate and iv) veratraldehyde.

5. Under the impact of UV-light, methanolic solution of azadirachtin degraded faster than as thin film on glass surface. The low concentration of recovered azadirachtin was attributed to the fact that azadirachtin was probably converted to a mixture of more polar materials from which no azadirachtin could be detected. Under sunlight irradiated conditions, methanolic solution of azadirachtin or its thin film on glass surface degraded comparatively slowly than in UV-light. Its half life ($t_{1/2}$) in solution and as thin film on glass surface were found to be 1.83d and 1.61d respectively.

On leaf surface the degradation was faster and its half life ($t_{1/2}$) was found to be 0.73 day. The faster degradation of azadirachtin on leaf surface was attributed to i) possible moisture content on leaf surface, ii) absorption/translocation of azadirachtin by leaves, iii) loss due to wind current and iv) chlorophyll sensitized phototransformations. In methanoic solution, photodegradation followed pseudo-first order kinetics, whereas, as thin film on glass and leaf surface it followed first order kinetics.

6. Since curcumin-I absorbs in UV and visible region, it is quite sensitive to both UV and visible light. Under the impact of UV-light, methanolic solution of curcumin-I degraded faster than its thin film on glass surface. Its half life ($t_{1/2}$) in methanolic solution and as thin film on glass surface was found to be 1.5 and 11.04 h respectively. The degradation followed pseudo-first order and first order kinetics in solution and as thin film on glass surface respectively. Under sunlight conditions, the half life of curcumin-I in methanolic solution and as thin film on glass and leaf surface was 4.77, 4.08 h and 0.73d respectively. The quick degradation of curcumin-I under sunlight condition is again due to the fact that volatilization losses in outdoor experiment (sunlight) are more than in experiments conducted indoor (UV-light). The degradation in thin film on either glass or leaf surface followed first order kinetics.

7. Photodegradation of three curcumins and two curcumin-I derivatives (methyl and butyl analogues) in combination with

azadirachtin (1:1) was more in solution than as thin film on glass surface.

8. In solution as well as in thin film on glass surface, azadirachtin content in azadirachtin : curcumin-I mixture (1:1, 2:1 and 3:1) was found to be highest in 1:1 followed by 2:1 and 3:1 proportions. Similar trend was observed with azadirachtin : curcumin-II and azadirachtin : curcumin-III mixtures. Quick degradation of methanolic solution of azadirachtin in combination with curcumin-III was due to the fact that curcumin-III molecule is more susceptible to UV-light than curcumin-I or curcumin-II.

9. Since azadirachtin molecule could not be stabilized with trimethyl curcumin-I and/or dibutyl curcumin-I, it was inferred that presence of free -OH group adjacent to $-OCH_3$ moiety in curcumin nucleus is important for imparting stability to azadirachtin molecule.

10. Under sunlight irradiated condition curcumin-I was able to stabilize azdirachtin upto seven days in solution phase (azadirachtin content 49.29%) and upto four days as thin film on glass surface (azadirachtin content 30 %).

11. Azadirachtin spiked neem oil in solution phase was more stable to sunlight than azadirachtin alone indicating that neem oil is capable of stabilizing azadirachtin content in the mixture. Similarly azadirachtin in azadirachtin : turmeric oil mixture was also stabilized and the extent of stabilization was more or less same as was achieved with neem oil. In the

presence of neem oil and turmeric oil, azadirachtin in solution phase did not degrade as rapidly as it would have degraded in their absence.

12. Of the various combinations, turmeric oil and neem oil mixture was found to be the best for stabilizing azadirachtin in solution phase. Unlike azadirachtin which under sunlight conditions degraded within 6 days to 9.49 % of its initial concentration in solution, turmeric oil-neem oil mixture could stabilize azadirachtin considerably as after 6 days 75.42 % of azadirachtin was still detectable. The extent of stabilization was more than either with neem oil or turmeric oil alone.

13. Under sunlight irradiated conditions disappearence of azadirachtin in various combinations (1:1, 2:1, 3:1) with neem oil, turmeric oil or neem oil-turmeric oil mixture was faster in thin film on glass/leaf surface than in solution phase. The rapid decrease in azadirachtin content was attributed mainly to vollatilization of turmerones, the chief constituent of turmeric oil.

14. Insect growth and moulting inhibition activity of azadirachtin : curcumin mixture (1:1, 2:1, 3:1) against 3rd instar larvae of *Splosoma obliqua* was better than azadirachtin alone. The dose dependant action was more pronounced in 3:1 followed by 2:1 and 1:1 proportion. At lower dose of 0.04 %, azadirachtin : curcumin-I mixture (2:1, and 3:1) imparted 80-90 % larval mortality and 10-20 % moulting abberations during pupal and adult emergence.

Against 5th instar larvae, the ecdysis inhibition activity was further enhanced following addition of curcumin-I. Here again, IGR activity was a shade better in 3:1 followed by 2:1 and 1:1 proportion. Unlike in the third instar larvae, mortality and moulting inhibition in the fifth instar larvae was less due to the fact that older fifth instar larvae being more tolerant are able to resist the effect. Prolongation of instar duration, delayed lethal effect and moulting abnormalities of azadirachtin with/without curcumins were attributed mainly to their interference with insect endocrine system.

15. Like insect growth inhibition activity, anti-feedant activity of azadirachtin and azadirachtin : curcumin-I mixtures were dose dependant. Following incorporation of curcumin-I, antifeedant activity of azadirachtin was further increased. The combination 3:1 provided the best anti-feedant activity. Since curcumin-I also exhibit antifeedant activity (>80% at 0.06% dose level), in combination with azadirachtin it presumably exert additive or stabilizing effect leading to increased bioavailabilitly of azadirachtin to the target pest.

It is thus apparent that turmeric products particularly curcumin-I and turmeric oil not only induce photostability but also cause significant enhancement in insect moulting inhibition and anti-feedant action of azadirachtin against 3rd/5th instar larvae of *S. obliqua*. Thus turmeric products can be effectively utilized to develop photostable azadirachtin based neem formulations for pest control.

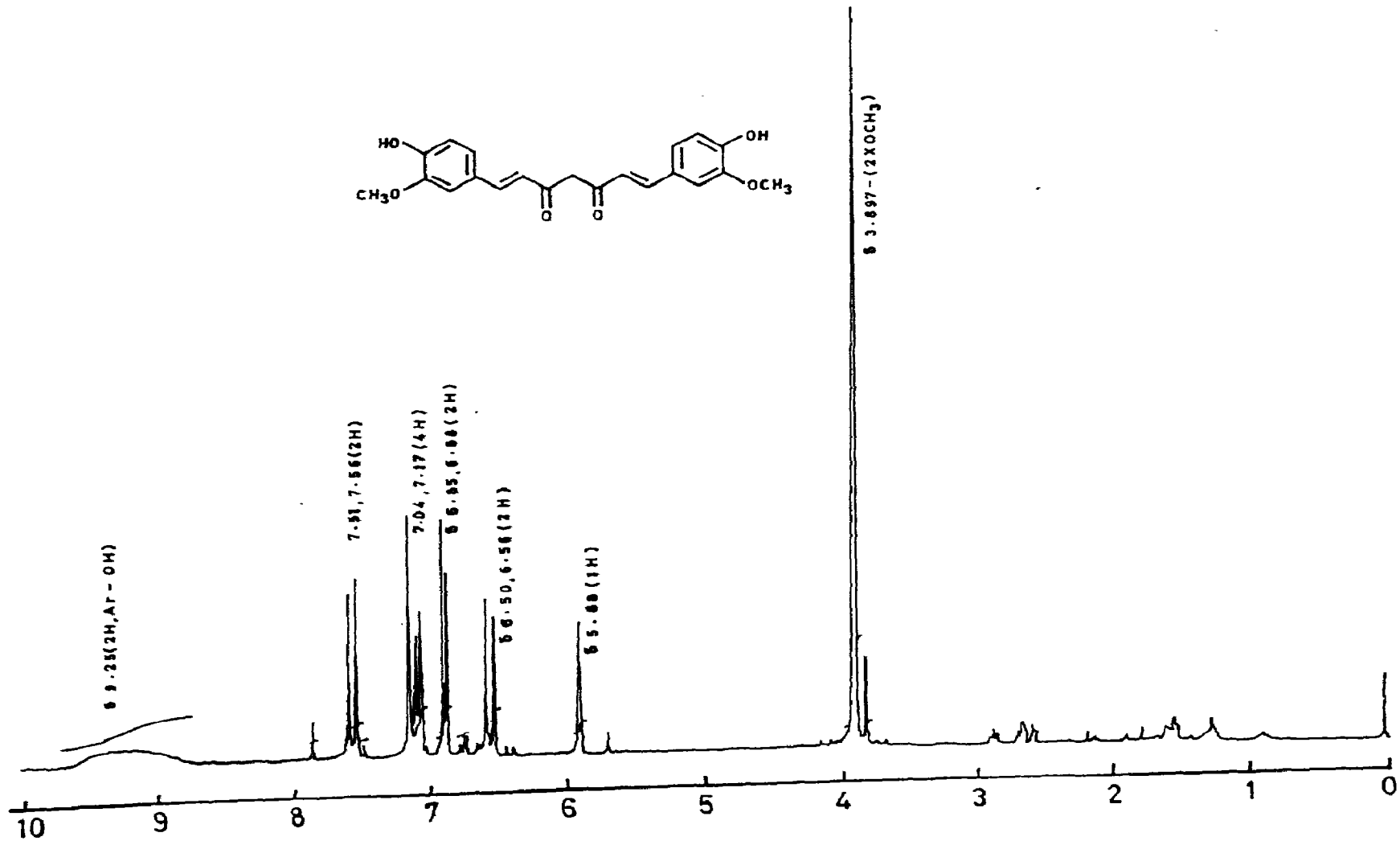


Fig. 26: ^1H NMR spectrum of curcumin – I

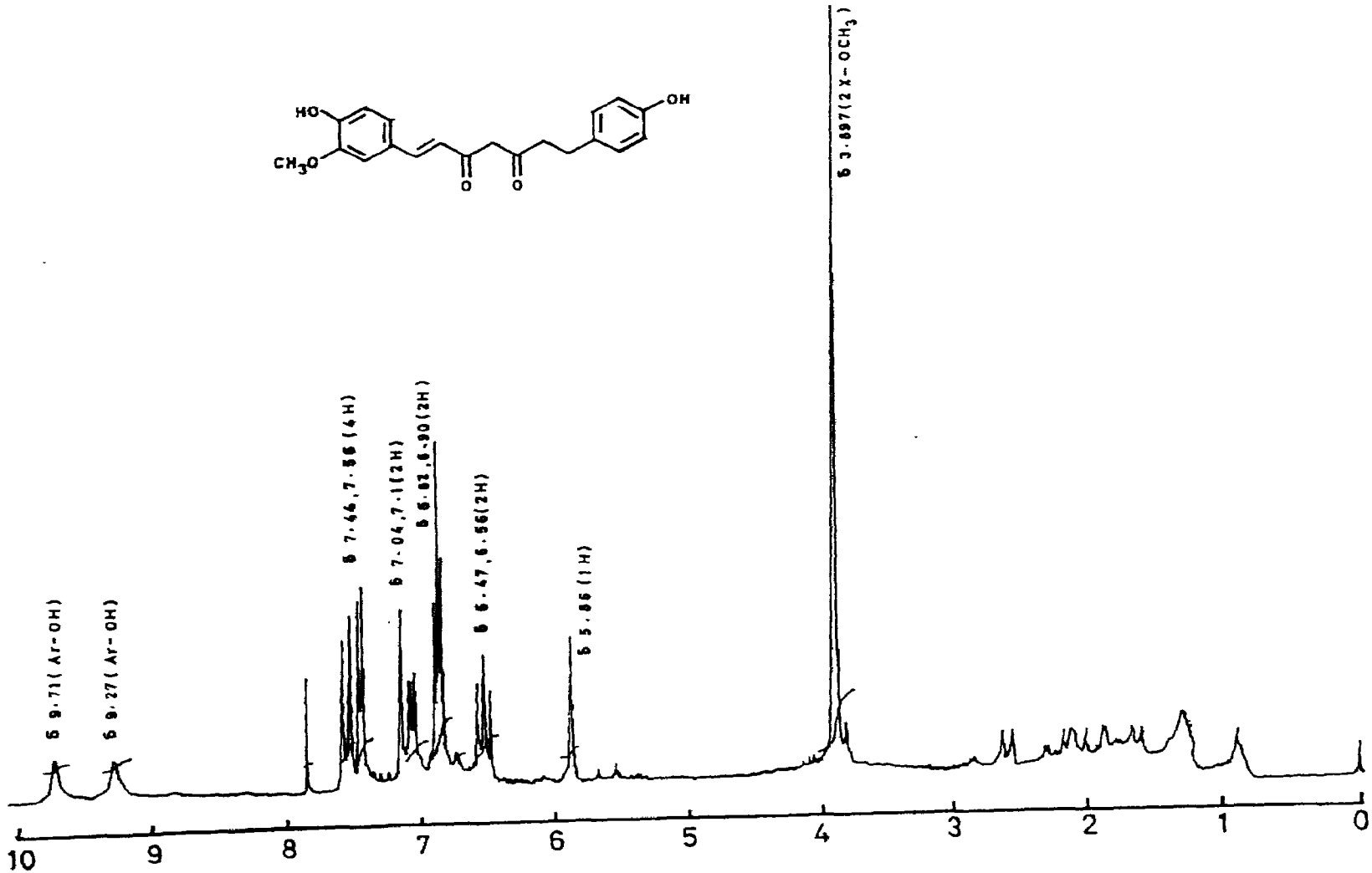


Fig . 27 : ^1H NMR spectrum of curcumin - II

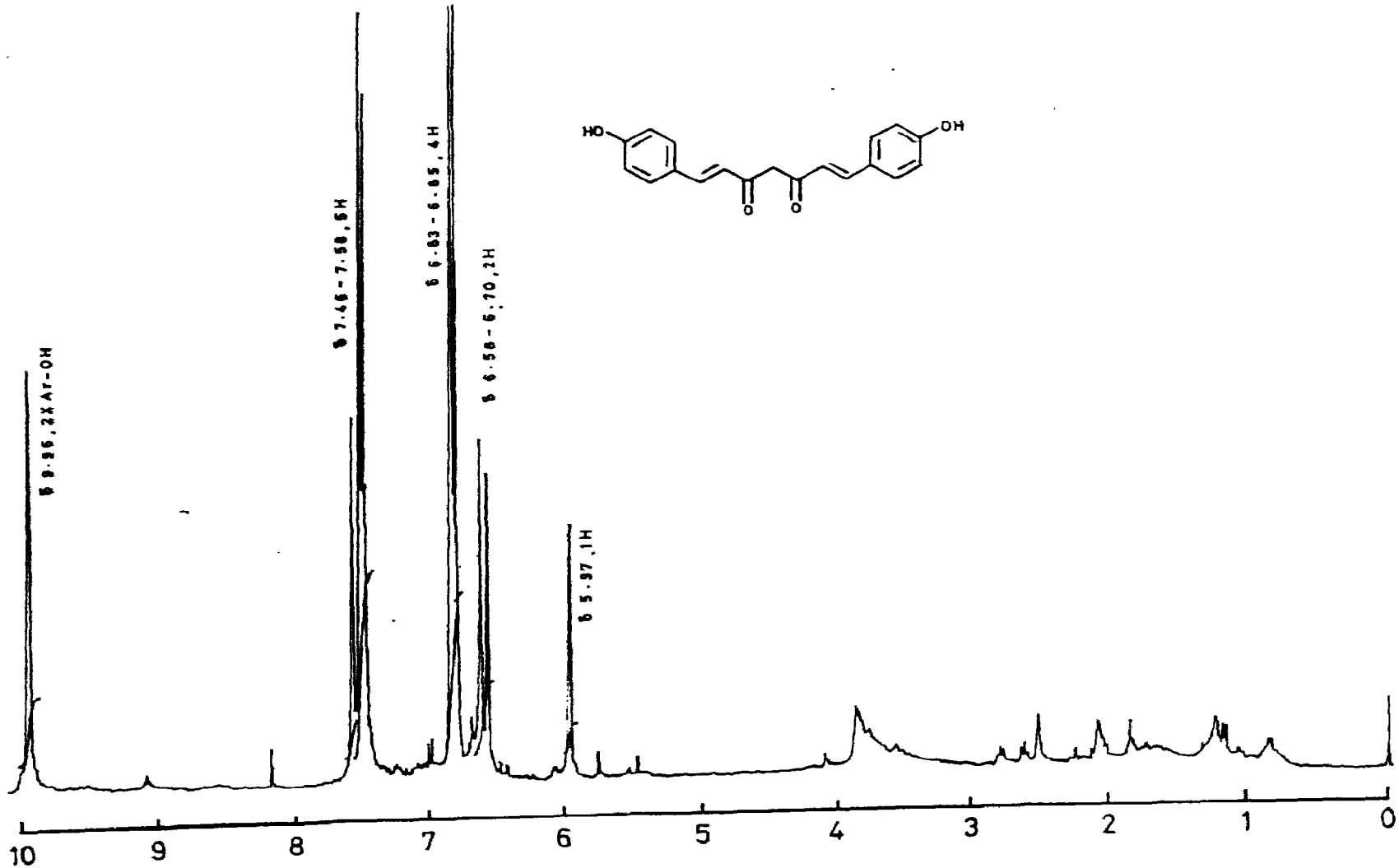


Fig. 28 : ^1H NMR spectrum of curcumin – III

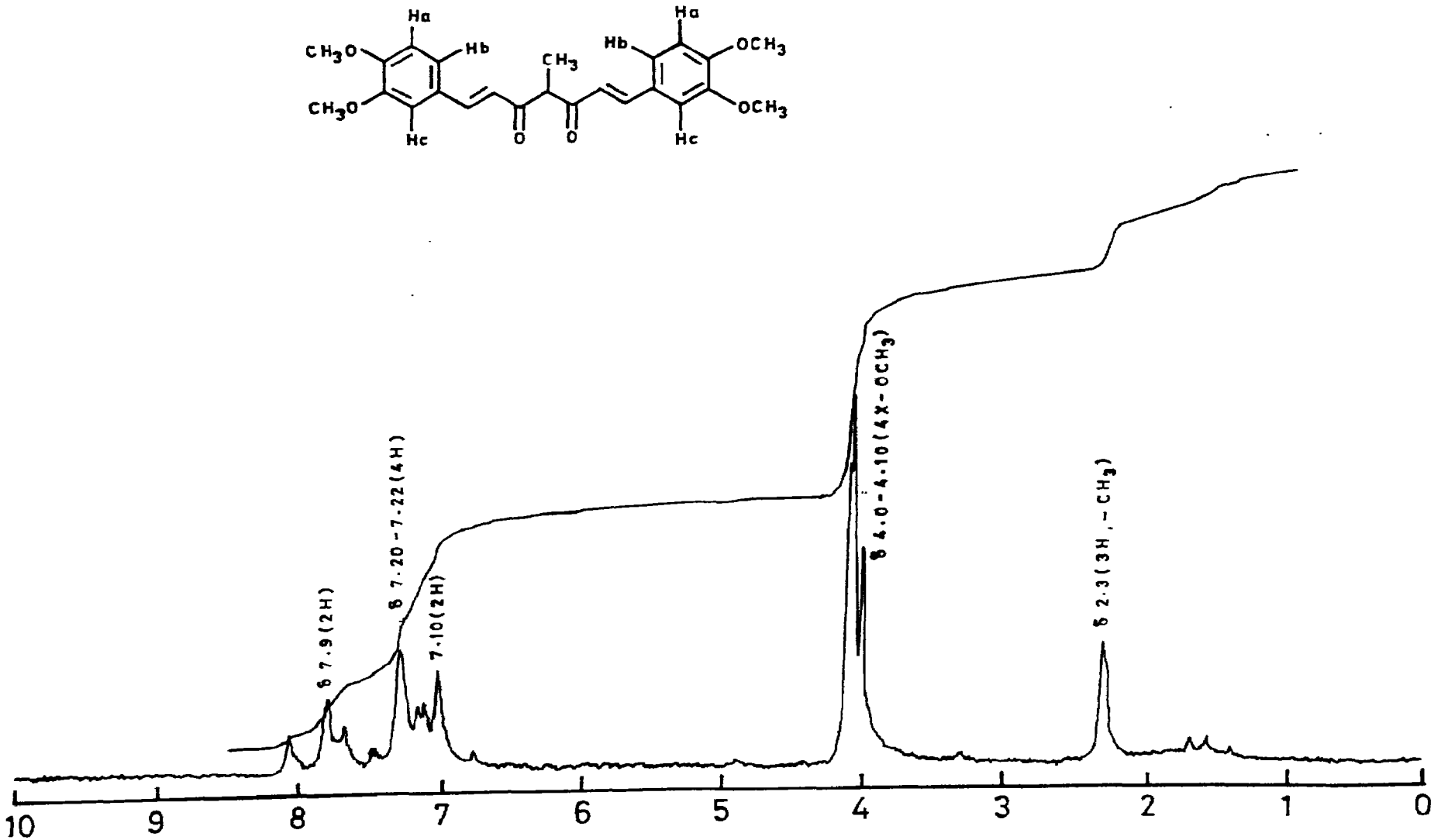


Fig. 29: ^1H NMR spectrum of trimethyl curcumin - I

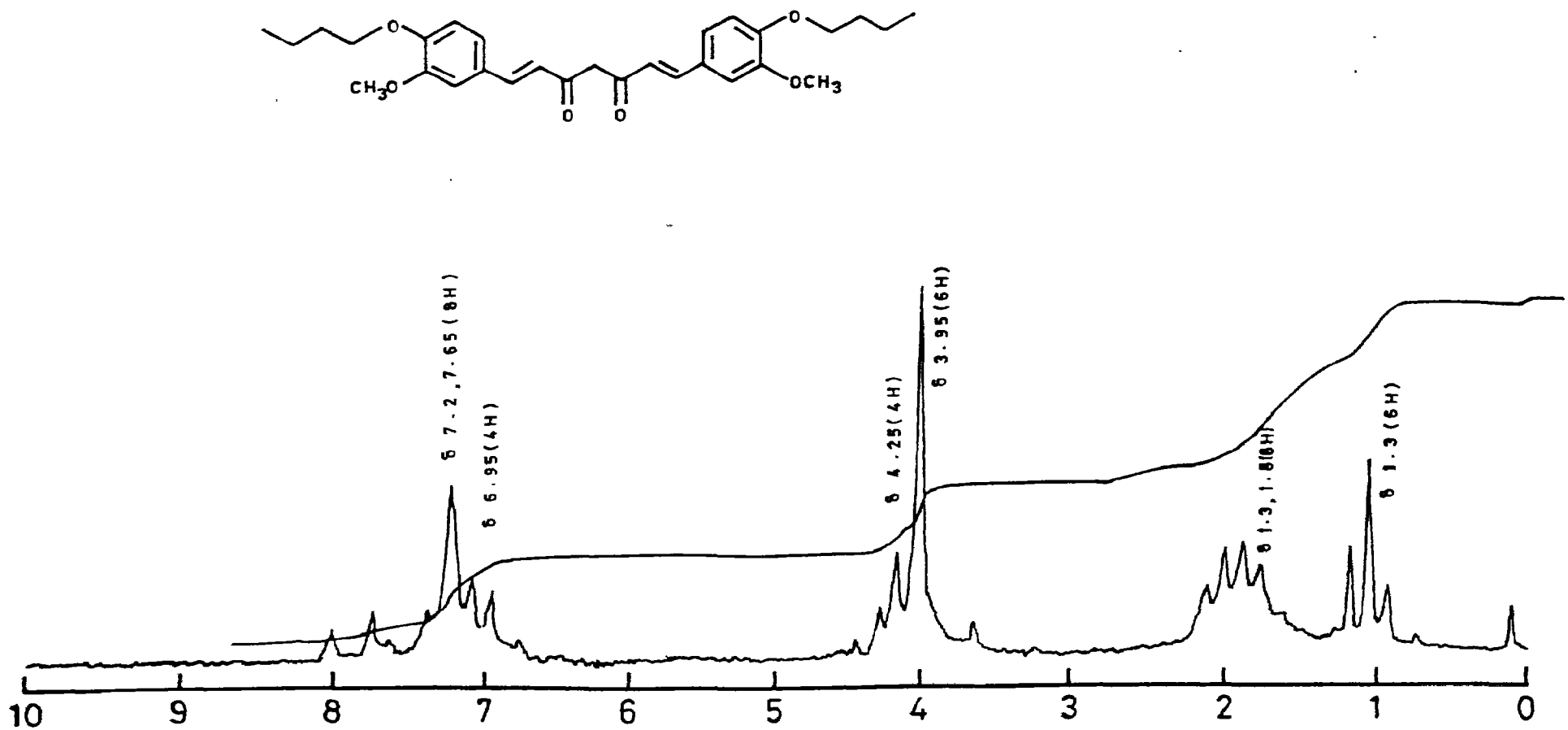


Fig. 30: ^{1}H NMR spectrum of dibutyl curcumin – I

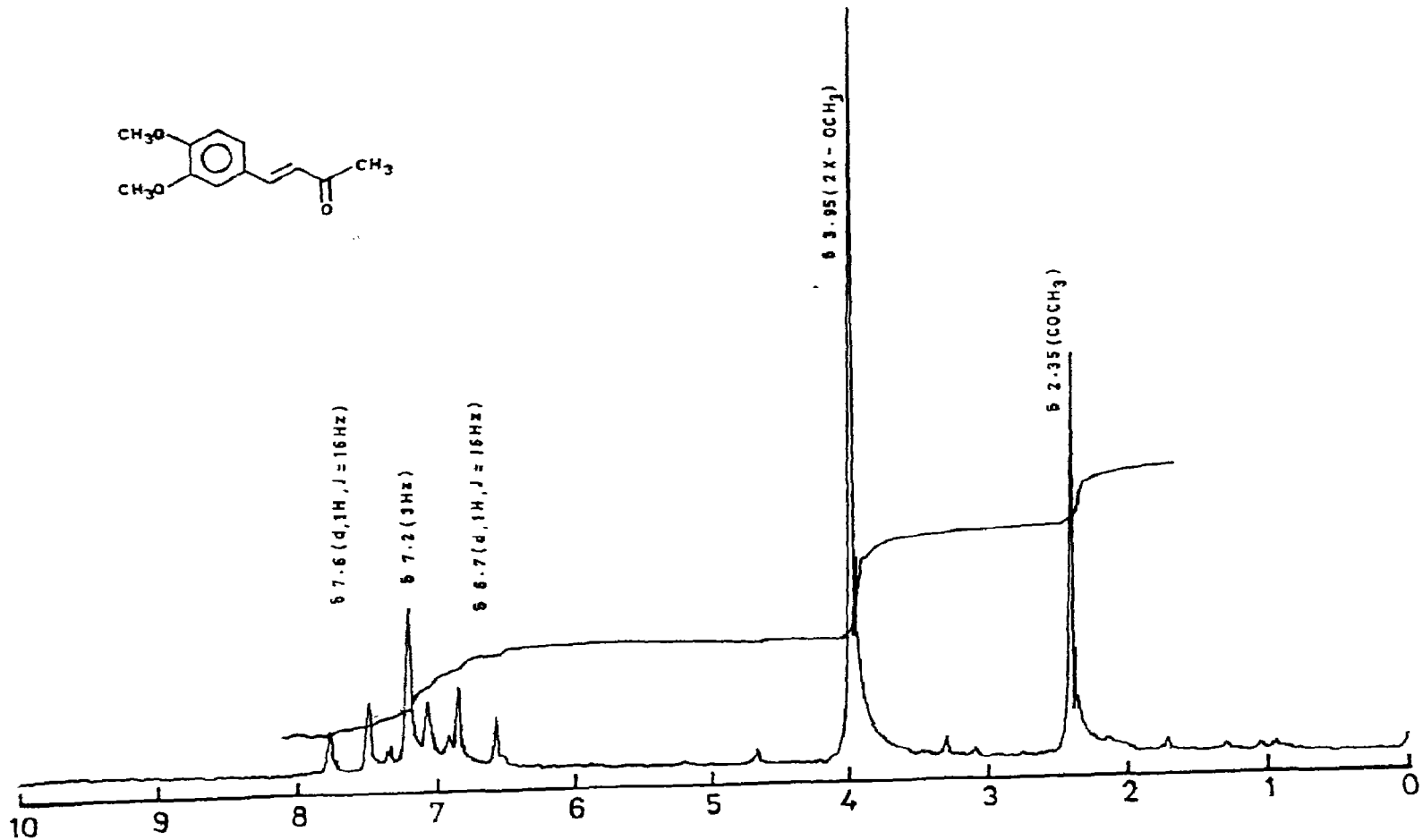


Fig. 31 : ^{1}H NMR spectrum of 3,4 – dimethoxy cinnamoyl methane.

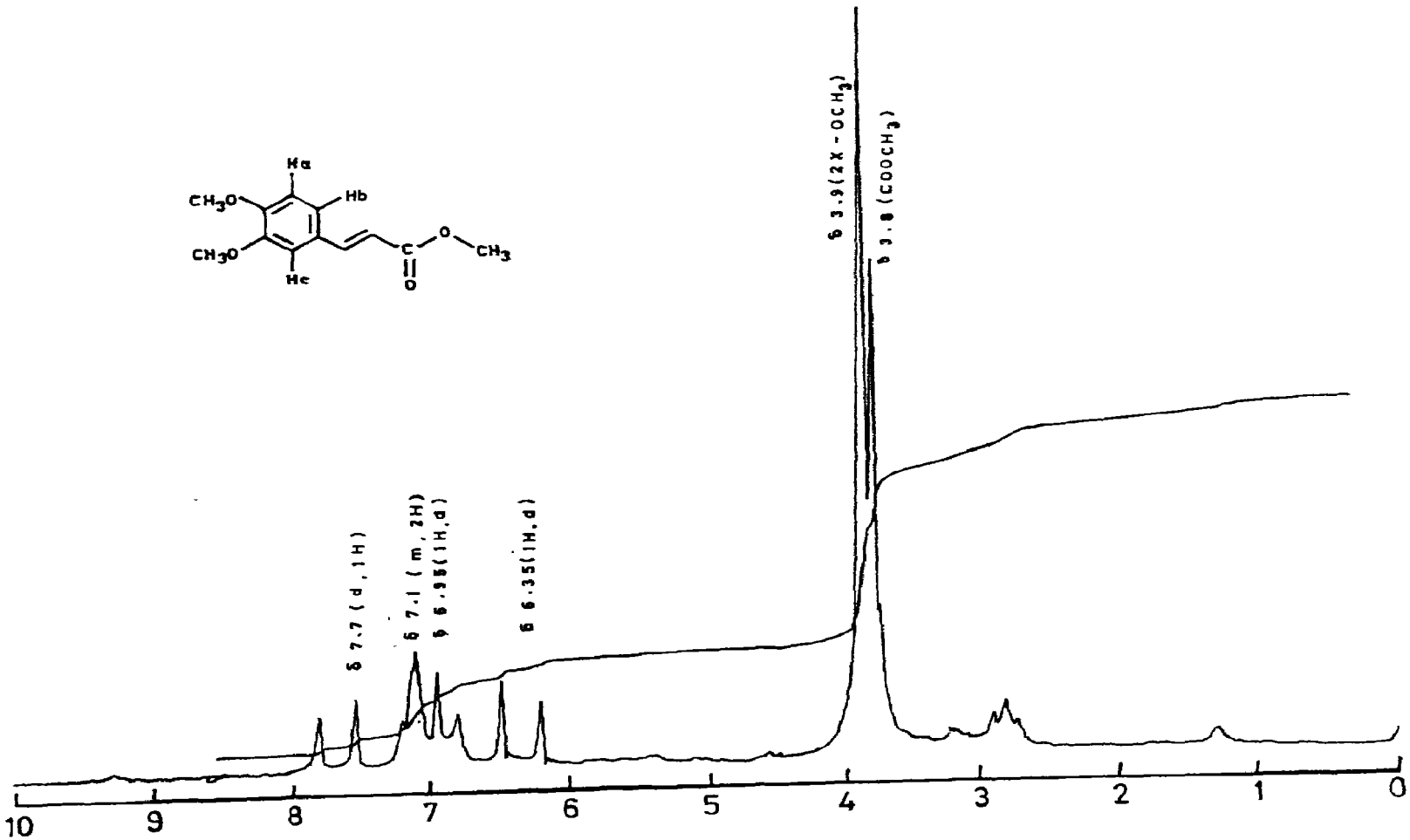


Fig. 32: ^1H NMR spectrum of 3, 4 - dimethoxy cinnamic acid methyl ester.

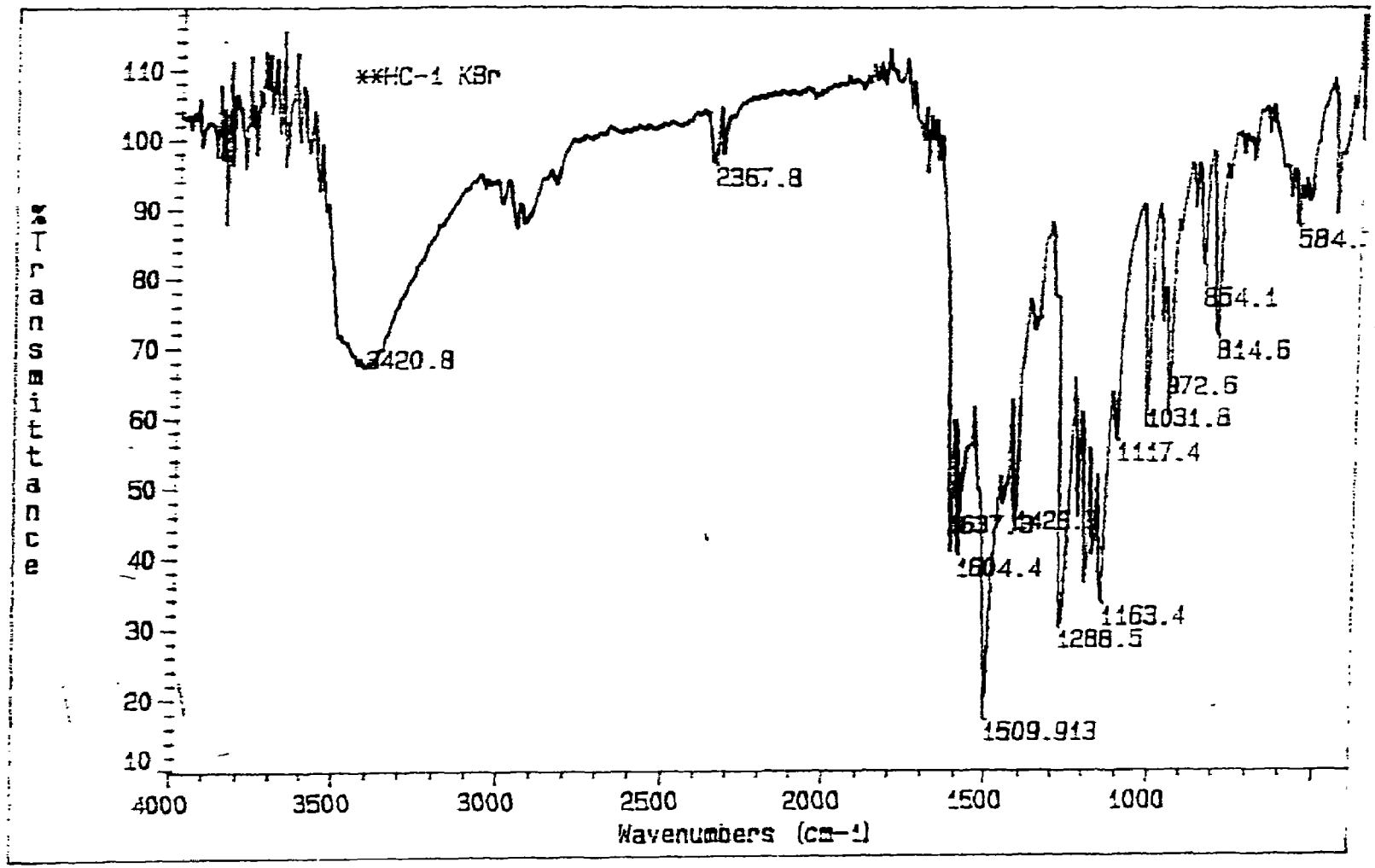


Fig. 38 : FT-IR spectrum of curcumin - I

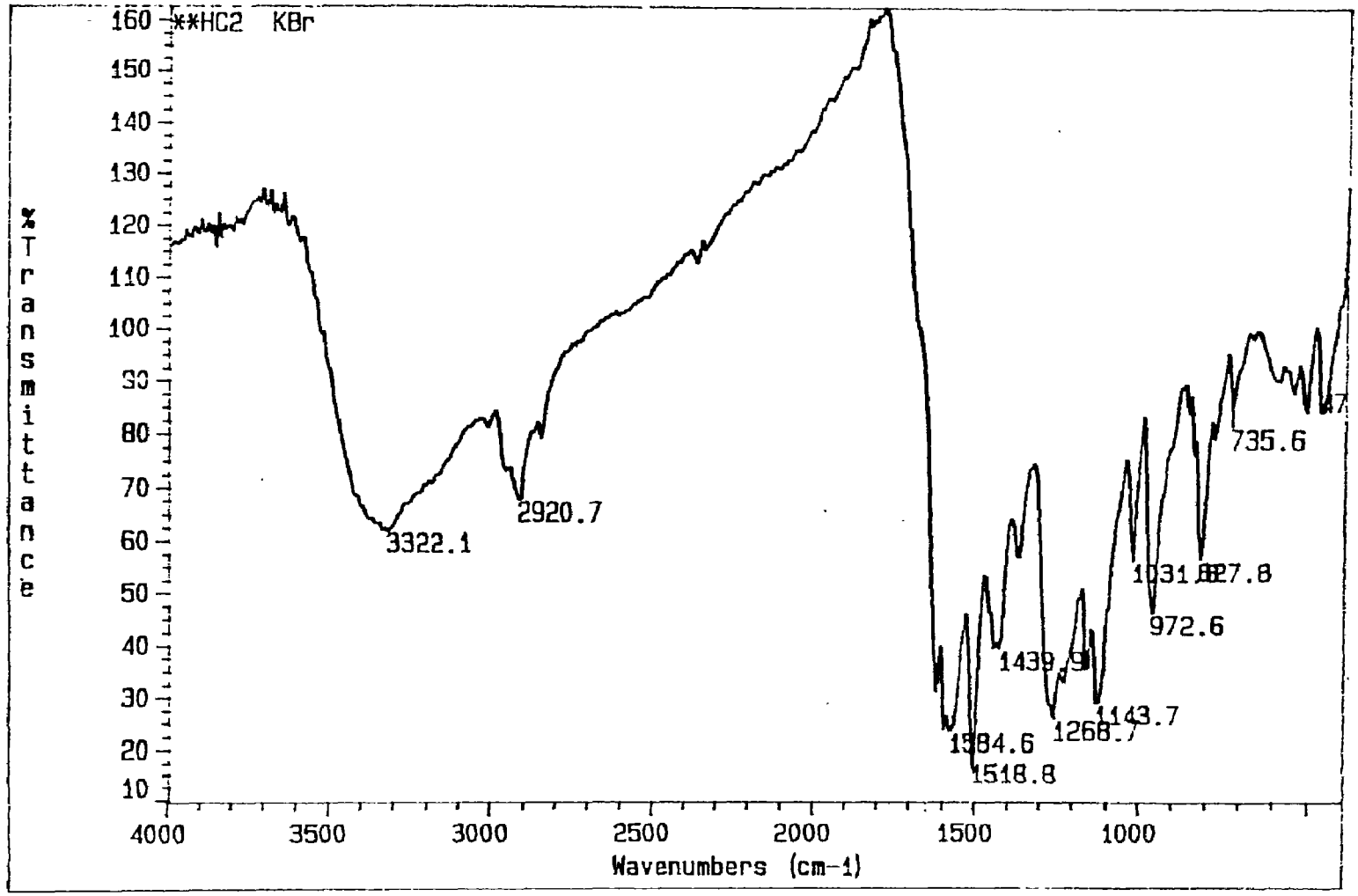


Fig. 34 : FT - IR spectrum of curcumin - II

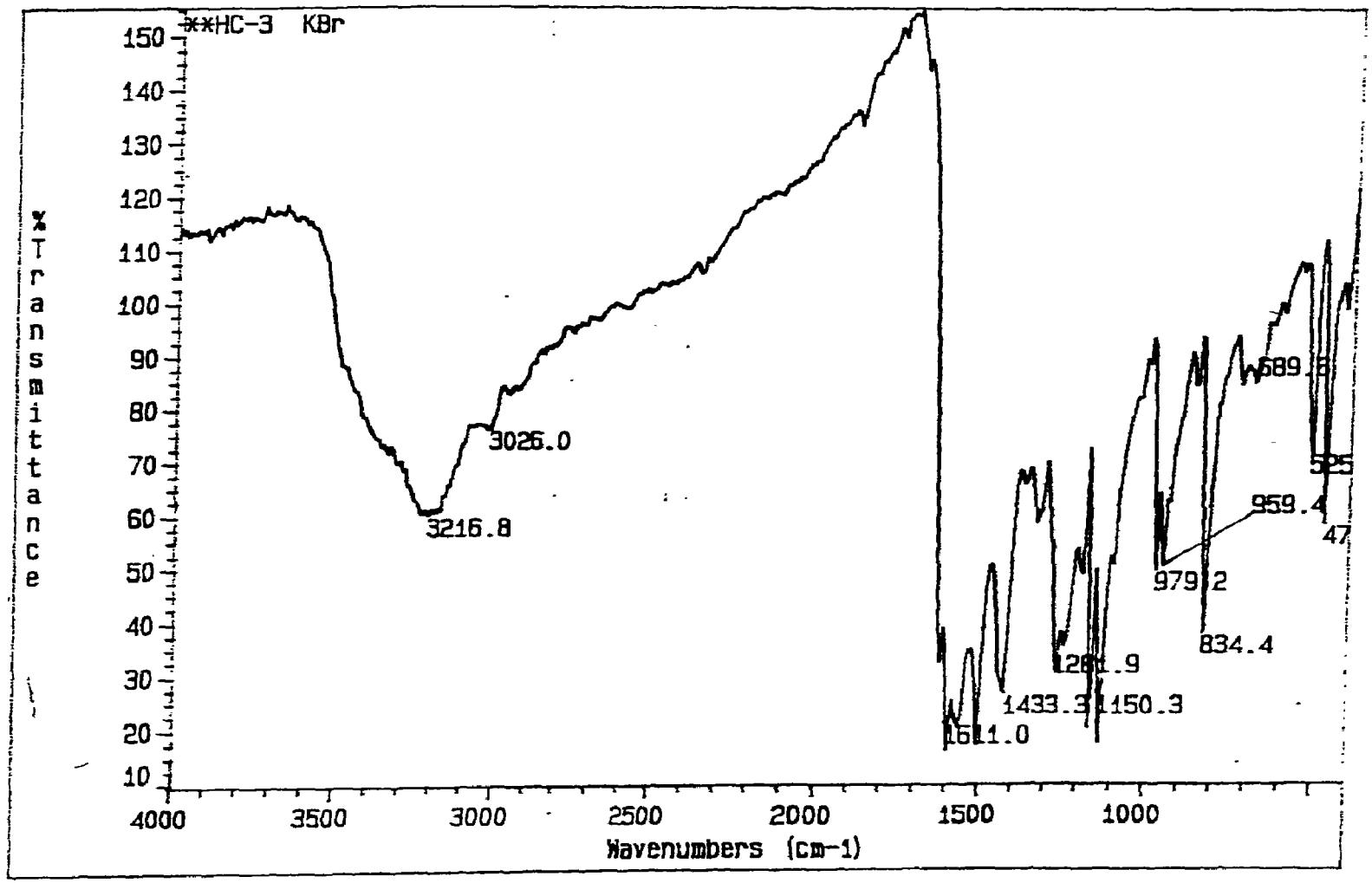


Fig. 35: FT-IR spectrum of curcumin-III

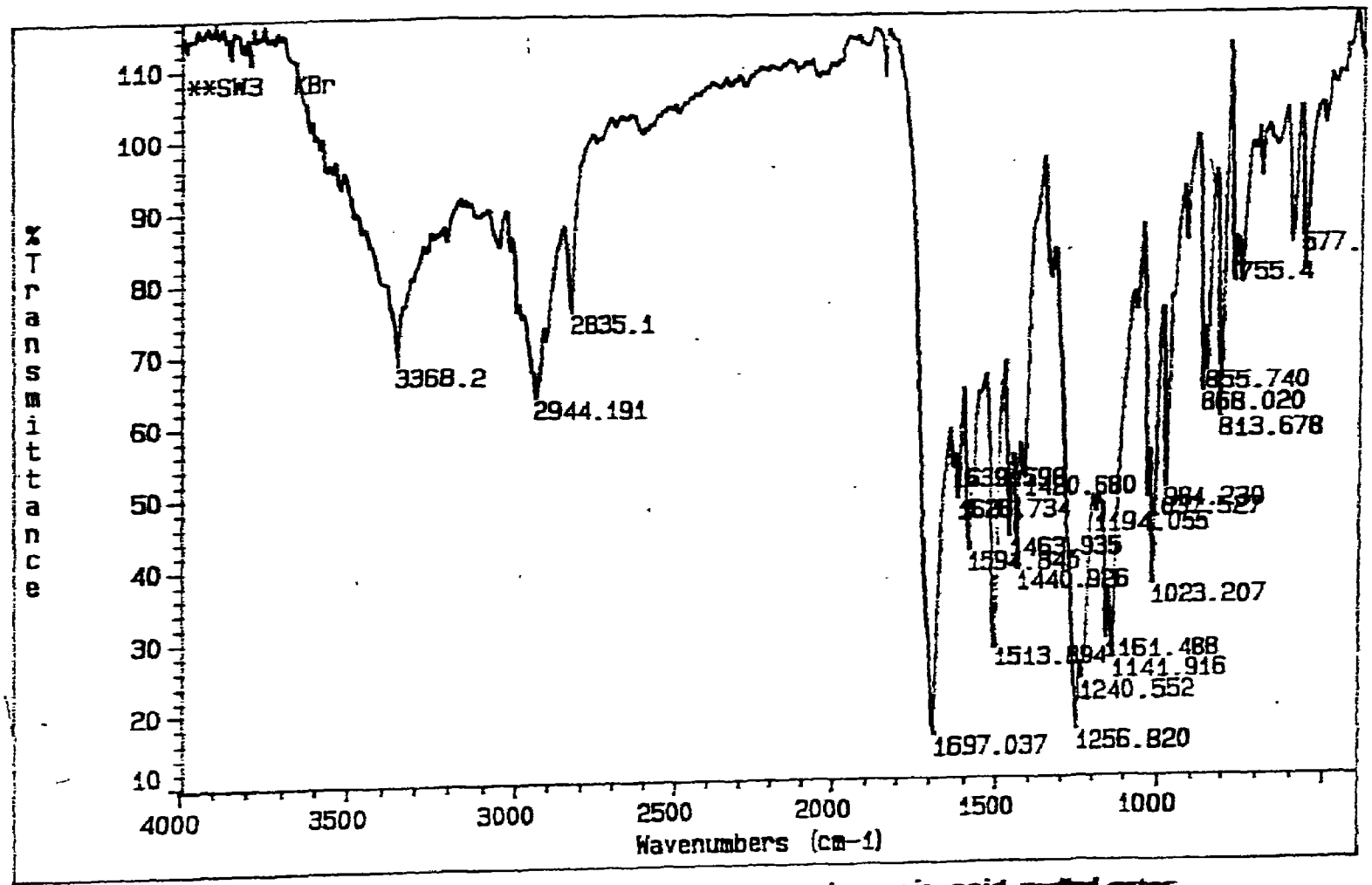
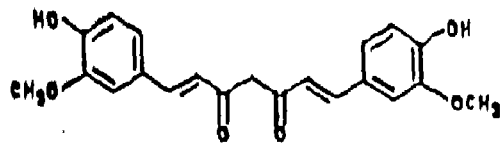


Fig. 36 : FT-IR spectrum of 3,4-dimethoxy cinnamic acid methyl ester

HP 5980 series
C:\VECTOR2\DATA\26749.TKF
Acquired on Fri Aug 30 18:24:53 1996
Sample RHC-1, Vol 1 Dil 1 Group 0
Probe of sample RHC-1 through 50-650
Instrument Parameters:
StartInjection
Scan Parameters:
SCAN every 1 secs for 30 min
50-650



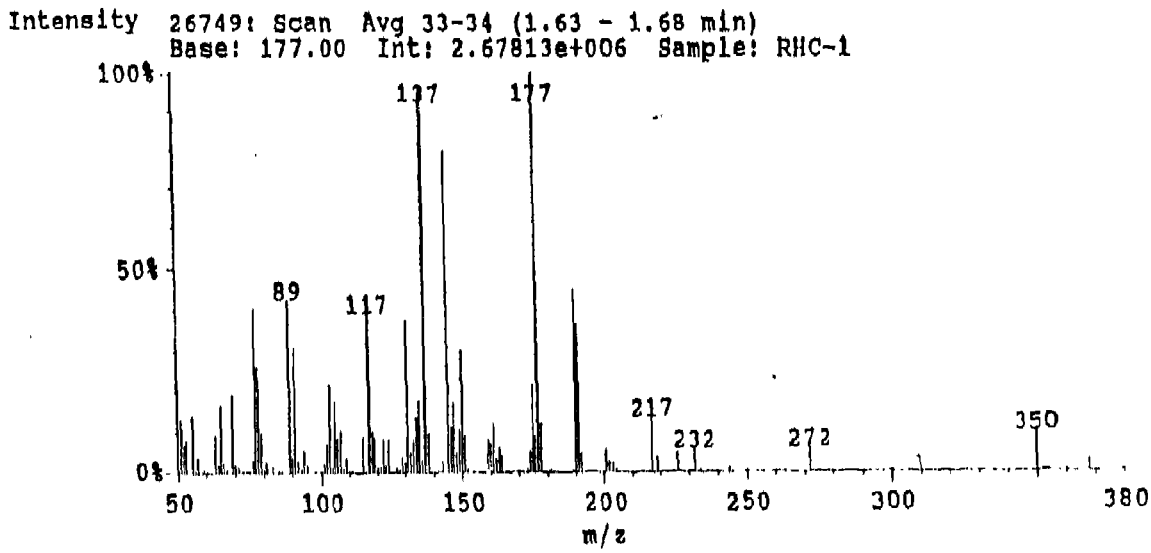
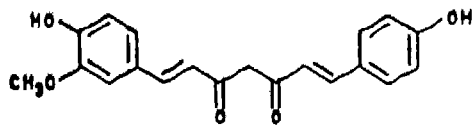


Fig. 37 : MS spectrum of curcumin - 1

HP 5980 series
C:\VECTOR2\DATA\RR009.TKF
Acquired on Tue Sep 17 17:31:47 1996
Sample Sample RHC-2, Vol 1 Dil 1 Group 0
EI SCANNED 50-650AMU
Instrument Parameters:
StartInjection
Scan Parameters:
SCAN every 1 secs for 20 min
50-650



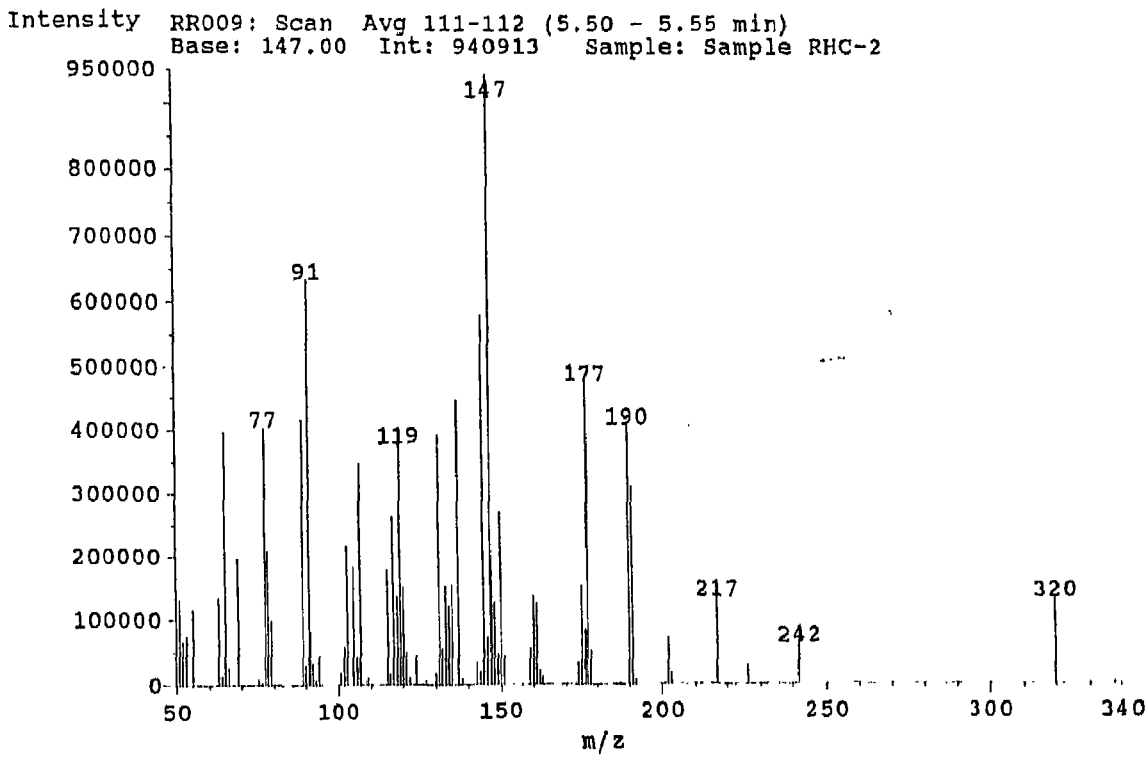
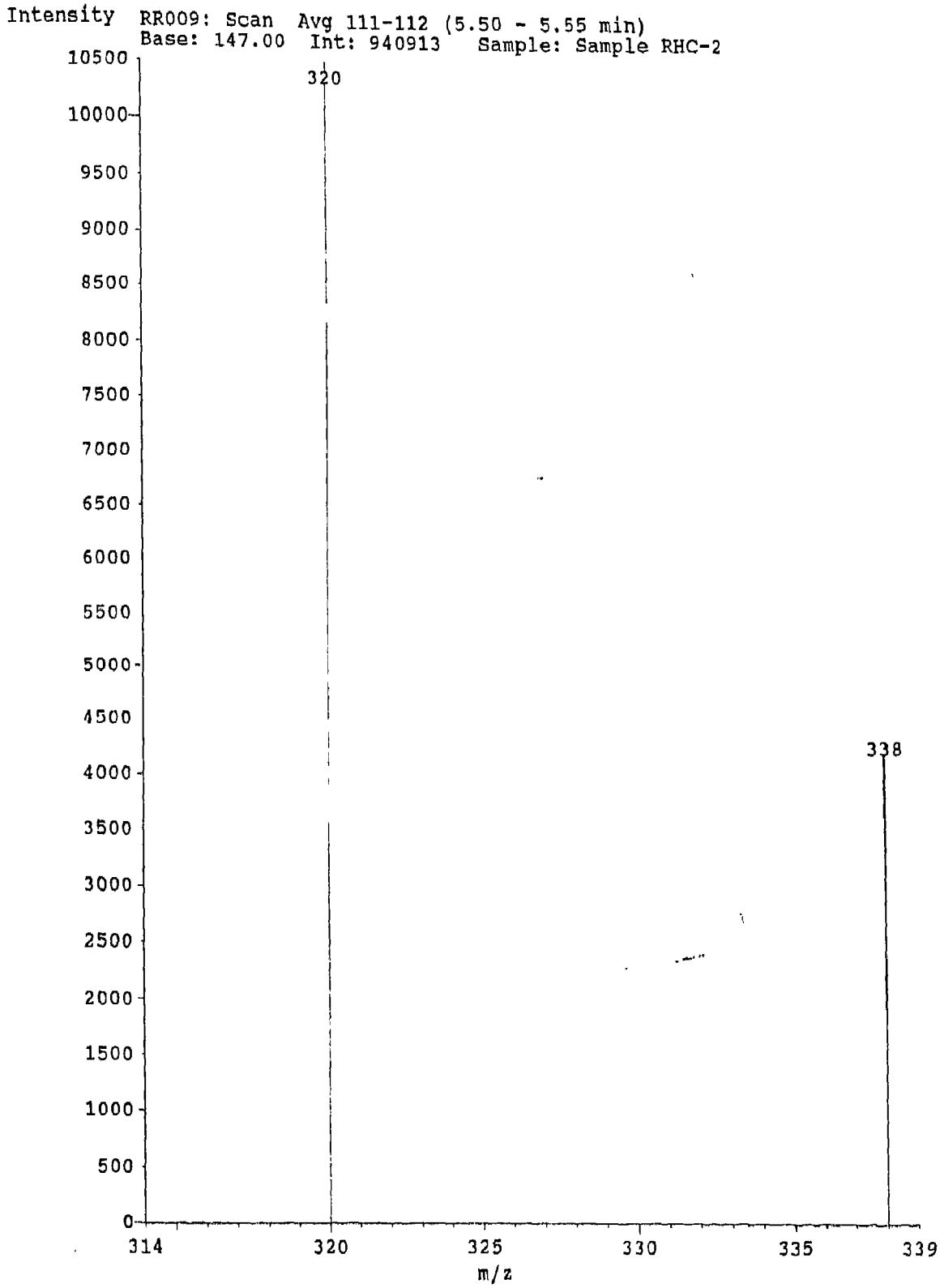
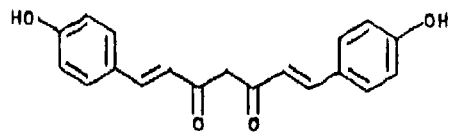


Fig. 38 : MS spectrum of curcumin - II


Intensity
RR009: Scan Avg 111-112 (5.50 - 5.55 min)
Base: 147.00 Int: 940913 Sample: Sample RHC-2
10500
10000
9500
9000
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
320
338
314
320
325
330
335
339
m/z

HP 5980 series
C:\VECTOR2\DATA\RR010.TKF
Acquired on Tue Sep 17 17:48:45 1996
Sample Sample RHC-3, Vol 1 Dil 1 Group 0
EI SCANNED 50-650AMU
Instrument Parameters:
StartInjection
Scan Parameters:
SCAN every 1 secs for 20 min
50-650



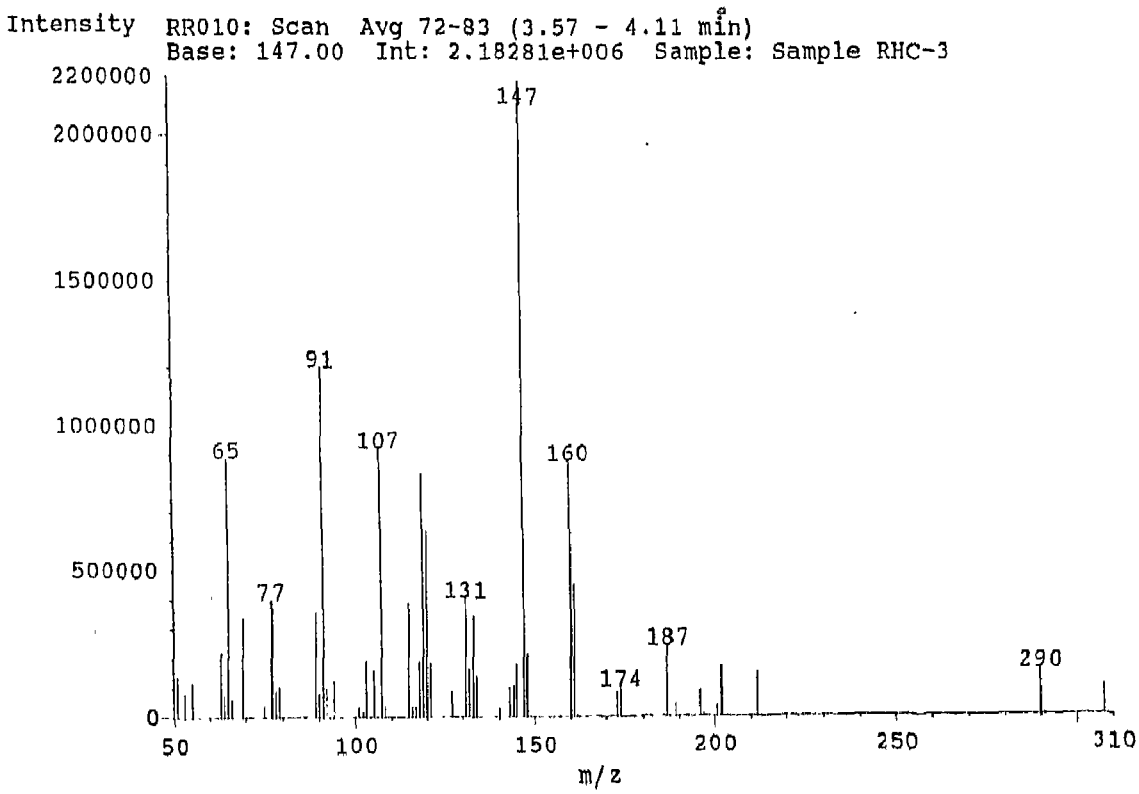
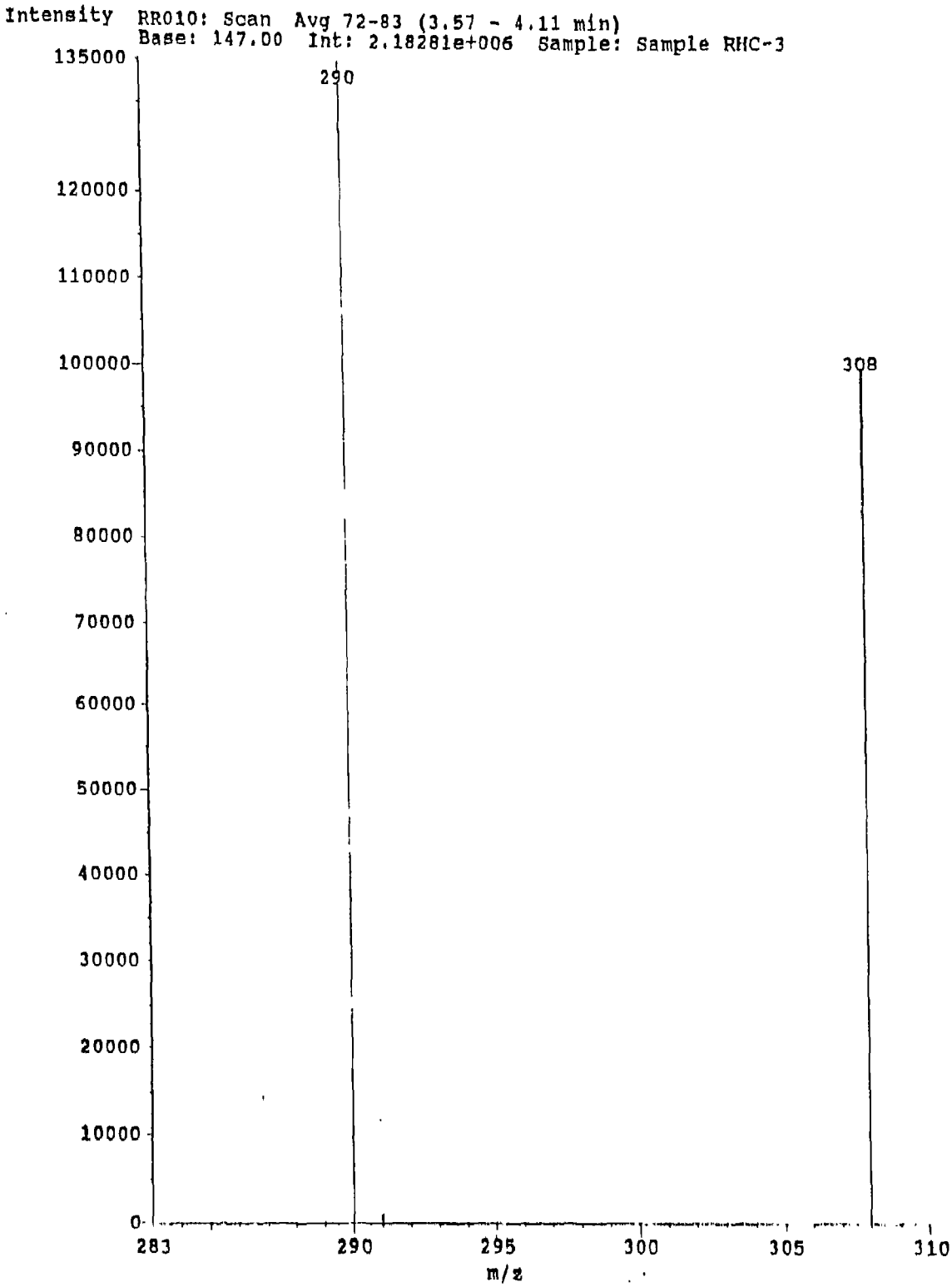


Fig. 39 : MS spectrum of curcumin -III


Intensity
RR010: Scan Avg 72-83 (3.57 - 4.11 min)
Base: 147.00 Int: 2.18281e+006 Sample: Sample RHC-3
135000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
290
308
283
290
295
300
305
310
m/z

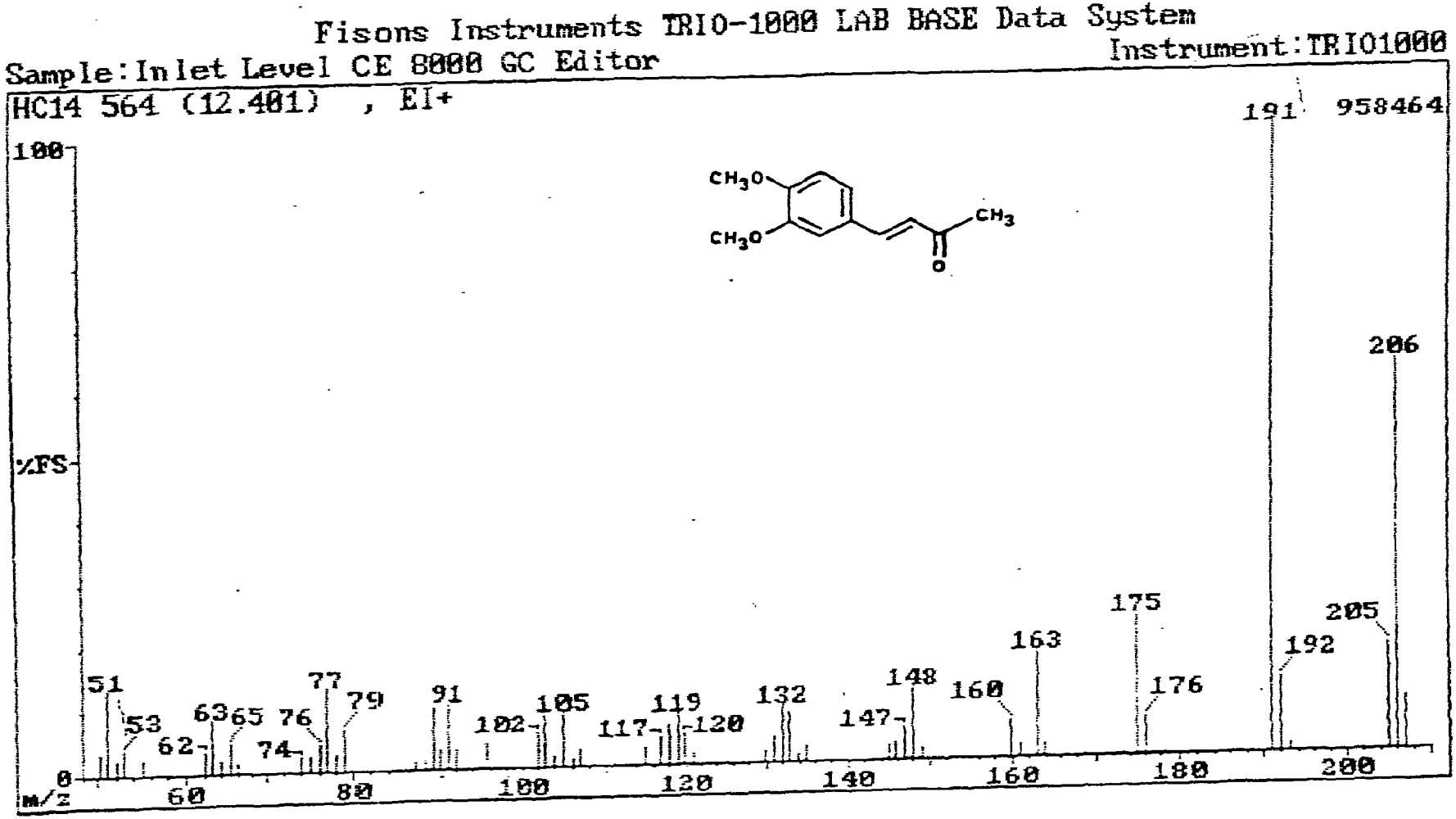


Fig. 40 : MS spectrum of 3,4-dimethoxy cinnamoyl methane

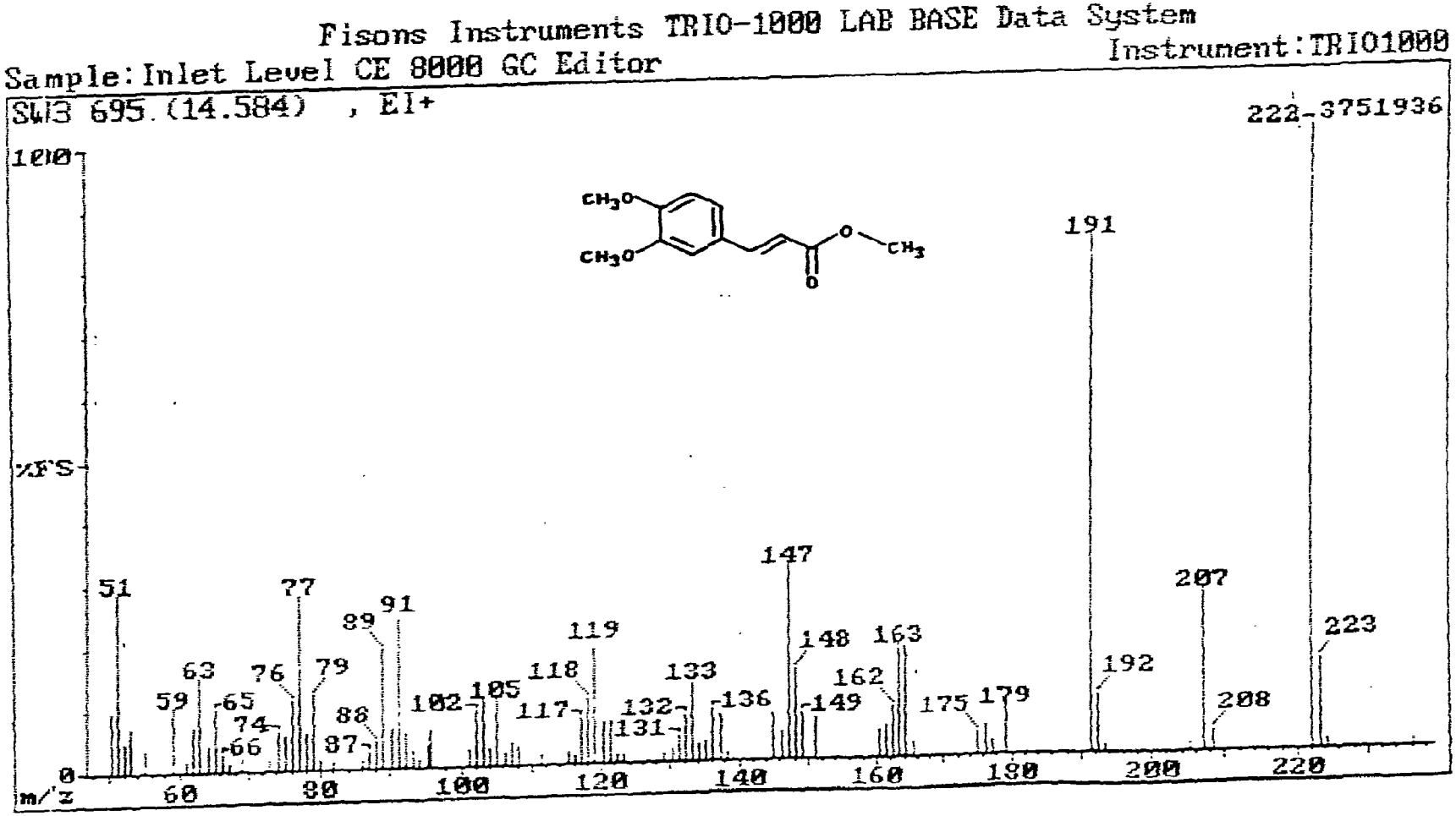


Fig. 41 : MS spectrum of 3,4-dimethoxy cinnamic acid methyl ester

BIBLIOGRAPHY

Ahmed SM, Ravindranath GM and Bhavanagary HM (1973a) Stabilization of pyrethrins for prolonged residual toxicity Part I : Screening of various ingredients *Pyrethrum post* **13**(3): 82-88.

Ahmed SM, Ravindranath GM and Bhavanagary HM (1973b) Stabilization of pyrethrins for prolonged residual toxicity Part-II : Development of new formulations *Pyrethrum post* **13**(4): 119-123.

*Anonymous (1980) *Firewood Crops* vol I National Academy of Sciences Washington DC.

Ara I, Siddiqui BS, Faizi S and Siddiqui S (1989a) Margosinone and margosinolone two new polyacetate derivatives from *Azadirachta indica* *Fitoterapia* **60**: 519-523.

Ara I, Siddiqui BS, Faizi S and Siddiqui S (1989b) Two new terpenoids from root bark of *Azadirachta indica* *J Nat Products* **52**: 1209-1213.

*Asakawa N, Tsuno M, Hattori T, Veyama M, Shinoda A, Miyake Y and Kagei K (1981) *Yakugaku Zasshi* **101**: 374.

Balandrin MF, Lee SM and Klocke JA (1988) Biologically active volatile organosulfur compounds from seeds of the neem tree *Azadirachta indica (Meliaceae)* *J Agric Food Chem* **36**: 1048-1054.

Barnby MA, Yamasaki RB and Klocke JA (1989) Biological activity of azadirachtin, three derivatives, and their ultraviolet radiation degradation products against tobacco budworm (Lepidoptera : Noctuidae) larvae *J Econ Entomol* **82**(1): 58-63).

Bilton JN, Broughton HB, Jones RS, Ley SV, Lidert Z, Morgan ED, Rzepa HS, Sheppard RH, Slawin AMZ and Williams DJ (1987) An X-ray crystallographic, mass spectroscopic and NMR study of the limonoid insect antifeedant, azadirachtin and related derivatives *Tetrahedron* **43**: 2805-2815.

Broughton HB, Ley SV, Slawin AMZ, Williams DJ and Morgan ED (1986) X-ray crystallographic structure determination of detigloyldihydroazadirachtin and reassignment of the structure of the limonoid insect antifeedant, azadirachtin *J Chem Soc Chem Comm* 46-47.

Brown GD (1993) Annulide, a sesquiterpene lactone from *Artimisia annua Phytochem* **32**(2): 391-393.

Bruhn A, Bokel M and Kraus W (1984) 4α, 6α Dihydroxy-A-homoazadiradione, a new tetranortriterpenoid from *Azadirachta indica* A. Juss. (Meliaceae) *Tetrahedron Letters* **25**: 3691-3692.

Butterworth JH and Morgan ED (1968) Isolation of a substance that suppresses feeding in locusts *J Chem Soc Chem Comm* 23-24.

Chander H, Kulkarni SG, Berry SK (1991) Effectiveness of turmeric powder and mustard oil as photoproducts in stored milled rice against the rice weevil *Sitophilus oryzae International Pest Control* **33**(4): 94-97.

Chander H, Kulkarni SG and Berry SK (1992) Studies on turmeric and mustard oil as protectants against infestation of red flour beetle, *Tribolium castaneum* (Herbst.) in stored rice *J Insect Sc* **5**(2): 220-222.

Chiu SF and Zhang X (1982) Experiments on the antifeedant and systemic principles of some botanical insecticides against the rice yellow stem borer *Scient Agric Sin* **2**: 55-59.

Chiu SF (1984) Recent research findings on Meliaceae and other promising botanical insecticides in China *Proc Res Plan Workshop on Indigenous Plant Materials for Pest Control* IRRI Los Banos Philippines 6-10 August 1984.

Choudhry GG, Roof AAM and Hutzinger O (1979) Mechanism in sensitised photochemistry of environmental chemicals *Toxicol Environ Chem* **2**: 259.

Choudhry GG (1984) Photophysical properties of soil and aquatic humic materials *Residue Rev* 92-59.

Crosby DG (1971) **In** *Naturally Occuring Insectcides* (Eds M Jacobson and DG Crosby) Marcel Dekker New York p 177-242.

Devakumar C and Mukerjee SK (1983) Chemistry of neem bitter principles *IARI Res Bull* **40**: 1-17.

Devakumar C, Goswami BK and Mukerjee SK (1985) Nematicidal principles from neem (*Azadirachta indica* A. Juss.) Part I: Screening of neem kernel fractions against *Meloidogyne incognita* *Indian J Nematol* **15**: 121-124.

Devakumar C, Riar SS, Illavazhagan G, Kain AK, Bardhan J, Thomas P and Singh B (1990) A modified process for *in vitro* spermicidal assay of neem products *Neem Newsletter* **7**: 13-15.

Devakumar C and Sukh Dev (1993) Chemistry **In** *Neem Research and Development* (Eds NS Randhawa and BS Parmar, Publication No.3 Society of Pesticide Science India 270-283.

Dorn A, Rademacher JM and Shen E (1986) Effects of azadirachtin on the moulting cycle, endocrine system, and ovaries in last instar larvae of the milk weed bug *Oncopeltus fasciatus* *J Insect Physiol* **32**(3): 231-238.

Dorn A, Rademacher JM and Sehn E (1987) Effects of azadirachtin on reproductive organs and fertility in the large milk weed bug, *Oncopeltus fasciatus* **In** *Natural Pesticides from the Neem Tree and other Tropical Plants Proc 3rd Int Neem Conf* Nairobi 1986 (Eds H Schmutterer and KRS Ascher) Eschborn, Germany : p 273-288.

Duke SO, Vaughn KC, Croom EM Jr and Elosohly HN (1987) Artemisinin, a constituent of annual worm wood *(Aretmisia annua)* is a slective phytotoxin *Weed Sci* **35**: 499-505.

Dureja P, Casida JE and Ruzo LO (1984) Dinitroanilines as photostabilizers for pyrethroids *J Agric Food Chem* **32**(2): 246-250.

Ekong DEU (1967) Chemistry of the meliacins (limonoids) The structure of nimbolide, a new meliacin from *Azadirachta indica J Chem Soc Chem Comm* 808.

Ermel K, Pahlich E and Schmutterer H (1987) Azadirachtin content of neem kernels from different geographical locations and its dependence on temperature, relative humidity and light **In** *Natural Pesticides from the Neem Tree and Other Tropical Plants Proc 3rd Inst Neem Confr* Nairobi 1986. (Eds H Schmutterer and KRS Ascher). Eschborn, Germany p 171-184.

*Ermel VK, Kalinowski HO and Schmutterer H (1991) *J Appl Ent* **112**: 512.

FAO(1971) Nutrition Meetings Report Series No. 48B World Health Organization and Food and Agriculture Organization of the United Nations Rome.

Feurhake KJ (1984) Effectiveness and selectivity of technical solvents from the extraction of neem seed components with insecticidal activity **In** *Natural Pesticides from the Neem Tree and other Tropical Plants Proc 2nd Int Neem Conf* Rauischholzhansen 1983 (Eds H Schmutterer and KRS Ascher) Eschborn Germany p 103-114.

*Fujio H, Hiyoshi T, Asari and Sumie K (1964) *Nippon Shokuhin-kogyo Gakkaishi* **16**: 241.

*Fujiwara T, Takeda T, Ogihara Y, Schimuzu M, Nomura T and Tomita Y (1982) Studies on the structure of polysaccharides from the bark of *Melia azadirachta* *Chem Pharm Bull* **30**: 4025-4030.

Fujiwara T, Sugishita E, Tokeda T, Ogihara Y, Shimizu M, Nomura T, and Tomita Y (1984) Further Studies on the structure of polysacharides from the bark of *Melia azadirachta* *Chem and Pharm Bull* **32**: 1385-1391.

Garg HS and Bhakuni DS (1984) An isoprenylated flavonone from leaves of *Azadirachta indica* *Phytochem* **23**: 2115-2117.

Govindarajan VS (1980) Turmeric chemistry, technology and quality *CRC Crit Rev Food Sci Nutr* p 199-301.

Govindachari TR, Sandhya G and Ganeshraj SP (1990) Simple method for the isolation of azadirachtin by preparative high performance liquid chromatography *J Chromatogr* **513**: 389-391.

Govindachari TR, Sandhya G and Ganeshraj SP (1991) Isolation of novel azadirachtin H and I by high performance liquid chromatography *Chromatographia* **31**: 303-305.

Govindchari TR (1992) Chemical and biological investigation on *Azadirachta indica* (the neem tree) *Current Science* **63**: 117-122.

Gujar GT and Mehrotra KN (1983) Juvenilizing effect of azadirachtin on a noctuid moth *Spodoptera liluta* Fab. *Indian J Exp Biol* **21**: 292-293.

Harris M, Henderson R, McCrindle R, Overton KH and Turner DW (1968) Tetranortriterpenoids VIII: The constitution and stereochemistry of nimbin *Tetrahedron* **24**: 1517-1523.

*Hasegawa T (1979) *Nippon Eiyo Shokuryo Gakkaishi* **32**: 267.

Hegnauer (1983) Chemical characters and the classification of the Rutales **In** *Chemistry and Chemical taxonomy of the Rutales* (Eds PG Waterman and MF Grundon) *Ann Proc Phytochemistry Soc* Europe No. 22 Academic Press New York.

Henderson R, McCrindle R, Melera A and Overton KH (1968) Tetranortriterpenoids-IX : The constitution and stereochemistry of salannin *Tetrahedron* **24**: 1525-1528.

Huang HP and Morgan ED (1990) Analysis of azadirachtin by super critical fluid chromatography *J Chromatogr* **519**: 137-144.

Huang RC, Zhou J, Suenaga H, Takezaki K, Tadera K and Nakatani M (1995) Insect antifeeding property of limonoids from Kinawan and Chinese *Melia azedarach* L. and from Chinese *Melia toosendan* (Meliaceae) *Biosci Biotech Biochem* **59**(9): 1757-1775.

Hwang HM, Hodson RE and Lee RF (1987) Photochemistry of environmental aquatic systems (Eds RG Zika and WJ Cooper) *ACS Symposium series 327* Washington DC p 27-43.

Isman MB, Koul O, Luczynski A and Kaminski J (1990) Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content *J Agric Food Chem* **38**(6): 1406-1410.

Jafferies PR, Toia RF and Casida JE (1991) Ryanodyl -3- (pyridine-3-carboxylate): a novel ryanoid from *Ryania speciosa* *J Nat Prod* **54**: 1147-1149.

Janaki N and Bose JL (1967) An improved method for the isolation of curcumin from turmeric *(Curcuma longa* L.) *J Indian Chem Soc* **44**: 985.

Jilani G and Su HCF (1983) Laboratory studies on several plant materials as insect repellents for protection of stored grains *J Econ Entomol* **76**: 154-157.

Jilani G, Saxena RC and Rueda BP (1988) Repellent and growth inhibiting effects of turmeric oil, sweetflag oil, and "Margosan-O" on red flour beetle (Coleoptera : Tenebrionidae) *J Econ Entomol* **81**(4): 1226-1230.

Jitoe A, Masuda T, Tenjah IGP, Suprapta DN, Gara W and Nakatani N (1992) Antioxidant activity of tropical ginger extracts and analysis of the contained curcuminoids *J Agric Food Chem* **40**: 1337-1340.

Johnson S, Morgan ED, Wilson ID, Spraul M and Hofmann M (1994) Photo-isomerization of azadirachtin studied by high performance liquid chromatography coupled to high field proton NMR spectroscopy *J Chem Soc Perkin Trans I* 1499-1502.

Jones PS, Ley SV, Morgan ED and Santafianos O (1990) The chemistry of neem tree **In** *Neem Tree : Focus on Phytochemical Pesticides (vol I)* (Ed M Jacobson) p 19-45 CRC Press Boca Raton FL (USA).

Ketkar CM (1976) Utilization of neem (*Azadirachta indica*) and its byproducts *Final Technical Report* Khadi and Village Industries Commission Bombay India.

Kleeberg H (1996) The NeemAzal Conception: Future possibilities of the use of neem in biological and integrated pest management **In** *Neem and Environment* (Eds RP Singh, MS Chari, AK Raheja and W Kraus) Oxford and IBH publication Co Pvt Ltd New Delhi India p 875-881.

Klenk A, Bokel M and Kraus W (1986) 3-Tigloyl azadirachtinol, an insect growth regulating constituent of *Azadirachta indica* *J Chem Soc Chem Comm* 523-528.

Koul O (1985) Azadirachtin interaction with development of *Spodoptera litura Fab.* *Ind J Exp Biol* **23**: 160-163.

Koul O, Isman MB, Ketkar CM (1990) Properties and uses of neem *Azadirachta indica* *Can J Bul* **68**: 1-11.

Kraus W, Bokel M, Klenk A and Pohnl HD (1985) The structure of azadirachtin and 22, 23-dihydro-23β-methoxy azadirachtin *Tetrahedron Letts* **26**: 6435-6438.

Kraus W (1986) Constituents of neem and related spices, A revised structure of azadirachtin **In** *New Trends in Natural Products Chemistry* (Eds A Rahman and PW Le Quesne) *Studies in Org Chem* Elsevier Science Publishers BV Amsterdam Netherlands vol 26 p 237-256.

Kraus W, Bokel M, Bruhn A, Cramer R, Klaiber A, Nagi G, Pohnl H, Sadio H and Vogler B (1987) Structure determination by NMR of azadirachtin and related compounds from *Azadirachta indica* A. Juss. (Meliaceae) *Tetrahedron* **43**: 2817-2830.

*Krishnamurthy N, Mathene AG, Nambudiri ES, Shivashankar S, Lewis YS and Natarajan CP (1976) *Trop Sci* **18**: 37.

Kubo I, Matsumoto A and Matsumoto T (1986) New insect ecdysis inhibitory limonoid, deacetyl azadirachtinol isolated from *Azadirachta indica* (Meliaceae) oil *Tetrahedron* **42**: 489-496.

Lauridsen EB, Kanchanaburagura C and Boonsemusk S (1991) Neem (*A. indica* A. Juss.) in Thailand *Forest Genetic Resources Information FAO Rome* **19**: 25-33.

Lavie D, Levy EC and Jain MK (1971) Limonoids of biogenetic interest from *Melia azadirachta* L. *Tetrahedron* **27**: 3927-3939.

Ley SV, Denholm AA and Wood A (1993) The chemistry of azadirachtin *Natural Products Reports* p 109-157.

Lowery DT and Isman MB (1996) Inhibition of aphid (Homoptera : Aphididae) reproduction by neem seed oil and azadirachtin *J Econ Entomol* **89**(3): 602-607.

Mariappan U and Saxena RC (1983) Effects of custard apple oil and neem oil on survival of *Nephotettix virescens* (Homoptera : Cicadellidae) and on rice tungro virus transmission *J Econ Entomol* **78**: 573-576.

*Mason RR (1970) USDA Forest Serv Res Note *PNW* - 120.

Masuda T, Jitoe A, Isobe J, Nakatani N and Yonemori S (1993) Anti-oxidative and anti-inflammatory curcumin-related phenolics from rhizomes of *Curcuma domestica Phytochem* **32**(6): 1557-1560.

Miskus RP and Andrews TL (1972) Stabilization of thin films of pyrethrins and allethrin *J Agric Food Chem* **20**(2): 313-315.

*Miyamoto J, Beynon KI, Roberts TR, Hemingway RJ and Swaine H (1981) *Pure Appl Chem* **53**, 1967.

Morallo-Rejesus B and Eroles L (1978) Two insecticidal principles from marigold *(Tagetes spp.)* roots *Philipp Ent* **4**: 87-91.

Morallo-Rejesus B and Decena A (1982) The activity, isolation, purification and identification of the insecticidal principles from *Tagetes Phil J Gop Sci* **7**: 31-33.

Mordue AJ and Blackwell A (1993) Azadirachtin: an update *J Insect Physiol* **39**: 903-924.

Mubarak AM and Kulatilleke CP (1990) Sulphur constituents of neem seed volatiles : A revision *Phytochem* **29**: 3351-3352.

Munrao HAU (1964) Manual of Fumigation for insect control Published by FAO of the United Nations Rome p 6-7.

Nakayama R, Tamura Y, Yamanaka H, Kikuzaki H and Nakatani N (1993) Two curcuminoid pigments from *Curcuma domestica Phytochem* **33**(2): 501-502.

Pabon HJJ (1964) Synthesis of curcumin and related compounds *Rec Trav Chim Pays-Bas* **83**: 379.

Pachapurkar PV, Kornule PM and Narayanan CR (1974) A new hexacyclic tetranortriterpenoid *Chem Lett* **4**: 357-358.

Pant N, Garg HS, Madhusudanan KP and Bhakuni DS (1986) Sulphurus compounds from *Azadirachta indica* leaves *Fitoterapia* **57**: 302-304.

Parmar BS, and Ketkar CM (1993) Commercialization **In** *Neem Research and Development* (Eds NS Randhawa and BS Parmar) Publication No 3 SPS India New Delhi p 270-283.

Parveen G, and Alam M (1993) Bioactivity against plant pathogens **In** *Neem Research and Development* (Eds NS Randhawa and BS Parmar Publications No 3) SPS India p 144-153.

Pieper GR, and Rappaport NL (1982) Photostabilization of bioethanomethrin resmethrin and natural pyrethrins by mixed diaryl-*p*-phenylenediamines *J Agric Food Chem* **30**: 405-407.

Pradhan S, Jotwani MG, and Rai BK (1962) The neem seed deterrant to locust *Indian Farming* **12**: 7-11.

Pradhan VR, Renapurkar DM and Antarkar DS (1983) Antimalarial activity of 'Sudarshan Ghan Vati'- an ayurvedic preparation *Bulletin-of-Haffkine-Institute* **11**: 3, 64-67.

Rao BS, Nazma and Rao JM (1977) Antifungal activity of gedunin *Curr Sci* **46**: 714-716.

Rao PJ, and Subrahmanyam B (1987) Effect of azadirachtin on *Achea janata* Linn. and *Spodoptera litura* F (Noctuidae : Lepidoptera) *J Entomol Res* **11**(2): 166-169.

Ray P (1956) History of chemistry in ancient and medieval India *Ind Chem Soc* **50**: 56-235.

Rembold H (1988) Focus on Phytochemical pesticides (Ed H Jacobson) CRC Press Inc Florida p 50.

Rembold H (1989) Isomeric azadirachtins and their mode of action **In** *The Neem Tree* (Ed M Jacobson) Boca Raton: CRC Press vol I p 47-67.

Rembold H (1990) Azadirachtin: Their structure and mode of action **In** *Insecticides of Plant Origin* (Eds JT Amason BJR Philogene and P Morad) *ACS Sym Ser 387 American Chemical Society* Washigton DC USA p 150-163.

Rembold H (1990) Isomeric azadirachtins and their mode of action **In** *Focus on phytochemical pesticides I: The Neem Tree* (Ed M Jacobson) CRC Press Boca Raton FI USA p 47-67.

Rengasmy, Kaushik N, Jitendra Kumar, Koul O and Parmar BS (1996) Azadirachtin content and bioactivity of some neem ecotypes of India **In** *Neem and Environment* (Eds RP Singh, MS Chari, AK Raheja and W Kraus) Oxford and IBH Publising Co Pvt Ltd New Delhi India Vol-I p 207-217.

Riar SS, DevaKumar C, Ilavazhagan G, Bardhan J, Kain AK, Thomas P, Singh R and Singh B (1990) Volatile fraction of neem oil as a spermicide *Contraception* **42**: 479-487.

Rajatkar SR, Joshi SP, and Nagasampagi BA (1996) Photo-oxidation of tetranortriterpenoids **In** *Neem and Environment,* (Eds RP Singh, MS Chari, AK Raheja and W Kraus) Oxford and 1B H Publishing Co Pvt Ltd New Delhi Vol I p 198-201.

Ruch B, Tross R and Kleeberg H, Environmental impact of the neem ingredient Neemazal *Abstract 5th World Neem Conference* Feburary 4-9, The University of Queensland Gatton College, Australia p-53.

Rukmini C (1987) Chemical and nutritional evaluation neem oil *Food Chem* **26**: 119-124

*Rupe H, Clar G, Pfau, A St and Plattner PI (1994) Volatile plant constituent II : Turmerone the aromatic principle of turmeric oil (in German) *Helv Chim Acta* **17**: 372 Chem, Abstr 28 47086.

Ruzo L (1983) Photodegradation of pesticides in solution: isomerization, dehalogenation and ester cleavage reaction of the pyrethroid pesticides **In** *Pesticide Chemistry*: Human Welfare and the Environment (Eds Miyamoto J and Kearney PC) Pergamon Press New York 1983 Vol 2 p 201.

Samuel R, Prabhu VKK and Narayanan CS (1984) Influence of spice essential oil on the life history of *Lasioderma serricorne* (F) *Indian Spices* **9** (3): 209-215.

Sankaram AVB, Mathanda Murthy M, Bhaskaraiah K, Subramanayam M, Sultana N, Sharma HC, Leuschner K, Ramprasad G, Sitaramiah S, Rukmini C and Rao PU (1987) Chemistry, biological activity and utilization aspects of some promising neem extractives **In** *Natural Pesticides from The Neem Tree and Other Tropical Plants* (Eds H Schmutter and KRS Ascher) GTZ Eschborn FRG p 127-148.

Saxena RC and Boncodin MEM (1988) Effect of neem seed bitters (NSB) on green leaf hoppers (GLH) *Int Rice Res News* **13**(1): 27.

Schluter U (1987) Effect of azadirachtin on developing tissues of various insect larvae **In** *Natural Pesticides from The Neem Tree and Other Tropical Plants Proc 3rd Int Neem Conf* Nairobi 1986 (Eds H Sehmutterer and KR Ascher) Eschborn:GTZ p 331-348.

*Schmutterer H and Remobold H (1980) *Z Angew Entom* **89**: 179.

Schmutter H (1987) Insect growth-disrupting and fecundity-reducing ingredients from the neem and Chinaberry trees **In** *Handbook of Natural Pesticides* (Eds ED Morgan and NB Mandava) (part B): 119-70, Boca Raton: CRC Press, pp 180.

Schmutterer H (1990) Properties and potential of natural pesticides from the neem tree *Azadirachta indica Ann Rev Entomol* **35**: 271-79.

Schrroeder DR and Nakarishi K (1987) A simplified isolation procedure for azadirachtin *J Nat Products* **50**(2): 241-244.

Sengupta P, Chudhuri SN and Khastiger HN (1960) Terpenoids and related compounds-I Constituents of the trunk bark of *Melia azadirachta* Linn. and the sturcture of the ketophenol, nimbiol *Tetrahedron* **10**: 45-59.

Shankaracharya NB and Natarajan CP (1973) Turmeric chemistry technology and uses *Indian Spices* **10**(3): 7.

Siddiqui S (1942) A note on the isolation of three new bitter principles from the neem oil *Curr Sci* **11**: 278-279.

Siddiqui S, Mahamood T, Siddiqui BS and Faizis (1985) Studies on the Monterpenoidal constituents of *Azadirachta indica* *Pak J Sc Ind Res* **28**: 1-4.

Siddiqui S, Faizi S, Mahamood T and Siddiqui BS (1986a) Two new insect growth regulator meliacins from *Azadirachta indica* A. Juss. (Meliaceae) *J Chem Soc Perkin Trans* **1**: 1021-1025.

Siddiqui S, Mahmood T, Siddiqui BS, and Faizi S (1986b) Isolation of a triterpenoid from *Azadirachta indica* *Phytochem* **25**: 2183-2185.

Siddiqui S, Mahamood T, Siddiqui BS and Faizi S (1986c) Two new tetranortriterpenoids from *Azadirachta indica* *J Nat Products* **49**: 1068-1073.

Siddiqui S, Siddiqui BS, Faizi S, and Mahmood T (1986d) Isoazadirrollide, a new tetranortriterpenoid from *Azadirachta indica* A. Juss. (Meliaceae) *Heterocycles* **24**: 3163-3167.

Siddiqui S, Siddiqui BS, Ghiasuddin and Faizi S (1991) Terpenoids from fruit coating of *Azadirachta indica* *Phytochem* **30**: 1615-1619.

Siddiqui S, Siddiqui BS, Ghiasuddin and Faizi S (1991) Tetracyclic triterpenoids of the fruit coats of *Azadirachta indica* *J Nat Products* **54**: 408-415.

Siddiqui S, Faizi S, Siddiqui BS, and Ghiasuddin (1992) Constituents of *Azadirachta indica*: Isolation and structure elucidation of a new anti-bacterial tetranortriterpenoid mahmoodin and a new protolimonoid naheedin *J Nat Products* **55**: 303-310.

Siddiqui BS, Faizi S, Ghiasuddin and Siddiqui S (1996) Chemistry of neem (*Azadirachta indica*), a sustainable source of naural pesticides **In** *Neem and Environment* (Eds RP Singh, MS Chari, AK Rahey and W Kraus) Oxford and IBH Publishing Co Pvt Ltd New Delhi Vol I p 187-198.

Siddiqui MA and Alam MM (1996) Evaluation of nematicidal potential in neem allelochemicals **In** *Neem and environment* (Eds RP Singh, MS Chari, AK Raheja and W Kraus) Oxford and IBH publishing Co Pvt Ltd New Delhi India p 669-678.

Sieber KP and Rembold H (1983) The effects of azadirachtin on the endocrine control of moulting in *Locusta migratoria J Insect Physiol* **29**: 523-27.

Singh S, Dube NK, Tripathi SC and Singh SK (1984) Fungitoxicity of some essential oils against *Aspergillus flavus Indian Perfumer* **28** (3/4) 164-166.

Sing RP (1993) Bioactivity against insect pests **In** *Neem Research and Development* (Eds NS Randhawa and BS parmar Publication No 3) SPS India p 109-122.

Singh RP and Raheja AK (1996) Strategies in management of insect pests with neem **In** *Neem and Environment* (Eds RP Singh, MS Chari, AK Raheja and W Kraus) Oxford and IBH Publishing Co Pvt Ltd New Delhi India Vol I p 103-120.

Sinha RC, Riar SS, Bardhan J, Thomas P, Kain AR and Jain RK (1984) Antiimplantation effects of neem oil *Indian J Med Res* **80**: 708.

*Smith HG and Hill ML (1949) To Gulf oil Corp US Patent 2, 421, 223 (May 27 1947): reissued 23 168 (Nov 15).

*Smith CW and Templin NM (1956) To Standard oil of Indiana US Patent 2 772 198 (Nov 27).

Smith RM and Witowska BA (1984) Comparison of detectors for the determination of curcumin in turmeric by high-performance liquid Chromatography *Analyst* **109**: 259-261.

Spencer K (Ed) (1988) *Chemical Mediation of Coevalution* Academic Press New York.

Sreenivasamurthy V and Krishnamurthy K (1959) Place of spices and aromatics in Indian dietary *Food Sci* (Mysore) 8: 289-288.

*Srinivasan KR (1953) *J pharm pharmacol* **5**: 448.

Subrahmanyam B and Rao PJ (1986) Azadirachtin effects on *Schistocerca gregaria* Forskal during ovarian development *Curr Sci* **55**(11): 534-538.

Subramanian SS and Nair AGR (1972) Melicitrin, a new myrecetin glycoside from the flowers of *M. azadirachta Indian J Chem* **10**: 452.

Subramaniam B, Sivaprasad B and Prasad VVLN (1996) RD-9 Repelin: An effective botanical for integrated pest management **In** *Neem and Environment* (Eds RP singh, MS Chari, AK Raheja and W Krous) Oxford and IBH Publication Co Pvt Ltd New Delhi India p 857-863.

Su HCF, Robert H and Jilani G (1982) Isolation, purification and characterization of insect repellents from *Curcuma longa* L *J Agric Food Chem* **30**: 290-292.

Szeto SY and Wan MT (1996) Hydrolysis of Azadirachtin in buffer and natural waters Vol I *5th World Neem Conference Abstract* February 4-9 University of Queensland Gatton College Australia p 4.

Taylor DAH (1984) The chemistry of the limonoids from Meliaceae *Fortschr Chem Org Naturst* **45**: 1-102.

Thakur DK, Misra SK and Choudhuri PC (1983) The use of *Leucas aspera* Spreng and *Curcuma longa* L. extracts in the treatment of *Trichophyton verrucosum* ringworm in cattle *Sri-Lanka Veterimary Journal* **31**(1/2): 37-39.

Tamizawa H, Sazuka Y. Komatsu A. Toda S and Takino Y (1983) A new efficacy test of antioxidants based on air-oxidation of linoleic acid *Chem Pharm Bull* **31**(11): 4139-4143.

*Toda S, Tanizawa H, Arichi S and Takino Y (1984) *Yakugaku zasshi* **104**: 394.

Toda S, Myase T, Arichi H, Tanizawa H and Takino Y (1985) Natural anti-oxidants III : Antioxidative components isolated from rhizome of *Curcuma longa* L. *Chem Pharm Bull* **33**: 1725-1728.

Tonnesen HH and Karlsen J (1982) High performance liquid chromatography of curcumin and related compounds *J Chromatogr* **259**: 367-371.

Turner CJ, Tempesta MS, Taylor RB, Zagorski MG, Termini JS Schroeder DR and Nakanishi K (1987) A NMR spectroscopic study of azadirachtin and its trimethyl ether *Tetrahedron* **43**: 2789-2803.

Ubel EC, Warthen JD and Jacobson M (1979) Preparative reversed phase liquid chromatographic isolation of azadirachtin from neem kernels *J Liq Chromatogr* **2**: 875-882.

Ueda K, Gaughan LC and Casida JE (1974) Photodecomposition of resmetherin and related pyrethroids *J Agric Food Chem* **22**(2): 212-220.

Van der Nat JM, 't Hart LA, Van der Sluis WG and Labadie RP (1987) Two functionally different immunomodulators from an aqueous bark extract of *Azadirachta indica* A. Juss. (Meliaceae) *Pharm Weekblad Sci Edition* **9**: 224.

Van der Nat JM, Van der Sluis WG 't Hart LA, Van Dijk H, de Silva KTD and Labadie RP (1991) Activity guided isolation and identification of *Azadirachta indica* bark extract constituents which specifically inhibit chemiluminesence production by activated human polymorphonuclear leukocytes *Planta Medica* **57**: 65-68.

Visan AC, Nirmala Menon A, Madhusudan Rao J, Narayana CS and Mathew AG (1989) Chemical analysis of some cultivars of *Curcuma longa* Linn. *J Fd Sci Technol* **26**(5): 293-295.

Warthen JD (1979) *Azadirachta indica* A source of insect feeding inhibitors and growth regulators Agric Rev Manuals ARM-NE-4 21 p USDA Beltsville MD USA.

Warthen JD, Stokes JB, Jacobson M and Kozempel MF (1984) Estimation of azadirachtin content in neem extract and formulations *J Liq Chromatogr* **7**: 591-598.

Watanabe K, Takada Y, Matsuo N and Nishimura H (1995) Rotundial, a new natural mosquito repellent from the leaves of *Vitex rotundifolia* *Biosci Biotech Biochem* **59**(10): 1979-1980.

WHO/FAO (1976) Add series No. 7 World Health Organization, Food and Agriculture organization of the united Nations Rome.

Xie YS, Fieds PG and Isman MB (1995) Repellency and toxicity of azadirachtin and neem concentrates to the stored product beetles *J Econ Entomol* 88(4): 1024-1031.

Yakkundi SR, Thejavathi R and Ravindranath B (1995) Variation of azadirachtin content during growth and storage of neem (*Azadirachta indica*) seeds *J Agric Food Chem* **43**: 2517-2519.

Yakkundi SR, Kumar SSR Ch and Ravindranath B (1996) Photochemistry of Azadirachtin *5th World Neem Conf Abstract* February 4-9 The University of Queensland Gatton College Australia p21.

Zanno PR, Miura E, Nakanishi K and Elder DL (1975) Structure of the insect phogorepellent azadirachtin Applications of PRFT/CWD carbon-13 nuclear magnetic resonance *J Amer Chem Soc* **97**: 1975-1977.

Zebitz CPW (1986) Effect of three different neem seed Kernel extracts and azadirachtin on larvae of different mosquito species *J Appl Ent* **102**: 455-463.

* Original not seen

1. Name of the student	:	**HEMANTA CHOWDHURY**
2. Roll No.	:	7873
3. Degree for which Thesis is submitted	:	Ph.D.
4. Discipline	:	Agricultural Chemicals
5. Date of joining the P.G. School	:	October 1, 1993
6. Date of submission of Thesis	:	November 18, 1996
7. Major Field	:	Agricultural Chemicals
8. Minor Fields	:	i) Entomology ii) Soil Science and Agriculture Chemistry
9. Name of the Chairman	:	Dr. Suresh Walia
10. Name of the Advisory Committee Members	:	Dr. B.S. Parmar Dr. (Ms.) P. Dureja Dr. P.J. Rao Dr. S.C. Dutta

11. Title of the Thesis : **Effect of Curcumins and Oil of Turmeric (*Curcuma longa*) on the Photostability and Efficacy of Azadirachtin and Azadirachtin Rich Neem Oil**

ABSTRACT

Neem based pesticides being environmentally benign are considered as potential alternative to the highly persistent and toxic synthetic pesticides. Like other botanicals, azadirachtin based neem products have limited stability and low persistence. Present investigation is thus aimed at exploring the possibility of stabilizing azdirachtin and azadirachtin rich neem oil with botanical stabilizers isolated from *Curcuma longa* (turmeric) rhizomes which are well known for their anti-oxidant properties. Neem oil and azadirachtin (45% purity) were isolated from neem seed kernel extract. Petroleum ether (40-60) extract of *Curcuma longa* rhizomes yielded turmeric oil, whereas, benzene extract of the deoiled turmeric powder yielded three yellow pigments which after purification were characterised as 1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin-I), 1-(4-hydroxy phenyl)-7-(4-hydroxy-3-methoxy phenyl)-1, 6-heptadiene-3, 5-dione (Curcumin-II), and bis (4-hydroxycinnamoyl methane (curcumin-III) by spectroscopic techniques (FT-IR, ^{1}H NMR and MS). Curcumin-I was converted to its two synthetic analogeous namely 1,7-bis (3,4-dimethoxyphenyl)-4-methylhepta-1,6-diene-

3,5-dione and 1,7-bis(3-methoxy, 4-*n*-butoxy)-hepta-1,6-diene-3,5-dione. These curcumin related products along with butylated hydroxy toluene (BHT) were evaluated as stabilizers for azadirachtin. Curcumin-I being sensitive to UV/ sunlight gets degraded to four photoproducts which after methylation with CH_2N_2 where identified as i) 4(3,4-dimethoxyphenyl)-but-3-ene-2-one, ii) 3,4-dimethoxy cinnamic acid methyl ester iii) methyl (3,4-dimethoxy) benzoate, and iv) veratraldehyde. Of the three curcumins, curcumin-I being a major component and more photostable, was selected for further investigations to stabilize azadirachtin in solution and as thin film on glass/leaf surface.

Under the impact of UV-light, azadirachtin degraded faster in solution than as thin film on glass surface. Reverse was however, true under sunlight irradiated condition wherein azadirachtin degradation was faster as thin film on both glass and leaf surface than in solution.

Stability of azadirachtin was enhanced following incorporation of curcumins, turmeric oil and neem oil in various (1:1, 2:1, and 3:1) proportions. Of the various combinations, highest degree of stabilization was achieved when azadirachtin : curcumin-I was combined in 1:1 ratio. Of the various combinations, turmeric oil and neem oil mixture was found to be the best for increasing residual life of azadirachtin in solution phase. Unlike azadirachtin which degraded within 6 days to 9.49 % of its initial concentration, azadirachtin in azadirachtin rich neem oil-turmeric oil mixture was detected to the extent of 75.42 % even after six days, thus providing considerable stability to azadirachtin. The stabilization was more pronounced in solution phase than as thin film on glass/leaf surface where quick dissipation of azadirachtin was attributed to possible losses due to volatilization, photodegradation, wind velocity and humidity etc. Since methyl and butyl derivatives of curcumin-I could not impart sufficient stability to azadirachtin, it was inferred that feruloyl moiety containing free -OH substituent adjacent to one methoxyl substituent is essential in cucurmin-I for imparting photostability to azadirachtin molecule.

When azadirachtin and curcumin-I mixture in various proportions were evaluated for possible growth and moulting inhibition against *Spilosoma obliqua* 3rd and 5th instar larvae, it was inferred that activity was more pronounced in 3:1 followed by 2:1 and 1:1 proportions. The prolongation of instar duration and delayed lethal effect or moulting abnormalilties with/ without curcumin-I were mainly attributed to their interference with insect endocrine system. Like insect growth inhibition, anti-feedant activity of azadirachtin was increased following incorporation of curcumin-I in 3:1 proportion. The comparable antifeedant action of both azadirachtin and azadirachtin : curcumin-I mixture was attributed to the fact that curcumin-I itself had antifeedant activity of more than 80 % at 0.06 % dose level.

It was thus concluded that turmeric products particularly curcumin-I and turmeric oil not only induce photostability to azadirachtin molecule but also cause significant enhancement in insect moulting inhibition and antifeedant action against 3rd and 5th instar larvae of Bihar hairy caterpiller, *S. obliqua*.