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DEVELOPMENT OF SLOW NITROGEN RELEASE UREA
FORMULATIONS EMPLOYING *Pongamia glabra* Vent.
ISOLATES AND SOME FURFURAL DERIVATIVES

ANIRUDDHA DATTA



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DIVISION OF AGRICULTURAL CHEMICALS
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IARI

2000

**DEVELOPMENT OF SLOW NITROGEN RELEASE UREA
FORMULATIONS EMPLOYING *Pongamia glabra* Vent.
ISOLATES AND SOME FURFURAL DERIVATIVES**

A THESIS

BY

ANIRUDDHA DATTA

**Submitted to the Faculty of Post Graduate School,
Indian Agricultural Research Institute, New Delhi,
in partial fulfillment of the requirements
for the award of the degree of**

DOCTOR OF PHILOSOPHY

IN

AGRICULTURAL CHEMICALS

2000

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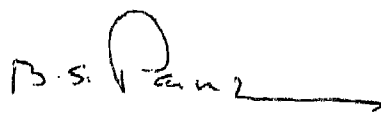
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This is to certify that the thesis entitled '**DEVELOPMENT OF SLOW NITROGEN RELEASE UREA FORMULATIONS EMPLOYING *Pongamia glabra* Vent. ISOLATES AND SOME FURFURAL DERIVATIVES**' submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY in AGRICULTURAL CHEMICALS**, embodies the results of a piece of *bona fide* research work carried out by **SHRI ANIRUDDHA DATTA** in the Division of Agricultural Chemicals, IARI, New Delhi, under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma. The assistance and help received by him during the course of this investigation and the source of literature drawn are duly acknowledged.



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ACKNOWLEDGEMENTS

I express my deepest sense of gratitude to Dr. B. S. Parmar, Head, Division of Agricultural Chemicals, and Chairman, Advisory Committee for suggesting the problem, valuable guidance, meticulous supervision, incessant encouragement and constructive criticism throughout this investigation and preparation of the manuscript.

I convey my profound sense of gratitude to Dr. Suresh Walia, Dr. B. Subrahmanyam and Dr. S. C. Datta, esteemed members of my Advisory Committee for their constructive and valuable suggestions and necessary assistance during my research work.

I express my profound gratefulness to Dr G. Kulshrestha, the professor of the Division of Agricultural Chemicals for providing necessary facilities and encouragement throughout this study.

I am very much obliged and grateful to Dr. A. K. Dikshit, Dr. P. Dureja and Dr. S. Rengasamy for providing necessary facilities and valuable advice during my research work. I express my sincere gratitude to Dr. M. Gopal and Dr. Jitendra Kumar for their valuable suggestion and cooperation.

I would like to record my special gratitude and heartfelt thanks to Sh. Shiv Dutta for his ungrudging help during the course of my research work. A word of appreciation goes to Ram Niwasji for his everwilling help and guidance.

The assistance offered by Mr K. K. Somayajulu, Mr. Mahesh Paswan and Mr. P. K. Singh is duly acknowledged.

Candid thanks to, Anil and Subhomay whose collective efforts have been reflected in the completion of this venture.

I am equally thankful to all my friends in IARI, in particular, Deepanjanda, Dipankar, Avijit, Gaur, Paraj, Samir, Uttam, Tirtha, Pratap, Kajal, Debi, Kali, Supra, Tarun, Deba, Anshuman and Sugata for their timely help and constant encouragement.

I gratefully acknowledge the help in statistical analysis provided by Computer Centre (BIC), IARI.

I also express my sincere thanks to Sushil for the cooperation in bringing out the manuscript in thesis form.

The encompassing love and affection bestowed on me by my parents and family members has played a key role in bringing me to this pursuit of studies.

The financial assistance received in the form of Senior Research Fellowship from ICAR, New Delhi, is gratefully acknowledged.

Aniruddha Datta
(ANIRUDDHA DATTA)

Date : 19th October, 2000

Place : New Delhi

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LIST OF ABBREVIATIONS AND SYMBOLS

δ	=	Chemical shift (ppm)
$^{\circ}\text{C}$	=	Degree Celcius
a.i.	=	Active ingredient
ACC	=	4-(Furan-1-yl)-1-but-3-en-2-one
AOE	=	N-O-allyl furfural oxime
APC	=	3-(Furan-1-yl)-1-phenyl-prop-2-en-1-one
AS	=	Furfurylideneaniline
av. wt.	=	Average weight
BOE	=	N-O-butyl furfural oxime
bp	=	Boiling point
C	=	Coal tar
CAS	=	Furfurylidene-4-chloroaniline
CDCl_3	=	Deutorated chloroform
D	=	Doublet
DCAS	=	Furfurylidene-3,4-dichloroaniline
dil	=	Dilute
EOE	=	N-O-ethyl furfural oxime
Fig	=	Figure
g	=	Gram
HAPC	=	3-(Furan-1-yl)-1-(4-hydroxyphenyl)-prop-2-en-1-one
HOE	=	N-O-hexyl furfural oxime
HPLC	=	High performance liquid chromatography
Hz	=	Hertz
J	=	Coupling constant
KA	=	Alcohol extract of karanj seed kernels
kg	=	Kilogram
KN	=	Karanjin
KO	=	Karanj oil

L	=	Litre
<i>m</i>	=	<i>Meta</i>
M	=	Molar
Mg	=	Megagram
mg	=	Miligram
Mol	=	Mole
mp	=	Melting point
N	=	Nitrogen
NA	=	Alcohol extract of neem seed kernels
NAS	=	Furfurylidene-3-nitroaniline
NI	=	Nitrification inhibition
NS	=	Nitrapyrin
<i>o</i>	=	<i>Ortho</i>
OE	=	Furfural oxime
<i>p</i>	=	<i>Para</i>
PMR	=	Proton magnetic resonance spectroscopy
POE	=	N-O- <i>isopropyl</i> furfural oxime
ppm	=	Parts per million
R _f	=	Retention factor
RH	=	Relative humidity
R _t	=	Retention time
S	=	Singlet
T	=	Triplet
Tg	=	Teragram
TLC	=	Thin layer chromatography
UV-VIS	=	Ultraviolet visible chromatography
w/w	=	Weight by weight
yr	=	Year

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**¹H NMR (PMR) SPECTRA OF SYNTHESIZED FURFURAL
DERIVATIVES**

Spectra No.	Compound
1	Furfural oxime
2	N-O-ethyl furfural oxime
3	N-O-isopropyl furfural oxime
4	N-O-butyl furfural oxime
5	N-O-hexyl furfural oxime
6	N-O-Allyl furfural oxime
7	3-(Furan-1-yl)-1-phenyl-prop-2-en-1-one
8	4-(Furan-1-yl)-1-but-3-en-2-one
9	Furfurylidene-4-chloroaniline
10	Furfurylidene-3,4-dichloroaniline
11	Furfurylidene-3-nitroaniline

Abstract

Nitrogenous fertilizers have made a major contribution towards improving the agricultural productivity the worldover. However, there is a continuous need to improve the efficiency of nitrogenous fertilizer use to achieve more efficient food production and minimize fertilizer related pollution of the environment. Various approaches have been suggested to achieve these objectives among which the use of nitrification inhibitors are prominent one.

In this study, an effort has been made to develop nitrification inhibitors based on furfural - a industrially available cheap raw material and karanj (*Pongamia glabra* Vent.) seed kernel extractives. Twelve furfural derivatives belonging to three series, namely, furfural oxime ethers (N-O-ethyl furfural oxime, EOE; N-O-isopropyl furfural oxime, POE; N-O-butyl furfural oxime, BOE; N-O-hexyl furfural oxime, HOE and N-O-allyl furfural oxime, AOE), furfural chalcones [4-(furan-1-yl)-1-but-3-en-2-one, ACC; 3-(furan-1-yl)-1-phenyl-prop-2-en-1-one, APC and 3-(furan-1-yl)-1-(4-hydroxyphenyl)-prop-2-en-1-one, HAPC] and furfural Schiff bases (furfurylideneaniline, AS; furfurylidene-4-chloroaniline, CAS; furfurylidene-3-nitroaniline, NAS and furfurylidene-3,4-dichloroaniline, DCAS) were synthesized and evaluated for nitrification inhibitory property in laboratory incubation studies in a typic Ustocrept soil.

Furfural oxime ethers and furfural Schiff bases showed potential activity as nitrification inhibitors and chalcones in general showed poor activity. Among the furfural oxime ethers EOE was the most active followed by BOE (N-O-butyl furfural oxime) and POE. HOE performed poorly whereas AOE performed well initially but its activity did not last for long. EOE, POE, BOE remained active upto the 45th day, after which the activity fell rapidly. On the 15th day, at higher dose (20%), EOE, POE and BOE approached nitrapyrin (5%) in activity. At lower dose (5%), the activity of these compounds though considerable was lower than nitrapyrin.

Among the Schiff bases, the chlorinated products showed considerable activity upto 45 days. At 20% dose, CAS was more active than nitrapyrin upto 30th day and was comparable to nitrapyrin upto the 60th day. Nonchlorinated Schiff bases like NAS and AS showed initially high activity but at lower doses their activity fell rapidly with time and at higher doses, their activity persisted upto the 45th day. None of the treatments accumulated nitrite to a toxic level.

Application of EOE and CAS coated urea failed to show improvement when compared with their application in solution form. EOE, POE, CAS and DCAS also did not show any adverse effect on the germination of chickpea seeds.

Six coating techniques of karanjin [a natural furanoflavonoid from karanj (*Pongamia glabra* Vent.) seeds] with urea on nitrification inhibition were evaluated in laboratory incubation studies. Among the coating aids evaluated the alcohol extracts of karanj (KA) and neem seed kernels (NA), at 5% dose imparted the desired degree of stickiness and could be advantageously utilised for further coating of the other ingredients and also imparted certain degree of nitrification inhibition of their own.

Coating of karanjin (KN) using coal tar (C) as a binder significantly improved its activity. Urea coated with alcohol extracts of karanj/neem and karanjin inhibited nitrification to a greater extent as compared to coal tar. Urea coated with 10% (of applied urea-N dose), karanjin employing 5% (of urea-N) alcohol extracts of karanj and neem (*Azadirachta indica* A. Juss) seed kernels as binders showed the best performance. These doses showed more than 40% inhibition on 45th day. Urea coated with karanjin employing karanj oil failed to improve the activity as compared to karanjin alone treatment. Nitrification inhibition increased with an increase in the dose of karanjin but the activity fell with the passage of time.

Mixture of synthetic furfural derivatives and karanjin coated urea generally showed additive activity. At lower dose of karanjin (2.5%), the mixture usually followed the activity pattern of the synthetic partner whereas at its higher doses (more than 5%) the additional effect due to karanjin was observed. Mixture of alcohol extract of karanj seed kernels and the synthetic derivatives showed almost similar trend as witnessed by their mixture with karanjin. Effect of alcohol extract was more prominent at lower dose of the synthetic derivative (2.5%).

The physical properties of the coated fertilizers matched the Fertilizer Control Order specifications except for the nitrogen content. Though some of the treatments contained as low as 43% nitrogen (as compared to the minimum requirement of 45%-N for coated urea products) but their nitrification inhibition values indicated that such products will ultimately supply substantially more nitrogen to plant as compared to the common urea prills.

INTRODUCTION

INTRODUCTION

In 1798, Thomas Robert Malthus, an English clergyman warned the mankind about the detrimental consequences of the unchecked population growth. At that time, the world population was estimated to be 980 million. At present the population of India alone has crossed the one billion mark and a billion person is estimatedly added every 12 to 13 years worldwide. Still the Malthus's prophecy hasn't come true. The spectacular progress in agriculture has helped mankind to keep pace with the ever increasing population. Most of this success is attributed to the high cost inputs such as chemical fertilizers, pesticides, improved seeds, irrigation, scientific management and labour saving and energy intensive farm machinery.

Fertilizer is one of the vital agro inputs which has helped the mankind to march ahead to achieve the food and nutritional security. Use of mineral fertilizers has become globalised in the past fifty years (Ayoub, 1999). Among the major fertilizer elements, nitrogen is the most limiting nutrient throughout the world and hence is applied in maximum amount. In India, the use of nitrogenous fertilizers has risen from 3.6 Tg yr⁻¹ in 1950 to 81.1 Tg yr⁻¹ in 1997-98 (Ayoub, 1999; FAI, 1999) and is estimated to be 90.4 Tg yr⁻¹ in 2003-2004. During this period the world cereal production has risen from 1.13 Mg ha⁻¹ to 2.76 Mg ha⁻¹. To sustain the current per capita consumption in the year 2030, a further doubling of the nitrogenous fertilizers is foreseen. T-6745

Nitrogen use efficiency of various nitrogenous fertilizers is very low because of its susceptibility to loss by various processes viz. leaching, volatilization, and nitrification-denitrification (Chart 1.1). Nitrogen use efficiency is reported to be between 30-50% by various authors

(Beauchamp, 1997; Prasad, 1998; Raun and Johnson, 1999; Mohanty and Sharma, 2000) and it may be as low as 21% in case of lowland rice (DeDatta, 1995). Impact of N loss from rice paddies can be cited as an example. At a rate of 40% N loss (N loss upto 79% is reported from rice fields) 1.6 Tg of N is lost out of approx. 4 Tg of N applied to rice annually. It will take four-fertilizer factories of approximately 1000 Mg yr^{-1} N production capacity to replenish this loss annually.

The world Nitrogen Use Efficiency (NUE) in cereals is estimated at approximately 33 per cent. Based on the present fertilizer use, 1% increase in NUE for cereal production worldwide leads to a saving of \$234.66 million in N fertilizer costs (Raun and Johnson, 1999).

Among the nitrogenous fertilizers urea ranks first in terms of production and consumption. When it is applied in soil it converts to ammonium via formation of ammonium carbamate within 2-7 days. This ammonium N can be taken up by the plant or it may volatilize to atmosphere or be converted to nitrate by group of chemoautotrophic bacteria (Family Nitrobacteraceae) to nitrate. This nitrate can be taken up by plant or may be lost by leaching (being negatively charged it is highly susceptible to leaching) or denitrification. Because of leaching and denitrification of nitrate, nitrogenous fertilizer is implicated in environmental contamination more often than other nutrient. Excessive nitrate in ground and surface water causes eutrophication of surface water and high level of nitrate (10 mg N L^{-1}) in drinking water causes methaemoglobinemia in infants and in ruminants (Newbold, 1989). Denitrification of nitrate gives rise to nitrous oxide which is known as greenhouse gas (Beauchamp, 1997) accounting for 2-6% of total global warming (Mosier, 1994). Nitrous oxide (N_2O) also acts as destructant of

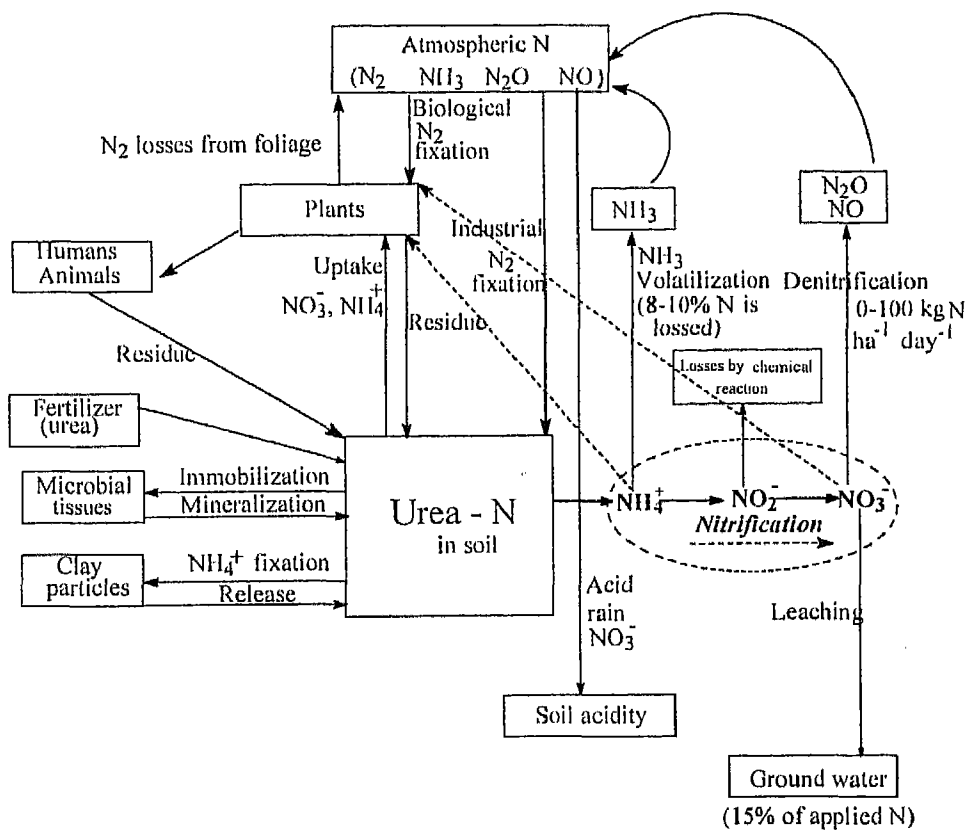


Chart 1.1 Urea - N dynamics in soil and environment

the protective ozone shield which saves living beings from the harmful effects of ultraviolet radiation (Prasad, 1998). Thus one third to one half of these applied nutrient is lost, only to create environmental problems.

Considering the above harmful effects of nitrogenous fertilizers and their low use efficiency, the need for improved fertilizer materials and new methods to manage nitrogen fertilizers to minimize the unwanted losses has been felt. Developed countries are increasingly adopting the principles of Low Input Sustainable Agriculture (LISA) and Integrated Plant Nutrient Supply System (IPNS) but still fertilizers form the most important constituent of such philosophy. Hence, various approaches have been suggested for increasing fertilizer nitrogen use efficiency (Chart 1.2). Among these, the use of nitrification inhibitors has attracted the attention of scientists throughout the world. Nitrification inhibitors inhibit the transformation of ammonium to nitrate and reduce the nitrate concentration and conserve ammonium, thus increasing the NUE.

Among nitrification inhibitors, Nitrapyrin [N-Serve, 2-chloro-6-(trichloromethyl)-pyridine], Etridiazole [Dwell, 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole], AM (2-amino-4-chloro-6-methyl-pyridine), DCD (dicyandiamide) are in practical use. A number of other compounds have also been tested as nitrification inhibitors, but these suffer from various drawbacks, including high cost, particularly for a developing country like India. On the contrary various indigenous compounds from natural sources as well simple synthetic derivatives of some easily available and cheap materials are reported to be possessing good nitrification inhibition property. Among these compounds some furfural derivatives have been tested and found to possess excellent nitrification inhibitory property (Sahrawat, 1973; Kuzvinzwa, 1974). Though their potential has been proved way back in

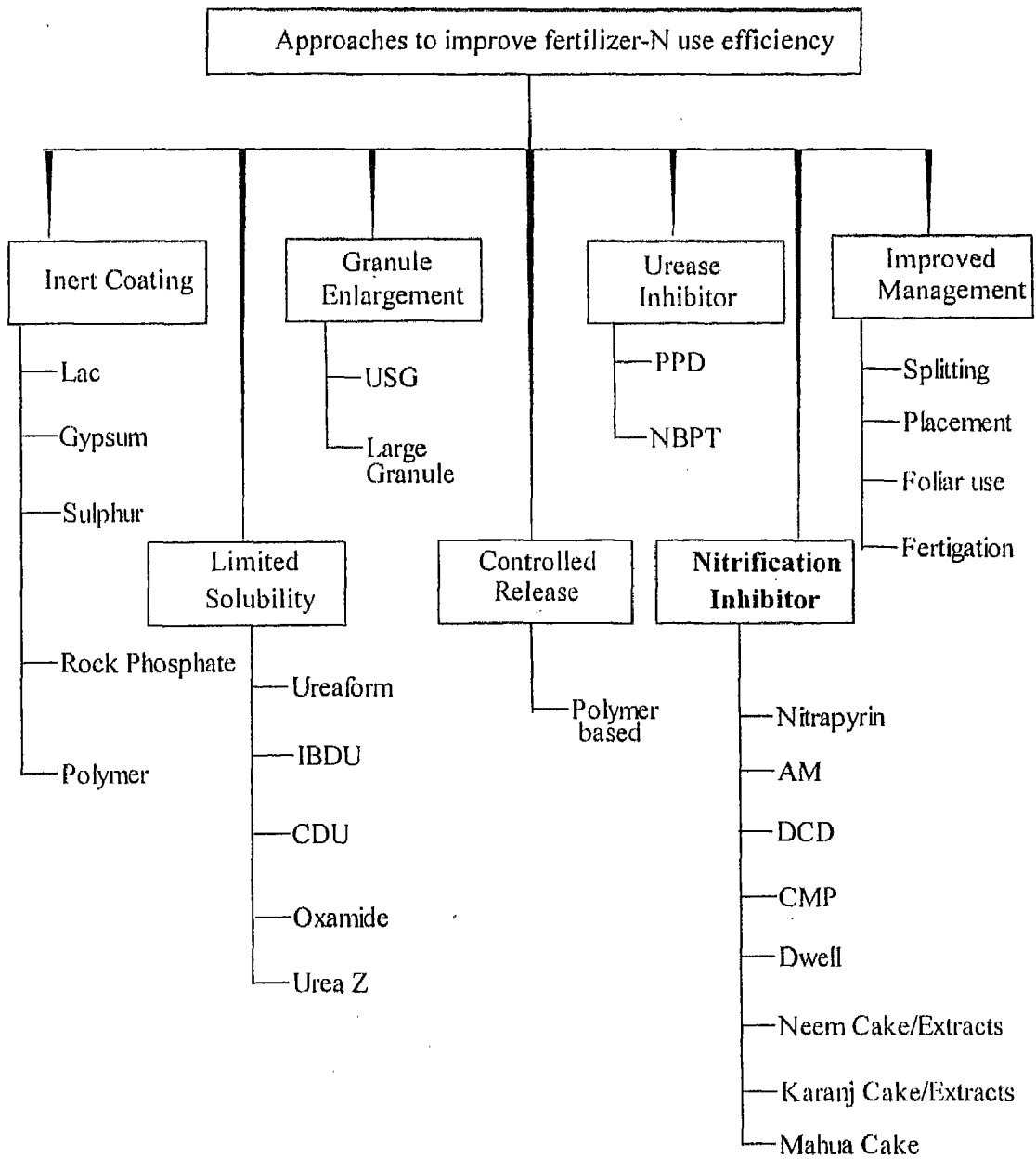


Chart 1.2 Approaches to improve fertilizer-N use efficiency (NUE)

70s, further evaluation to evaluate their practical use has not been carried out subsequently. Since furfural is cheaply available industrial byproduct any simple compound developed based on furfural may prove cost effective. This thinking framed the basis of choosing the present problem.

Numbers of natural products have been reported to possess nitrification inhibitory property. Neem (*Azadirachta indica* A. Juss) and karanj (*Pongamia glabra* Vent.) have been evaluated by several researchers. Neem bitters/extracts such as Nimin, Neemex (Prasad, 1998) etc. are already available in the Indian market and several such products are in the process of development such as neem coated urea by KRIBHCO. In the literature many researchers have reported that karanj cake and karanjin are even more effective as nitrification inhibitor than neem extractives (Kamire and Sonar, 1979; Sahrawat, 1982). But till today there is not a single karanj or karanjin based product available in the market probably because the required developmental and standardization work has not been carried out. Any slow release urea product based on karanj seed extracts or karanjin may prove economically viable because karanj plant is widely distributed throughout the country and its seed is cheaply available. So the available information on these materials need to be pursued further to develop worthwhile products.

Another important aspect of research on nitrification inhibitor studies is the application technology. It is important to see that these material or compounds are intimately mixed with the fertilizer. It has been suggested that coating of fertilizer with the solution of nitrification inhibitor is by far more efficient and effective than their simple mixing (Sahrawat, 1980; Suri, 1995). Ineffectiveness of nitrification inhibitors in many cases may be due to a dissimilar mobility of the fertilizer and nitrification inhibitor. Hence mobility of fertilizer and inhibitor with the soil solution needs to be taken into account.

With this background this research work entitled "Development of slow nitrogen release urea formulations employing *Pongamia glabra* Vent. isolates and some furfural derivatives" has been carried out with the following objectives :

- I. Development of slow nitrogen release urea products utilizing *Pongamia glabra* Vent. isolates and some furfural derivatives.
- II. Evaluation of physicochemical properties and performance of the developed products.

The results are contained in this dissertation.

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

Food and nutritional security, sustainability and profitability are the key thrusts of the present and future agricultural development strategies. The food production in India has increased from 50.8 Tg yr⁻¹ in 1950-51 to 203 Tg yr⁻¹ in 2000 and this increase has transformed India from a begging bowl status of 1950s to today's bread basket status. Various agroinputs like improved seeds, irrigation, improved farm machinery, fertilizers, pesticides etc have helped us to attain food security. Use of fertilizers has revolutionised modern agriculture. In fact nearly 50 per cent gain in food grain production seen in recent times has come through fertilizer alone (Singh and Biswas, 2000). Since the beginning of the green revolution, fertilizer (NPK) use has risen from 1.54 Tg yr⁻¹ in 1967-68 to 16.75 Tg yr⁻¹ in 1998-99, and is estimated to reach 45.48 Tg yr⁻¹ at the end of 11th plan in 2011-12 (FAI, 1999). India currently enjoys third position in the world only after China and USA in terms of fertilizer production and consumption. In the absence of fertilizers India would have required 2-3 times more land to produce the same quantity of food grains.

Among fertilizer nutrient elements, nitrogen is applied in maximum amount (67.59%, FAI, 1999). It is the major nutrient limiting agricultural production in most soils and N-fertilizers have made major contribution towards improving agricultural productivity the world over. Urea, because of its low cost and high analysis (46% N) is the most popular (83% of total nitrogenous fertilizers in 1998-99, FAI, 1999) nitrogenous fertilizer.

The Green Revolution has come to be associated with not only higher production through enhanced productivity, but also with several negative ecological and social consequences. The application of high input technologies has undoubtedly increased the production, but there is a

growing concern over the adverse effects of the use of these chemicals on soil productivity and environmental qualities. All the commonly used nitrogenous fertilizers especially urea suffer from two main drawbacks – low nitrogen use efficiency and contribution towards environmental pollution.

2.1 Nitrogen use efficiency (NUE) of urea

The nitrogen use efficiency hardly exceeds 50% and in case of low land rice culture, NUE even drops below 30% (DeDatta and Buresh, 1989; Raun and Johnson, 1999; Mohanty and Sharma, 2000). Thus more than 50% of the applied nutrient is lost, causing economic loss and environmental pollution.

2.2 Environmental pollution

Excessive use of nitrogenous fertilizers has raised environmental concerns, chief among these being eutrophication of fresh water bodies, global warming and stratosphere ozone depletion, proliferation of algal blooms in surface water, overloading of drinking water with harmful nitrates and contribution towards acid rain (Amsterl and Swart, 1994; Eichner, 1990). Thus the need for improved fertilizer materials and new methods to manage nitrogenous fertilizers has always been of interest to growers.

2.3 Approaches to increase NUE of applied nitrogenous fertilizers

Various approaches have been suggested to improve the NUE of the applied nitrogenous fertilizers. These include the use of coatings, chemical additives and the various chemical and physical modifications.

2.3.1 Inert coating : Soluble urea is coated with an insoluble but mostly biodegradable and slowly permeable material to achieve controlled and delayed release of urea-N. It is possible to prepare coated urea with a desired N release rate (Stangel *et al.*, 1993). Several organic and inorganic coating

materials such as sulphur, gypsum, lac, latexes, polyolefins, resins, plastics, polyurethanes, rock phosphates etc. have been attempted using three types of coating processes—rolling bed, falling curtain and fluidized bed.

2.3.2 Granule enlargement : This approach resulted in larger granules of urea and urea super granule (USG). The USG consisting of 1-2 discrete urea particles is not so efficient, but its proper deep placement (1 USG for 4 hills at 7-10 cm soil depth with the hole at the placement site closed) makes it efficient (Savant *et al.*, 1983).

2.3.3 Limited water soluble urea fertilizers : This group of compounds consisting of ureaforms (38% N), oxamide (30% N), isobutylidenediurea (IBDU, 30%N), urea-Z (35% N) etc are only slightly soluble in water. The rate of nitrogen release from these compounds depends upon water solubility, rate of microbiological action and chemical hydrolysis.

2.3.4 Urease inhibitors : These reduce the hydrolysis of urea by inhibiting soil urease activity and result in preventing rapid development of high partial pressure of NH_3 and high pH of flood water in rice fields and eventually reduce NH_3 volatilization losses (Phongpan *et al.*, 1995). Numerous compounds have been reported in literature as urease inhibitors, like phosphoramides, quinones, hydroxamates etc. (Chai and Bremner, 1987; McCarty *et al.*, 1989).

2.3.5 Nitrification inhibitors : Considerable research carried out throughout the world shows that nitrification inhibitors can reduce loss of applied nitrogen and increase NUE. These are discussed in detail under Section 2.4.

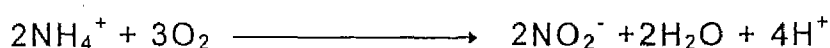
2.3.6 Improved management : Split application, placement, foliar application, fertigation etc are some of the agronomic techniques by which NUE of nitrogenous fertilizers can be increased (Yadvinder-Singh *et al.*, 1994, Raun and Johnson, 1999).

2.4 Concept of nitrification inhibitor

2.4.1 The nitrification process

Nitrification is a microbiological process by which ammonium is converted to nitrate via the intermediate formation of nitrite in a two step reaction mediated by two different groups of obligate chemolithoautotrophic bacteria (Family Nitrobacteraceae). Some heterotrophs are also involved in the process but they are of minor importance.

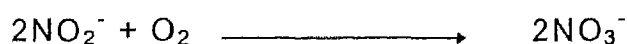
1st step :



Microorganism : *Nitrosomonas* (major) and also

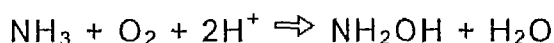
Nitrosolobus, *Nitrosospira*, *Nitrosovibrio* (minor)

2nd step :

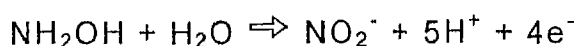


Microorganism : *Nitrobacter*

In-depth study (Bedard and Knowles, 1989) of ammonia oxidation revealed that hydroxylamine is first produced by a complex of membrane bound proteins that include ammonia monooxygenase (AMO) enzyme :



Monooxygenase activity requires a source of two electrons, which drive the reaction. The source of electrons is the further oxidation of the hydroxylamine within the organism by the following reaction, catalyzed by hydroxylamine oxidoreductase (HAO) :



The normal substrate for AMO has been found to be NH_3 rather than NH_4^+ . The ionic state of NH_4^+ reflects the active site properties such as hydrophilicity, which in turn affects the nature of other substrates and the inhibitors, that have a high affinity for the active AMO (McCarty, 1999).

2.4.2 Fate of nitrate

2.4.2.1 Leaching loss : As nitrate is negatively charged, it is more susceptible to leaching loss. As a result, relatively high fractions of the applied nitrogen may potentially be leached down from the root zone into the surface and ground waters (Smith *et al.* 1990). Deng and Tabatabai (2000) reported that 15% of the annually applied N-fertilizers and even greater percentages of residual N were leached into ground water.

High nitrate levels in drinking water may cause :

- ❖ Methaemoglobinemia in infants and in ruminants (Newbold, 1989).
- ❖ A possible link between nitrites and nitrosamines with gastric cancer, goiter, birth defects and heart diseases has been suggested (Forman, 1989; Black, 1989).
- ❖ Eutrophication of surface water.

Due to these undesirable effects, in USA the upper limit of nitrate in drinking water has been fixed at 10 mg N L^{-1} .

In many European countries the ground water is increasingly becoming loaded with nitrates (Spalding and Exner, 1993; Prasad and Power, 1995). Investigations carried out by several workers in India established that the upper aquifer (phreatic aquifer, watertable or unconfined aquifer) in many places contained high concentration of nitrates (Handa, 1987). Although our present level of N-consumption is rather low, there are reports of rising level of nitrate-N in ground water in few areas due to nitrification and subsequent leaching of nitrate-N from applied nitrogenous fertilizers (Singh and Biswas, 2000). In Ludhiana district of Punjab, the highest consumer of nitrogenous fertilizers in India, monitoring of shallow well waters from 1975 -1988 revealed that nitrate – N in well waters has increased considerably with the increase in fertilizer consumption from 56 kg ha^{-1} to 188 kg ha^{-1} (Prasad, 1998).

2.4.2.2 Denitrification : Denitrification is one of the major mechanisms of nitrogen loss from the agricultural fields, ranging from negligible to as high as 100 kg N ha^{-1} (Prasad, 1998). During denitrification process, nitrous and nitric oxides (N_2O and NO) are formed which are environment pollutants because of their role in ozone layer destruction which subsequently leads to atmospheric holes, thus exposing humans and animals to excessive ultraviolet radiation (Newbold, 1989). Nitrous oxide is also known as green house gas (Stangel *et al.*, 1993). It contributes upto 2-6% of the total global warming (Mosier *et al.*, 1996). Rodhe (1990) indicated that N_2O is 300 times more radioactive than CO_2 . The nitrous oxide fluxes from climate contribute to the greenhouse warming by about 4% (UNEP, 1992). It has been estimated that the fertilizer nitrogen contributes $1.5 \text{ Tg N}_2\text{O-N yr}^{-1}$ (Mosier *et al.*, 1996). Beauchamp (1997) reported that N_2O emission from chemical fertilizers varies from 0.03 to 2.0 Tg yr^{-1} . Fertilizer derived emission of N_2O in the year 2000 will account from 0.1 to 1.5% of the global source and the agriculture's contribution to global N_2O loading from the year 1986 to 2026 may increase by 90% with increased N fertilizer consumption (Prasad, 1998; Iserman, 1994).

Various oxides of nitrogen accumulated in the atmosphere from denitrification and volatilization form nitric acid, which forms acid rain ($\text{pH} < 5.7$). Though major contributing factor of acid rain is sulfuric acid, but nitric acid adds to the acidity and is an oxidiser. This acid rain causes corrosive damage and kills aquatic life.

The global consumption of nitrogen fertilizer, which was 81.1 Tg yr^{-1} in 1997-98 is estimated to increase to 1225 Tg yr^{-1} by the end of 21st century (Kawashima *et al.*, 1997). This estimate clearly indicates the possible threat to our environment from N-fertilizers in the future if serious steps are not taken to overcome the maladies. There is a continuing need to improve the efficiency of N-fertilizer use to achieve more efficient production of food crops and minimize the fertilizer-related pollution of the environment.

Table 2.1 : Some of the potentially adverse environmental and health effects caused by the different forms of nitrogen

Causative agent	Effect
Environment quality	
Nitrogen sources in surface water	Eutrophication (algal bloom)
Nitric acid in rainfall (acid rain)	Corrosive damage
Oxides of nitrogen (NO, N ₂ O)	Ozone layer depletion, global warming
Human health	
Excess NO ₃ ⁻ and NO ₂ ⁻ in food and water	Methemoglobinemia
Peroxy acetylnitrates and other nitrous oxides	Respiratory illness
Nitrosamines from NO ₂ ⁻ and secondary amines in food	Cancer

As a partial remedy to the problem, the concept of nitrification inhibition came in vogue. It addressed to the slowing down of the nitrification process. Nitrification inhibitors are compounds or materials that specifically retard the oxidation of ammonium (NH_4^+) to nitrite without affecting the subsequent oxidation of nitrite to nitrate. NH_4^+ being positively charged is less prone to leaching and is not of much environmental concern. Thus NUE of urea is increased and the environment is safeguarded.

An ideal nitrification inhibitor should possess the following characteristics (Slangen and Kerkhoff, 1984) :

- ❖ Nontoxic to plants, soil organisms, fishes and mammals.
- ❖ Specific activity against *Nitrosomonas* group of bacteria.
- ❖ It should move with the fertilizer so as to ensure uniform distribution in the soil.
- ❖ It should be able to maintain the inhibitory action for periods ranging from several weeks to months.
- ❖ Be relatively inexpensive.

2.5 Synthetic nitrification inhibitors

A number of synthetic chemicals have been reported as effective nitrification inhibitors. Among these few have been extensively tested. Some of the commercial and widely tested chemicals are listed in Table 2.2.

2.5.1 Nitrapyrin : Nitrapyrin [2-Chloro-6-(trichloromethyl)-pyridine, I], is a nitrification inhibitor developed by Dow Chemical Company. This product is marketed under the trade name of "N-Serve 24 Nitrogen Stabilizer" (a.i. 240 g L^{-1}) and "N-Serve 24E Nitrogen Stabilizer" (a.i. 240 g L^{-1}). The rates of application advised by Dow Chemical Company for band and row placement are 1.125-1.25 L ha^{-1} of N-Serve 24 E for cotton, maize, sugerbeet, sorghum and wheat and 4.50 – 6.75 L ha^{-1} for potatoes before or after planting or sowing. For

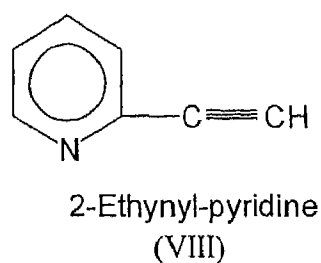
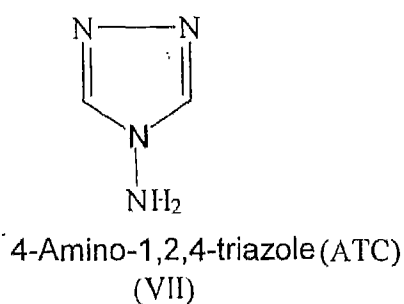
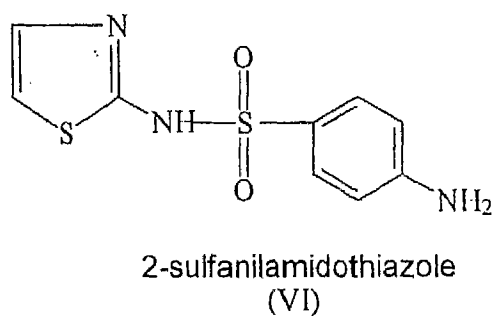
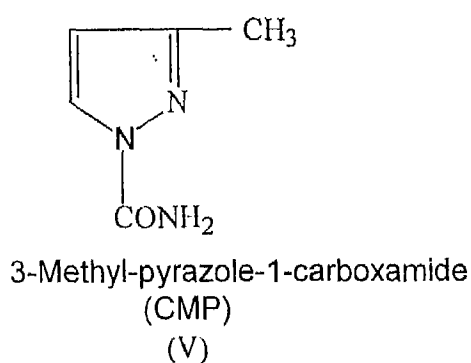
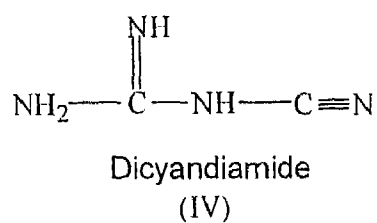
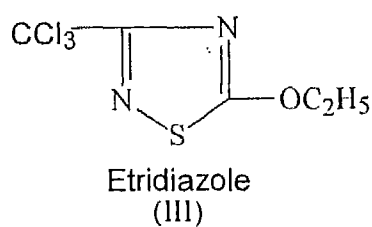
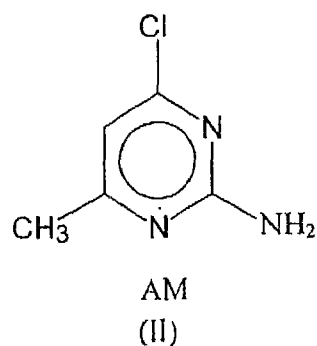
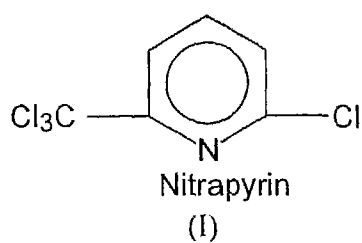


Chart 2.1 Some commercial nitrification inhibitors

broadcasting, the rate of application has to be increased considerably. When granulated fertilizer is used, it can be applied at 0.2-1% of the amount of the fertilizer N (Goring 1962ab, Goring and Scott, 1976). Because of its high vapor pressure, nitrapyrin cannot be granulated with solid-N fertilizer like urea without loss of the inhibitor during processing, storing and handling (Yadvinder-Singh *et al.*, 1994). Nitrapyrin sometimes shows poor activity due to sorption on soil colloids, hydrolysis to 6-picolinic acid and loss by volatilization.

2.5.2 AM : 2-Amino-4-chloro-6-methylpyrimidine (II), is another well-known nitrification inhibitor developed by Toyo Kaotsu Industries Inc. (now Mitsui Toatsu) of Japan (Toyo Kaotsu Industries Inc., 1965). Pure AM is a white crystalline substance (mp, 182°C) and is soluble in water but unlike nitrapyrin, it is relatively insoluble in organic solvents. AM is less volatile and less effective than nitrapyrin. AM is effective when applied @ 5-6 kg ha⁻¹ (Prasad *et al.*, 1971).

2.5.3 Etridiazole : 5-Ethoxy-3-trichloromethyl-1,2,4thiadiazole (Terrazole, Etridiazole, Dwell, III) is an effective nitrification inhibitor developed by Olin Corporation, Baltimore, USA (Olin Corporation, 1976ab). This product is available as a wettable powder or technical grade liquid with 35% and 95% a.i., respectively. As a coating on ammonium sulphate and urea, terrazole 95% a.i. is used upto 1.5% by weight. Rates of application advised by Olin Corporation are 0.6-1 kg ha⁻¹ (Turner and McGregor, 1978). Beside this compound some other thiodiazoles are also known to inhibit ammonia oxidation. Among them 3,4-dichloro-1,2,5-thiadiazole is noteworthy.

2.5.4 Dicyandiamide (DCD) : It (IV) has been developed both as a slow release nitrogen source as well as nitrification inhibitor (Amberger and Guster, 1978; Rochester *et al.*, 1994). In Japan it is added to mixed fertilizer and a product containing ureaform plus 10% by weight of DCD is produced. A fertilizer containing urea and DCD in a 4:1 ratio is commercially available in West Germany. DCD is toxic to plants but the effects differ with plant species

Table 2.2 : Some commercial and extensively tested synthetic nitrification inhibitors

Nitrification Inhibitor	Reference
Nitrapyrin [2-Chloro-6-(trichloromethyl)-pyridine]	Goring 1962ab
AM [2-Amino-4-chloro-6-methyl pyrimidine]	Toyo Koatsu Industries, 1965
DCD [Dicyandiamide]	Amberger and Guster, 1978
Terrazole or Dwell or Etridiazole [5-Ethoxy-3-trichloromethyl 1,2,4 thiadiazole]	Olin Corporation, 1976ab
DCS [N- (2,5-Dichlorophenyl succinamic acid)]	Namioka and Komaki, 1975
KN ₃ (Potassium azide)	Huges and Welch, 1970
ATC (3-Amino 1,2,4 triazole)	Guthrie and Bomke, 1980
TU (Thiourea)	Goring, 1962b
MBT (2-Mercaptobenzothiazole)	Hauck, 1972
2-Ethynyl pyridine	McCarty and Bremner, 1986
MPC (3-Methyl-pyrazole-1-carboxamide)	McCarty and Bremner, 1990a
ST (2-Sulfanilamidothiazole)	Mitsui Toatsu chemical inc., 1968
Carbondisulfide	Ashworth <i>et al.</i> , 1977
3-Mercapto 1,2,4 triazole	McCarty and Bremner, 1989
Sodium diethylthiocarbamate	Prasad and Power, 1995
Acetylene	Hynes and Knowles, 1981
Gaseous hydrocarbon such as ethane, ethylene, methane	McCarty and Bremner, 1991
Ammonium thiosulfate	Goos and Johnson, 1992
Thiophosphoryl triamide	Radel <i>et al.</i> , 1992
4-Mesyl benzotrichloride	Hauck, 1972
4-Nitrobenzotrichloride	Heytler <i>et al.</i> , 1984
Guanyl thiourea	Hauck, 1972
2,4-Diamino-6-trichloromethyl triazine	Hauck, 1972
Potassium trithiocarbonate	Zourarakis and Killorn, 1990
Sodium thiocarbonate	Ashworth <i>et al.</i> , 1977
2-Amino-4-methyl-6-trichloromethyltriazine (MAST)	Hauck, 1972
2-Benzothiazole-sulfane-morpholine	Hauck, 1972

(Gasser, 1970). This compound is effective over a period of 1-3 months. A comparatively higher rate of the compound is needed. When applied @ 10-15% of applied nitrogen the compound remains active for a period of 2 months. Cost of production of DCD is however lower than the corresponding cost of nitrapyrin and etridiazole and DCD has the advantage that it completely decomposes in soil to CO_2 and NH_4^+ over several weeks and thereby acts as a high analysis (66.7% N) slow release N fertilizer. Compounds containing ammonium sulphate and dicyandiamide are available in granulated and coated form from Suddesche Kalkstickstoffwerke AG, Trostberg and Sisco Corporation Japan and recommended as slow release fertilizers. Increase in NUE of urea by DCD in field studies on different crops have been reported by various authors. (Sharma and Kumar, 1998; Montemurro *et al.*, 1998).

2.5.5 Pyrazoles : CMP [1-carbamoyl-3-methylpyrazole, V] has been found to be an effective nitrification inhibitor (McCarty and Bremner, 1990a). Under flooded conditions, CMP affected almost total inhibition of nitrification and prevented build up of nitrite and nitrate in floodwater (Blaise *et al.*, 1999). In pure culture studies, CMP inhibited the growth of *Nitrosomonas* at concentrations as low as 1 ppm. Researchers in Germany have screened over 25000 compounds for inhibition of NH_3 oxidation in soil and found that CMP was the most promising (Walter *et al.*, 1985). McCarty and Bremner (1989) tested 11 pyrazoles, most of which exhibited strong nitrification inhibition property.

2.5.6 Sulfathiazoles : A group of thiazoles such as sulfathiazoles and especially 2-sulfanilamidothiazole (ST, VI) were introduced as nitrification inhibitor by Mitsui Toatsu Chemicals Inc., Japan (1968). ST is more stable than AM and can be formulated with both acidic and basic fertilizers. It is apparently more volatile than AM. Prasad and Reddy (1977) tested six sulpha

drugs in soil incubation studies and found that 2-sulphanilamidothiazole was most effective in inhibiting nitrification at 2 ppm level. ST was commercially used in Japan at a limited scale.

2.5.7 Triazoles : Many triazoles particularly 1,2,4-triazoles are reported to have nitrification inhibition activity. Among these compounds 4-amino-1,2,4-triazole (ATC, VII) is the most potent inhibitor but not as effective as nitrapyrin. ATC was produced from formic acid and hydrazine by Ishihara Industries, Japan. It completely checks nitrification of urea for four weeks at a concentration of 5% by weight of urea (Hauck, 1972).

2.5.8 Pyridines : Among the various pyridines tested, 2-ethynylpyridine (VIII) and nitrapyrin are the most potent inhibitors of nitrification but other compounds also possess this activity. Among those, 2-chloropyridine, 2,6-dichloropyridine, 6-chloro-2-picoline significantly inhibited ammonia oxidation in soil whereas compounds containing carboxylic group (6-chloro-picolinic acid) had little effect (McCarty and Bremner, 1989).

2.5.9 Acetylenic compounds : Studies on several acetylenic compounds showed that these inhibited oxidation of ammonia to nitrate in soil and could be developed as a fertilizer amendment for retarding nitrification. Among these, 2-ethynylpyridine is a potent inhibitor of nitrification (McCarty and Bremner, 1986; McCarty and Bremner, 1990b). When applied @ 5 g kg⁻¹ of soil, 2-ethynylpyridine showed better activity than etridiazole and nitrapyrin. Besides 2-ethynylpyridine, phenylacetylene is also a potent nitrification inhibitor. Other gaseous hydrocarbons like ethylene, ethane and methane also exhibited nitrification inhibitory property in the order acetylene > ethylene > ethane > methane (McCarty and Bremner, 1991).

2.5.10 Furan derivatives : Furan derivatives are known for their antibacterial action since 1925. Novikov *et al.* (1963) studied the physiological activity of simple nitrofuran derivatives. A compound of structure IX was found to be

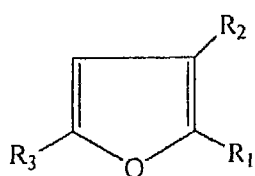
bactericidal, fungicidal and herbicidal.

Nardi *et al.* (1967) synthesized a series of 5-nitrofurfural semicarbazones and thiosemicarbazones (X) and screened for antibacterial activity and some of the thiosemicarbazone showed antifungal activity.

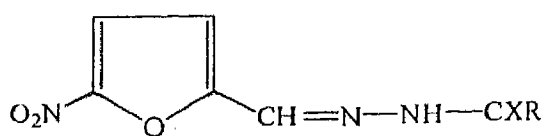
Powers and Mertes (1970) observed antibacterial action of nitro derivatives of benzofurans. They reported that 3,7-dinitrobenzofuran (XVII) was as potent as furazin (XI). Thirty-five new nitrofurans were synthesized and their bacteriostatic and fungistatic properties were studied and compared with furazin, 3-(5'-nitrofurfurylidene)-amino-2-oxazolidinone (furazone, XII), 5-nitrofurfurylidene-phenyl hydrazine (XIII) and its *p*-nitro analogue (XIV). The derivatives of aliphatic and alicyclic ketones were found to be bacteriostatic. The 3-(5'-nitrofurfurylidene)-2-butanone (XV) and the pentanone (XVI) were most active equaling furazin (Endra and Tibur, 1967). The compound 5'-nitrofurfurylidene-2,4-thiazolidinedione (XVIII) is also known to possess bactericidal property (Nguyen *et al.*, 1993).

Sahrawat and Mukherjee (1977b) after observing the effect of furan ring on nitrification inhibition screened some furano compounds like furfural (XIX) and furfural alcohol (XX) for possible effect on nitrification. They observed that 20% to 30% concentrations of these compounds matched 5% karanjin (the major furanoflavonoid constituent of *Pongamia glabra*) in nitrification inhibition during 45-60 days. The inhibition of nitrification decreased after 45 days while karanjin remained effective even after 45 days. Furfural alcohol was found to be a better inhibitor of ammonium sulphate than that of urea.

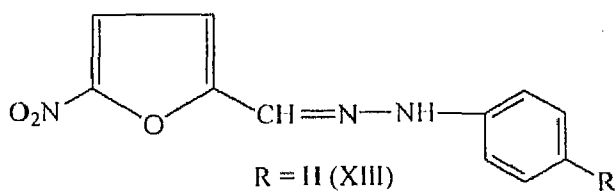
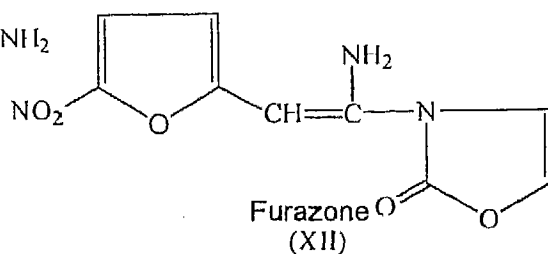
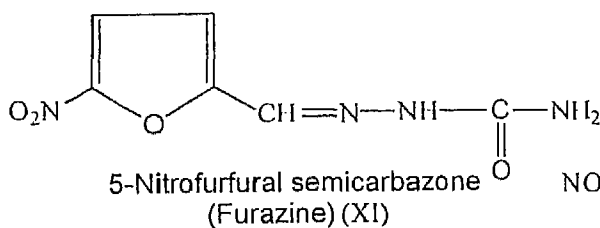
Mutinsky and Svehla (1976) evaluated the effect of urea-furfural condensates with urea-furfural ratios varying from 1 : 1 to 3 : 1 on the yield of oat in pot trials. Grain yields increased with increase in the ratio from 1 : 1 to 3 : 1 upto a maximum of 19.9 % more than the control where N was applied as urea alone.



$R_1 = \text{H, Br, NO}_2, \text{CH=NOH or COOEt}$
 $R_2 = \text{H, NHCOOEt, NHCOOCH}_3 \text{ or COOH}$
 $R_3 = \text{H, NO}_2, \text{CH=CHNO}_2, \text{Br or NHCONH}_2$
 (IX)



$X = \text{S or O}$
 $R = \text{NEt}_2 \text{ or piperidino}$
 (X)



$R = \text{H (XIII)}$
 $R = \text{NO}_2 \text{ (XIV)}$
 5'-Nitrofurfurylidene phenyl hydrazine

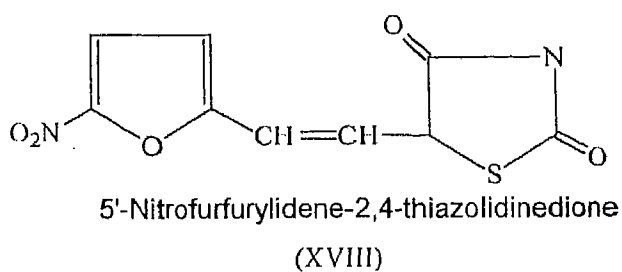
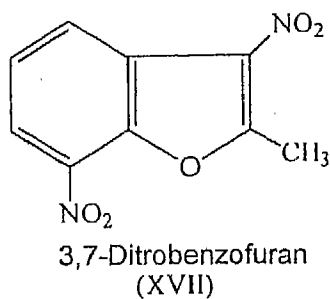
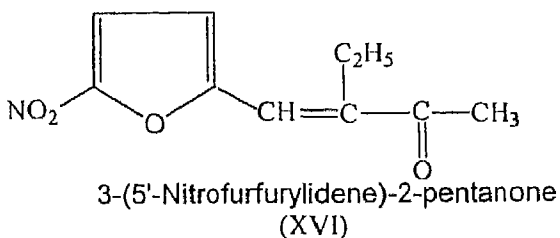
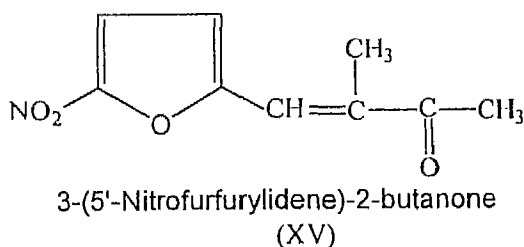


Chart 2.2 Bactericidal furan derivatives

Kuzvinzwa (1974) tested eight compounds mostly derivatives of furfural along with a natural furanocoumerin, psoralen for nitrification inhibition activity in laboratory incubation studies. The test chemicals were applied at 5, 10 and 15% concentration of applied nitrogen (200 ppm N) using ammonium sulphate and urea as N sources. He reported that three compounds namely 5-nitrofurfural oxime (XXI), furfural oxime (XXII) and furfural semicarbazone (XXIII) were most effective followed by 5-nitrofurfural semicarbazone (XXIV), 5'-nitro-3-chloro-2-furanilide (XXV) and psoralen (XXVI). Among this compounds only 5-nitrofurfural oxime approached nitrapyrin in effectiveness. It was further observed that the nitro derivatives tended to become general bactericides and became effective against *Nitrobacter* species also, thereby causing accumulation of nitrite nitrogen. 3-chlorofurananilide and furfural oxime caused very little accumulation of nitrite even at the highest concentration (15 %, N-basis).

Starting material for the synthesis of the above-mentioned compounds was furfural. Furfural is a cheaply available industrial byproduct manufactured from agricultural waste materials such as corncobs, oat hulls, wood wastes and bagasse, which are rich in pentosans (xylan, arabinan). The pentosans on acid hydrolysis release pentose units (mainly xylose) that dehydrate to yield furfural on steam distillation. The economics of furfural production is highly dependent on pentosan content, availability and cost of raw materials as well as the expense of collecting, shipping and handling of material (Kirk-Othmer Encyclopedia of Chemical Technology, 1980).

The question of the conformational preference of furfural has been studied using a wide variety of methods. The *trans* form is more preponderant in vapour phase, but the *cis* isomer is undoubtedly the more abundant in dipolar solvents (Cheng *et al.*, 1975).

It is reasonable to assume that any simple synthetic product

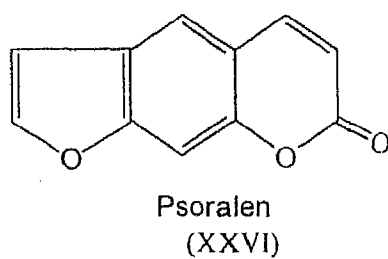
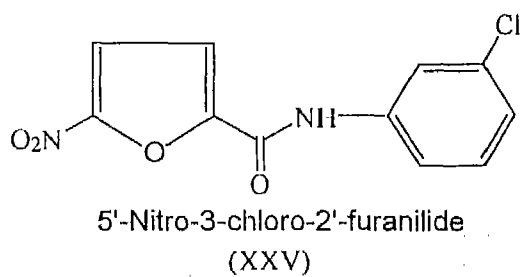
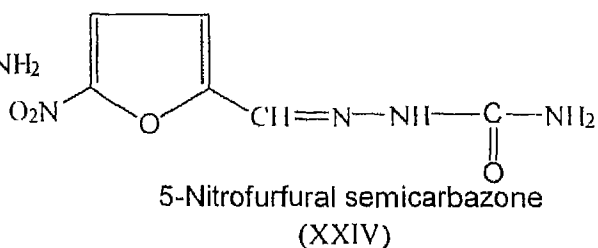
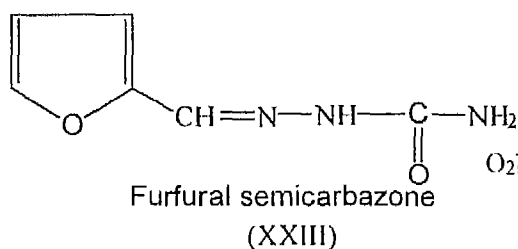
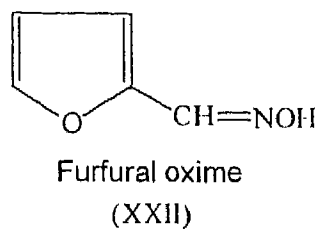
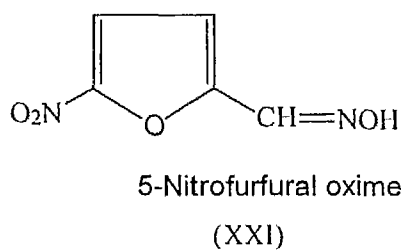
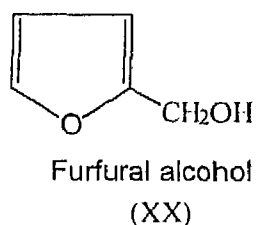
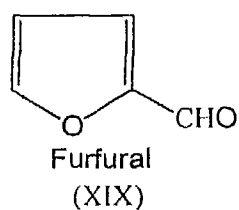


Chart 2.3 Some furan derivatives having nitrification inhibition property

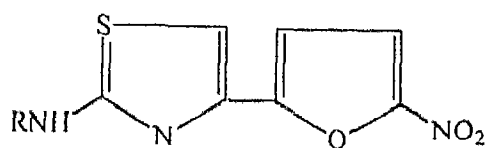
developed from furfural, even if endowed with moderate activity, can be used as an economically viable and environmentally acceptable adjuvant in urea formulations.

Besides simple furan based compounds, a number of complex furan derivatives have been reported to be general bactericides. Some of these compounds include 2-aryl-amino-4-(5-nitro-2-furyl)-thiazoles ($R = 2\text{MeC}_6\text{H}_4$, $4\text{-MeC}_6\text{H}_4$, $4\text{-O}_2\text{NC}_6\text{H}_4$, $2\text{-ClC}_6\text{H}_4$; Shivarama *et al.*, 1992, XXVII), compounds containing iso-oxazoles (XXVIII), triazoles (XXIX) and 1, 4-diketones (Bratenko, *et al.* 1991, XXX), benzofuranyl hydrazides (Nasef *et al.*, 1991, XXXI) and furansulfonyl amino acid derivatives (Badies, 1991, XXXII).

2.5.11 Miscellaneous : Among the unsubstituted heterocyclic nitrogenous compounds, those containing two or three adjacent ring nitrogens [pyrazole (XXXIII), 1,2,4-triazole (XXXIV), pyridazine (XXXV), benzotriazole (XXXVI), indazole (XXXVII), thiadiazole (XXXVIII)] significantly inhibited nitrification in soil, whereas those containing two or three non-adjacent ring nitrogen atoms [pyrimidine (XXXIX), imidazole (XL), s-triazine (XLI), benzimidazole (XLII)] or only one ring N atom [pyridine (XLIII), pyrrole (XLIV), indole (XLV)] had little or no effect on nitrification (McCarty and Bremner, 1989).

Goos and Johnson (1992) studied the effect of ammonium thiosulphate (ATS), dicyandiamide (DCD) and ATS/DCD mixtures on the nitrification of banded urea solution or urea ammonium nitrate (UAN) in field and laboratory conditions. The laboratory study demonstrated that adding 6.7% (v/v) ATS and DCD showed better effect than DCD alone. In field microplot studies, the addition of ATS to urea solution significantly increased the residual soil ammonium levels over urea alone at 6 of 11 trials and ATS + DCD outperformed DCD alone at only 1 of the 11 trials.

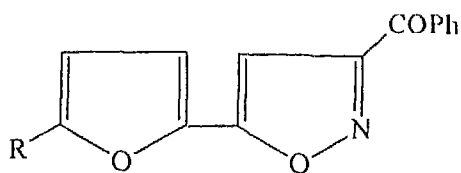
Guanidine sulphate was tested as a slow release nitrogen fertilizer as well as a nitrification inhibitor (Zhang *et al.*, 1992). It was concluded that guanidine sulphate had little effect in inhibiting nitrification and showed a



R = 2C₆H₄CH₃
 4C₆H₄CH₃
 C₆H₄NO₂
 2C₆H₄Cl

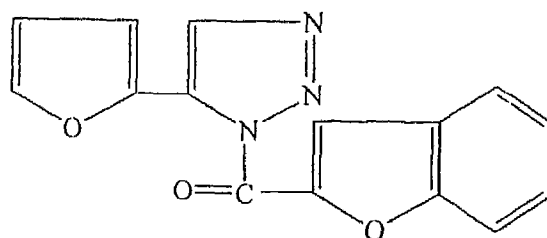
2-Aryl-amino-4-(5-nitro-2-furyl)-thiazoles

(XXVII)

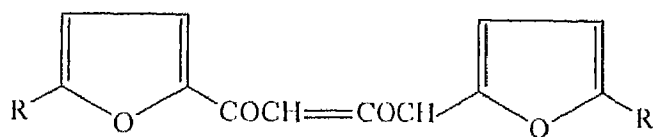


R = PhCH : CH
 4-BrC₆H₄CH : CH
 4-ClC₆H₄CH : CH

Iso-oxazoles
 (XXVIII)

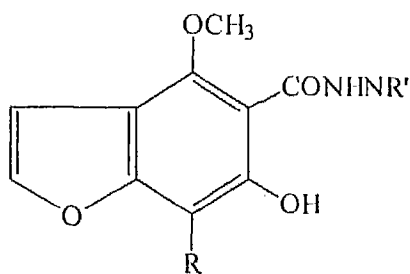


Triazoles
 (XXIX)

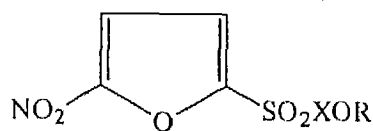


1,4-Diketone
 (XXX)

R = PhCH : CH
 4-BrC₆H₄CH : CH
 4-ClC₆H₄CH : CH

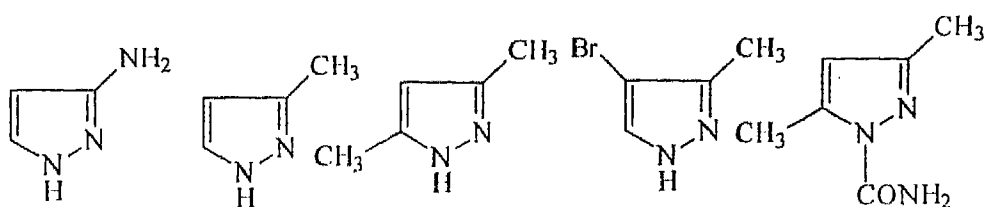


R = H, O, CH₃
 Benzofuranyl hydrazides
 (XXXI)

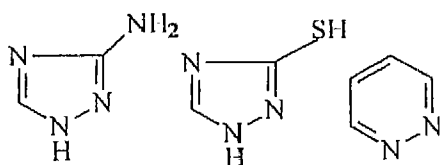


R = H
 X = Gly, β Ala, Val, Leu, Phe, Tyr
 Furansulfonyl aminoacid derivatives
 (XXXII)

Chart 2.4 Some complex furan based compounds with general bactericidal activities

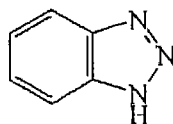


Pyrazoles (XXXIII)

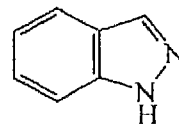


1,2,4 Triazoles
(XXXIV)

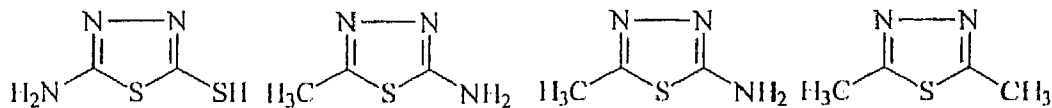
Pyridadazine
(XXXV)



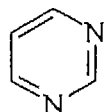
Benzotriazole
(XXXVI)



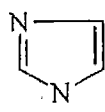
Indazole
(XXXVII)



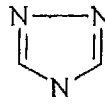
Thiadiazoles (XXXVIII)



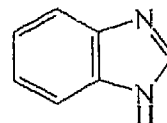
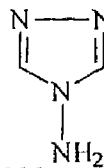
Pyrimidine
(XXXIX)



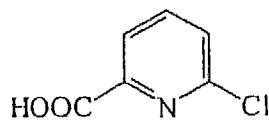
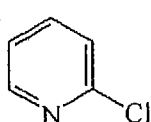
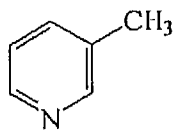
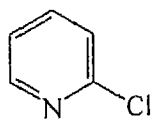
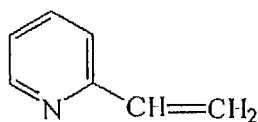
Imidazole
(XL)



Triazines
(XLI)



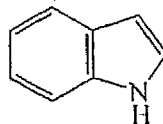
Benzimidazole
(XLII)



Pyridines (XLIII)



Pyrrole
(XLIV)



Indole
(XLV)

Chart. 2.5 Some aromatic heterocyclics studied for nitrification inhibition properties

marked short term slow release of mineral N, especially when banded.

Dow Elanco Company has developed a new nitrification inhibitor XDE-474 (Kpombekou-A and Killorn, 1995). The authors found that XDE-474 in general was more effective than nitrapyrin when applied at a dose of 0.05, 0.10, 0.20 and 0.40 active ingredient g^{-1} of soil. Tsai and Huber, 1996 observed that XDE-474 was more effective than nitrapyrin in maize crop.

In an experiment conducted by Mandal and Parsons (1996) in rye crop, potassium ethyl xanthate a nitrification inhibitor when applied with NH_4Cl and NaNO_3 increased N, Fe and Mn contents and uptake and decreased P level and uptake.

Kholdebarin *et al.* (1998) tested uncouplers of oxidative phosphorylation, DNP (2,4-dinitrophenol) and CCCP (carbonylcyanide-*m*-chlorophenylhydrazone) and one inhibitor of terminal oxidase – KCN (potassium cyanide) for their possible effect on nitrification inhibition. Due to its rapid hydrolysis, DNP was inactive whereas KCN reduced and CCCP fully inhibited the oxidation of NH_4^+ .

Sharma and Tomar, (1998) tested α -HCH and its two derivatives 1,2,4 trichloro-5-nitrobenzene (TCNB) and 1,2-dichloro-4-(2-hydroxy-ethyl-amino)-5-nitrobenzene (DCAB) for their possible effect as nitrification inhibitor in pot culture study with rice wheat cropping sequence. N-uptake showed that both α -HCH and DCAB were comparable to DCD in regulating the efficacy of soil applied urea.

Tomar *et al.* (1998) evaluated four heterocyclic derivatives viz. 4-methyl-6-phenyl-2-oxo-1,2,5,6-tetrahydropyrimidine (MPDP), 4-methyl-6-phenyl-2-thio-1,2,5,6-tetrahydropyrimidine (MPTP), 1-H-3-methyl-5-phenyl-2-pyrazoline (PMP) and 1-acetyl-3-methyl-5-phenyl-2-pyrazoline (APMP) for nitrification inhibition activity in pigeonpea-wheat cropping sequence in green house experiments. These compounds applied at 5% of nitrogen in an Inceptisol, invariably showed better N uptake in both the crops as compared

to control and urea alone.

Beside these compounds, various other compounds namely, potassium chloride, chlorates, sodium cyanite, quinhydrone, formaldehyde, guanidine, hydroxylamines, hydrazines, ethyl urethrene etc are known to possess nitrification inhibition activity.

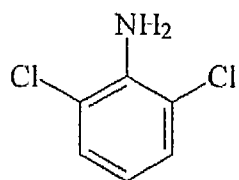
Anilines are also known to possess nitrification inhibition property. Their nitrification inhibition property increased with increasing chlorine substitution. 2,5-dichloroaniline (Thompson and Corke, 1969; XLVI), N-(2,5-dichlorophenyl) succinamic acid (Namioka and Komaki, 1975; XLVII), *m*-chloroacetanilide (Andreeva and Schcheglova, 1968; XLVIII) and furan-3-chloro-anilide (Kuzvinzwa *et al.*, 1984) have been shown to possess nitrification inhibition property.

Various compounds, which can form chelates with copper, are reported to have nitrification inhibition property. Allylthiourea and thiourea (Weir, 1966; Lees, 1963), potassium-ethyl-xanthate, sodium dithiocarbamate, salicyladoxime inhibited nitrification by chelation of copper. The *m* and *p*-chloro-phenylisocyanates (XLIX, L) have been found to be effective retarders (Harada *et al.*, 1964) and 2,5-dichlorophenylisocyanate (LI) was very effective, next only to nitrapyrin in increasing rice yield (Nishihara and Tsunyoshi, 1968). The nitrification inhibitory action of some volatile 'S' compounds are also reported (Maddux *et al.*, 1985). Carbondisulfide (CS₂), a highly effective nitrification inhibitor can be generated from trithiocarbonate and application of such compounds is possible as they are not volatile, inflammable and have less offensive smell than CS₂ (Ashworth *et al.* 1977). Potassium and sodium azides are also effective nitrification inhibitors (Bundy and Bremner, 1973).

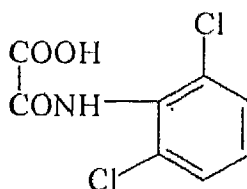
2.6 Nitrification inhibitors from natural sources

2.6.1 Biological nitrification inhibitors

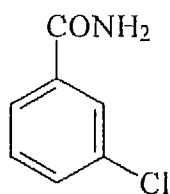
Synthetic nitrification inhibitors can efficiently inhibit



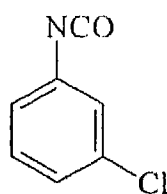
2, 5-Dichloroaniline
(XLVI)



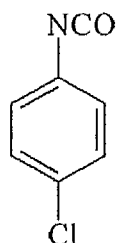
N--2,5 Dichlorophenyl
succinamic acid
(XLVII)



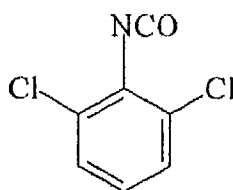
m-Chloroacetanilide
(XLVIII)



m-Chloro
phenylisocyanate
(XLIX)



p-Chloro
phenylisocyanate
(L)



2,5-Dichlorophenyl
isocyanate
(LI)

Chart 2.6 Some aromatic compounds tested for nitrification inhibition activity

nitrification process but these compounds are relatively expensive and not available in number of countries. Some naturally occurring materials mostly of plant origin have been tested and among them neem *Azadirachta indica* A. Juss based products like Nimin, Neemex and NeemGold-A are already available in the Indian market for improving the N-use efficiency. In the following section, some of the work carried out with various plant products have been discussed.

2.6.1.1 Nonedible oil seeds

Nonedible oil seeds like neem (*Azadirachta indica* A. Juss.), karanj (*Pongamia glabra* Vent.), mahua (*Madhuca indica*, *M. latifolia*) have been extensively studied for nitrification inhibition properties (Sahrawat *et al.*, 1974; Sahrawat and Parmar, 1975; Reddy and Prasad, 1975; Parmar *et al.*, 1976; Sahrawat, 1981; Sahrawat 1982b). Oil cakes in general and nonedible oil cakes in particular have been known to possess certain non fatty minor physiologically active constituents which make these oilseeds unsuitable for human consumption. These oilseeds have been traditionally used as slow nutrient release manures or in admixture with manures to regulate the nutrient release. Several of their biologically active constituents have been separated and tested for wide range of activity including pesticidal and nitrification inhibitory activity (Sinha, 1964; Singh, 1966; Vimal and Naphade, 1980).

Phyman and Bal (1919) studied the nitrifiability of oil cakes like karanj and til and reported that the presence of oil retarded the rate of nitrification in case of til cake. But this couldn't claim the superiority of neem and karanj cake over others. Later it was thought that the beneficial effects of nonedible oilseeds were because of their ability to control soil microbes due to presence of bitter compounds in them.

2.6.1.1.1 *Pongamia glabra* (Syn. *P. Pinnata*, pongam, karanj, karanja, sukhchein, Indian Beech) : *Pongamia glabra* Vent. (Legumoinaceae, papilionaceae), a medium sized glabrous tree, popularly known as karanj is

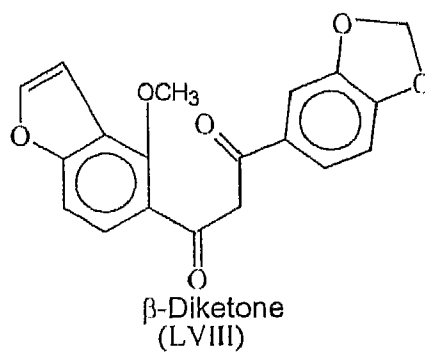
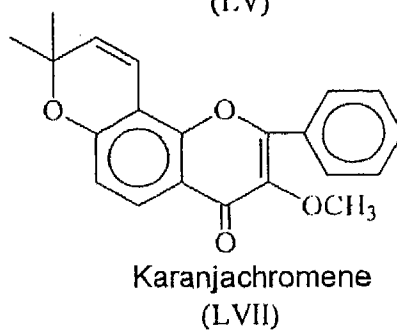
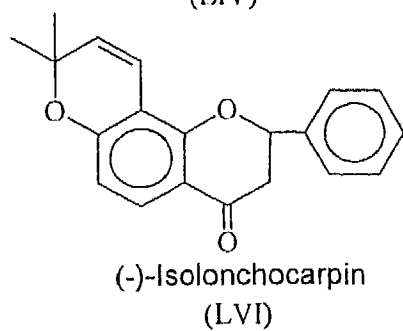
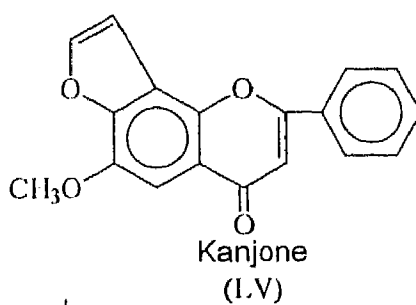
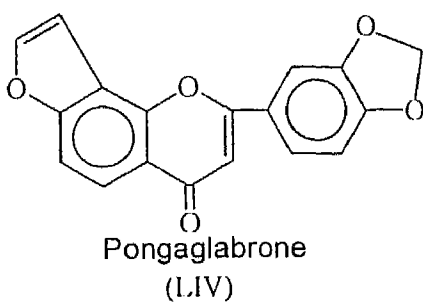
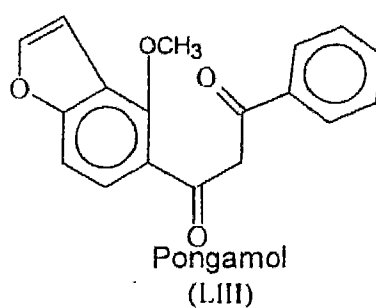
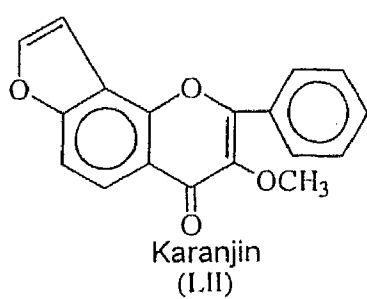
an important shade tree in India. The plant includes perplexingly variable forms, sometimes classified as different varieties and species. A comparative study of the chemical components of the various parts of the Indian and Australian materials suggested that they may be distinct. The tree is considered native to Western Ghat but it can be grown in wide range of agroclimatic conditions and upto the elevation of 1000 m. It is commonly found along the banks of streams, rivers, tidal forest and sea coast. It starts bearing at the age of 4-7 years. The pod becomes ready to harvest generally between November to May-June depending upon agroclimatic conditions of a particular region (Parmar *et al.*, 1976; The wealth of India, 1969). The yield usually varies from 9-90 kg per tree. Karanj (*Pongamia glabra/pinnata*) is different from karanjwa (*Caesalpinia bonducella*) though both are used in Unani medicines (Afaq *et al.*, 1997)

2.6.1.1.1.1 Chemical composition of karanj seeds: Karanj is one of the most extensively investigated plants because of its bioactive properties. A large number of biogenetically related compounds has been isolated, characterised and evaluated for biological properties.

The mature karanj seeds (av. wt. 1 g) consists of about 5% of total shell and 95% of oleaginous kernel. Air dried sample on an average consists of 19% moisture, 27.5% fatty oil, 17.4% protein, 6.6% starch, 7.3% crude fiber and 2.4% ash. The seeds contain mucilage (13.5%), traces of essential oil, complex amino acid glabrin ($C_{21}H_{42}O_{12}N_3$, mp, 290° C) and some distinctive chemical constituents.

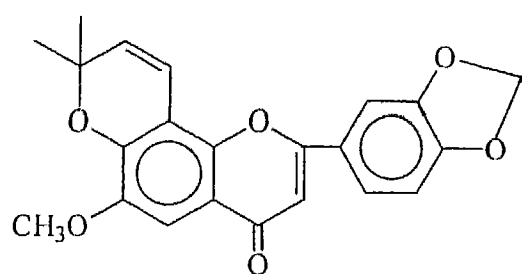
The seeds contain 27-39% fatty oil, which is used for leather dressing, soap making, lubrication, illumination and for medical purposes. Crude pongam oil has a yellowish orange to brown colour, which darkens on storage. It has a disagreeable odour and bitter taste with the following characteristics :

Specific gravity at 30°C : 0.9373, saponification value 181.5°,

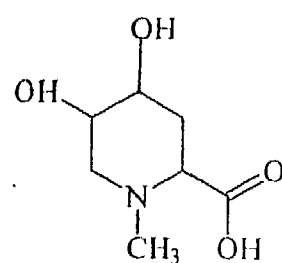


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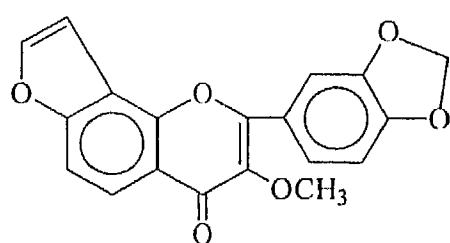
Chart 2.7 Compounds identified in karanj seeds



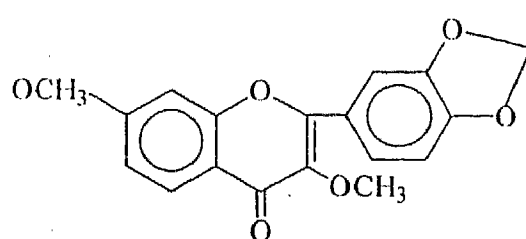
Isopongachromene
(LIX)



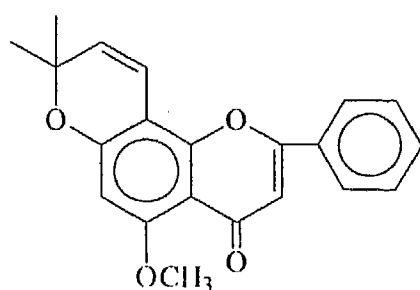
Glabrin
(LX)



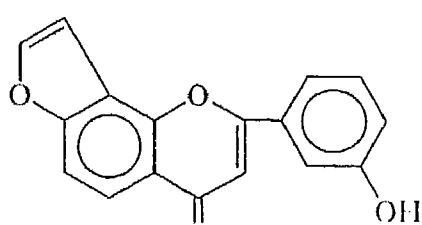
Pongapin
(LXI)



Desmethoxykanugin
(LXII)



Isopongaflavone
(LXIII)



Pongal
(LXIV)

iodine value 89.1, acid value 6.3 and unsaponifiable matter 4.2 per cent.

Karanjin [3-methoxyfurano-(2'', 3'' : 7',8')-flavone (LII)] is the principle nonfatty constituent (approximately 1.25% oil basis) of the seeds of the plant. It was first reported by Limaye in 1925. It was isolated from seed oil and root (Manjunath *et al.*, 1939) and its constitution was established by Limaye (1926) and Manjunath *et al.*, 1939. Synthesis of karanjin has been attempted by Rangaswami and Seshadri (1939). Aneja *et al.*, (1958) gave a new method of the synthesis of karanjin.

Karanjin (mp, 158°C) on demethylation gave a hydroxy compound, called karanjonol (C₁₇H₁₀O₄) mp, 192-193°C. It is slightly bitter in taste and insoluble in water and aqueous dil. sodium hydroxide. It gives orange-yellow coloration on reduction with magnesium and hydrochloric acid.

After the discovery of karanjin, Rangaswami and Seshadri reported pongamol (C₁₈H₁₄O₄, LIII) in 1942. It was a minor component of oil (0.85%, oil basis) and was latter found to be a novel diketone [4-methoxy-5-(ω-benzoylacetyl)-coumerone, mp, 128°C]. Its structure was latter confirmed by Narayanaswamy *et al.*, (1954).

Khanna and Seshadri (1963) isolated a new furanoflavone-pongaglabrone [3', 4'-methylenedioxyfurano-(2'',3''-7,8)-flavone, LIV] from the light petroleum extract of the seeds of karanj. It is a colourless and fat-soluble compound melting at 233°C with a molecular formula C₁₈H₁₀O₅ and no methoxy group.

Aneja *et al.*, 1963 reported a new furanoflavonoid – kanjone [6-methoxyfurano-(2'',3'' : 7,8)-flavone, (LV)], mp, 191°C, from the light petroleum extract of karanj seeds. An optically active dimethylchromenoflavone, isolonchocarpin, mp, 25°C (LVI) and desmethoxykanugin (LXII) were isolated by Satam and Bringi (1973a) from karanj seed oil. The seed oil also contains a chromenoflavone - karanjachromene (LVII) (Satam and Bringi, 1973b).

Table. 2.3 : Various chemical constituents of karanj seed

	Constituent	Structure
	Mature seed	
LII	Karanjin	3-Methoxyfurano-(2'', 3'' : 7',8')-flavone
LIII	Pongamol	4-Methoxy-5-(ω -benzoyl acetyl)-coumerone
LIV	Pongaglabrone	3', 4'-Methylenedioxyfurano-(2'',3''-7,8)-flavone
LV	Kanjone	6-Methoxyfurano-(2'',3'' : 7,8)-flavone
LVI	(-) – Isolonchocarpin	2'',2''-Dimethylchromeno-(6'', 5'' : 7,8)-flavanone
LVII	Karanjachromene	3-Methoxy-2'',2''-dimethylchromeno-(6'', 5'' : 7,8)-flavone.
LVIII	β -Diketone	
LIX	Isopongachromene	6-Methoxy-6'',6''-dimethyl-3',4'-methylenedioxychromeno-(7,8 : 2'',3'')-flavone
LX	Glabin	4,5-Dihydroxy-N-methyl pipecolic acid
LXI	Pongapin	3-Methoxy-3,4-methylenedioxyfurano-(2'',3'' : 7,8)-flavone.
LXII	Desmethoxykanugin	3,7-Dimethoxy-3',4'-methylenedioxyflavone
	Immature seeds	
LXIII	Isopongaflavone	5-Methoxy-6'',6''-dimethylpyrano-(2'',3'' : 7,8)-flavone
LXIV	Pongol	3' Hydroxyfurano (2'',3'' : 7,8) flavone
	Karanjin, pongapin, lanceolatin B, kanjone	
	Seed shells	
	Pongamol, karanjin, desmethyl kanugin, kanugin, pongapin, kanjone, pinnatin	

Sharma and Parthasarthy (1977) reported a new diketone from karanj oil. The new compound (mp, 117-20°C) is similar to pongamol in structure with an additional methylenedioxy at 3',4' position (LVIII).

Pathak *et al.* (1983a) reported a new chromenoflavone - isopongachromene [6-methoxy-6'',6''-dimethyl-3',4'-methylenedioxychromene -(7,8 : 2'',3'')-flavone] (LIX) from the ethanolic extract of mature karanj seed oil by column chromatography on silica gel. Pathak *et al.*, (1983b) further reported the presence of new chromenochalcone – glabrachalcone from the ethanolic extract of the seed oil of karanj.

The alcoholic extract of defatted seeds contains glabrin (LX) a nitrogen containing compound mp, 290°C and gives colourless crystal cubes from water-ethanol-ether (Aneja *et al.*, 1963).

In mature karanj seeds, karanjin and pongamol are the major constituents in contrast to the immature seeds where karanjin, kanjone and lanceolatin B predominate. It appears that lanceolatin B undergoes conversion to pongamol during the course of ripening of seeds (Roy *et al.*, 1977a). Immature seeds of karanj contain chromenoflavone - isopongaflavone [5-methoxy-6'',6''-dimethoxypyrano-(2'', 3'' : 7',8')-flavone,(LXIII)] (Roy *et al.*, 1977b), furanoflavone - pongol [3'-hydroxyfuran-(2'',3'' : 7', 8')-flavone, LXIV] (Roy and Khanna, 1979).

Karanj oil is extracted with petroleum ether from karanj seeds. The oil obtained from the petroleum ether extract of the seeds gives karanjin on cooling. The remaining oil on silica gel column chromatography gives pongamol in earlier fractions using petroleum ether as eluant and a diketone in latter fractions of the same solvent as eluant. Karanjin and pongapin were obtained from benzene elutant. Pongaglabrone and kanjone were obtained from benzene – chloroform eluant (Sharma and Parthasarathi, 1977).

Numerous reports are available in the literature regarding the occurrence of physiologically active constituents in defatted karanj seed kernels (Singh, 1966). The extraction of defatted karanj seed kernels with alcohol removes large portion of these constituents. Toxicants are partly lost in the oil fraction leaving subsequently obtained alcoholic extract less potent. The amount of nonfatty minor constituents as well as karanjin co-extracted with lipid during petroleum ether extraction depends upon the intensity of extraction (time) to which the powdered seed kernels are subjected. Hence the quality of alcoholic extracts depends much upon preliminary defatting step. Alcoholic extracts from defatted karanj seed kernels are also known to contain karanjin (Singh, 1966).

Beside seeds, flowers (Talapatra *et al.*, 1980; Talapatra *et al.*, 1982; Dashkanungo *et al.*, 1987; Ganguly *et al.*, 1987), leaves (Malik *et al.*, 1976; Malik *et al.*, 1977a; Malik *et al.*, 1977b), heartwood (Subrahmanyam *et al.*, 1973; Subrahmanyam *et al.*, 1977), bark (Mukherjee *et al.*, 1969, Garg *et al.*, 1978) of *Pongamia glabra* contains several distinctive compounds. These have been reported in literature since 1940s. The names of these compounds are listed in Table 2.4.

2.6.1.1.1.2 Karanj seed as a source of nitrification inhibitor : Singh (1966) carried out nitrification inhibition studies on karanj seed, cake and their extractives. The effect of karanjin, hot alcohol extractives of cake and defatted seeds, cold alcohol extractives of the cake and defatted seeds, and mixtures of these extracts on nitrification was studied. The results showed that the presence of fatty materials and nonfatty minor constituents exerted an inhibitory effect on nitrifiers, leading to accumulation of ammonium. Alcohol extracts, both hot and cold, removed these active constituents, which retarded nitrification. It was further observed that the addition of karanjin also brought about ammonium accumulation, practically to the same extent, as the

Table 2.4 : Chemical constituents of flowers, leaves, stembark and heartwood of karanj plant

Flowers
<u>Furanoflavones</u> Pongaglabol, isopongaglabol, 6-methoxyisopongaglabol, karanjin, kanjone, lanceolatin B, 5-methoxyfurano-(8,7 : 4'',5'') -flavone, 6-methoxyfurano-(2'',3'' : 7,8)-flavone, 5-methoxy-3',4'-methylenedioxyfurano-(8,7 : 4'',5'')-flavone, pinnatin, glabone, pongone <u>Simple flavones</u> Tetra-O-methyl fisetin , kanugin, desmethoxykanugin, kaempferol. <u>Chromenoflavanones</u> (-)-Isolonchocarpin, ovalichromene B <u>Furanodiketones</u> Pongamol, ovalitenone <u>Triterpenes</u> Cycloart-23-ene-3β-25 diol, friedelin <u>Dipeptide</u> Aurantiamide acetate <u>Miscellaneous</u> β sitosterol, γ sitosterol
Leaves
<u>Furanoflavones</u> Karanjin, 3'-methoxy pongapin, kanjone, 8-methoxyfurano-(4'',5'' : 6,7)-flavone <u>Chalcones</u> Glabrachalcone-I, glabrachalcone-II
Heart wood
<u>Chromenochalcones</u> Ionchocarpin, pongachalcone-I, pongachalcone-II, glabrachromene-I, glabrachromene-II <u>Flavones</u> Pongaglabrone, kanugin, pongachromene, desmethoxykanugin
Stem bark
Pongapin, desmethoxykanugin, kanugin, pinnatin, tetra-O-methyl fisetin, gamatin, 5-methoxyfurano-(2'',3'' : 7,8)-flavone, 5-methoxy-3',4'-methylene dioxyfurano-(2'',3'' : 7,8)-flavone, glabra-I , glabra-II, pongachromene

addition of crude extractives. The method of extraction did not cause any significant difference in activity. The oil also exhibited some nitrification inhibition property (Singh *et al.*, 1970)

Sahrawat *et al.* (1974) investigated the nitrification inhibitory activity of the seeds, barks and leaves of karanj plant. The results showed that the hot ethanol extract of the defatted seeds (applied @ 20 and 30%) had maximum nitrification inhibitory activity followed by bark extracts (extracted with 40 : 60, v/v, mixture of petroleum ether and acetone) and leaves had negligible effect. The alcohol extract of the seeds was effective in retarding nitrification for 60 days when applied at the rate of 20% of the fertilizer nitrogen dose. The percentage inhibition of nitrification of urea in soil was 47-55% even after 45 days of application of seed extract.

Sahrawat and Mukherjee (1977a) observed that karanjin present in seed is a potent inhibitor of nitrification. The inhibition of nitrification of urea or ammonium sulfate was found to be 43% even after 8 weeks of incubation when karanjin was applied at 5% of applied fertilizer nitrogen (Sahrawat, 1973).

Comparative evaluation of karanjin and three patented nitrification inhibitors nitrapyrin, AM and DCD when applied in a sandy loam soil at 5 mg kg⁻¹ of soil reduced the nitrification rate in the following order (Sahrawat, 1981) :

Nitrapyrin > Karanjin > AM > DCD

Sahrawat and Mukherjee (1977a) reported that furan ring of karanjin was responsible for nitrification inhibitory property. They synthesized some structural analogues of karanjin namely karanjketone (4-hydroxy-5- ω -methoxyacetyl coumarine), karanjonol [3-hydroxy-furano-(2', 3' : 7, 8)-flavone] and dihydrokaranjin and tested their nitrification inhibition properties. Except dihydrokaranjin, with hydrogenated furan ring, other compounds showed nitrification inhibition of varying degree.

In pot culture experiment conducted to study the effect of karanjin on rice yield karanjin competed with nitrapyrin upto 5 weeks when added @ 5% of urea and ammonium sulphate – N.

Several reports are available in the literature regarding the effect of karanj cake in increasing NUE of applied nitrogenous fertilizer (Sahrawat, 1982b; Sathinathan, 1982; Sinha *et al.*, 1982; Ketkar, 1983; Patra and Sahu, 1983; Prasad *et al.*, 1989; Yadav and Singh, 1991; Turker *et al.*, 1989; Shivaprakash, 1991). Most of these experiments were carried out by coating and blending karanj cake with urea or applying the cake directly in soil. In a field trial, Singh and Sharma (1989) applied karanj and neem cake blended urea as a nitrogen source to wheat crop. They observed that a mixture of 75 kg ha⁻¹ urea + 32 kg ha⁻¹ karanj cake gave wheat yields comparable to those obtained from 100 kg ha⁻¹ urea alone. Ketkar (1983) observed that urea blended with karanj seed cake containing 15.4% oil was the most effective for increasing the NUE in sugarcane crop. Patra and Sahu (1983) observed that karanj cake coated urea gave maximum yield of rice as compared to lac coated urea and isobutylidenediurea (IBDU).

Ahmed *et al.*, (1978) studied efficiency of karanj cake and its lipid associates in nitrification regulation under submerged rice cultivation in calcareous soil in North Bihar. Lipid associates (obtained by alcohol extraction of defatted karanj cake) when applied @ 20 ppm reduced the number of *Nitrosomonas* to the maximum extent upto 4 weeks after application. Higher amount of lipid associates was required to increase the concentration of NH₄⁺ after the lapse of 4 weeks of its application.

2.6.1.1.2 Neem

2.6.1.1.2.1 Neem as a source of nitrification inhibitor : Experiments conducted at Pratapgarh and Gorukhpur from 1916 to 1940 indicated superiority of neem cake in increasing the yield of sugarcane. Sinha (1960,

1964) seems to be the first to show that neem seed cake exerted beneficial effects by decreasing the rate of nitrification of ammoniacal fertilizers. Bains *et al.* (1971) were the first to show under field conditions that treatment with urea and acetone extracts of dried and crushed neem kernel compared well with proven nitrification inhibitors. Sahrawat and Parmar (1975) showed that the alcohol extract of neem cake was effective in retarding the conversion of ammonium to nitrate and that the higher levels of the extract (20-30% of fertilizer-N) were effective in conserving ammonium upto 75 days of incubation. The effectiveness of neem cake as a nitrification retarder was subsequently reported by various authors (Reddy and Prasad, 1975; Mishra *et al.*, 1975; Sharma *et al.*, 1981; Hulagur and Shinde 1982; Sudhakara and Prasad, 1986; Saranom, 1988; Singh and Sharma, 1989; Joseph, 1991; Parashar *et al.*, 1980; Jain *et al.*, 1994; Kurup, 1994; Shirale and Nayeem, 1999; Kumar *et al.*, 1999).

Thomas and Prasad (1982) observed that urea coated with the cake was more effective than the straight mixing of the cake powder and urea. After 3 weeks of incubation, inhibition of nitrification was 63%, 46%, 29% and 12% with neem blended with nitrapyrin, commercially available neem coated urea (15%), IARI made neem-coated urea and neem mixed urea (20%), respectively. Patel *et al.*, (1995) compared the nitrification inhibition property of neem oil, acetone extract of neem oil, neem cake, ether extract of neem oil, hexane extract of neem oil and found the activity in the following order :

Neem oil > acetone extract of neem oil > neem cake > ether extract of neem oil > hexane extract of neem oil

Similar results were obtained by Kumar and Thakur (1993).

Neem meliacins are normally responsible for nitrification inhibition property. Pure neem oil devoid of bitter principles is inactive and the activity is shown by fractions containing meliacins (Devakumar, 1986). At 30%

of N level meliacin fractions caused substantial conservation of ammoniacal nitrogen. Devakumar (1986) evaluated various meliacins and observed that among these 4-epinimbin was the most active followed by 6-desacetylnimbin and salannin but nimbin had poor activity. Vyas *et al.*, 1991 on the other hand reported that nimbin and other meliacins namely nimbinin, nimbidin, nimbininin possessed nitrification inhibition property.

Singh *et al.* (1997) reported that coating of urea with neem oil increased NUE by 13.8% as compared to control in rice – wheat cropping system.

2.6.1.1.2.2 Activity of karanj products vis-à-vis neem products : Kamire and Sonar (1979) found that karanj cake was better than neem cake in delaying the availability of urea-N. Similarly Sinha *et al.*, (1982) reported that *Pongamia* cake was better than neem cake in delaying the availability of urea-N.

Sahrawat (1982a) studied the activity of alcohol extract of karanj and neem vis-à-vis karanjin in an incubation study. Urea treated with the extracts @ 30% of N applied were comparable to karanjin applied at 5 and 10% concentrations. The extracts were effective upto 45 days whereas karanjin was effective upto 60 days. There was difference in the pattern of inhibition of nitrification by karanj and neem seed extracts in that the extract of the former was more effective on the 15th day (71% and 43% NI at 30% application rate for karanj and neem seed extracts, respectively on the 15th day) whereas the neem seed extract was either at par or a better inhibitor of nitrification on the 45th and 60th day (5% and 20% NI at 30% application rate for karanj and neem seed extracts, respectively on the 60th day). However, Surve and Daftarder (1985) reported that neem products such as neem cake, neem oil and neem oil extractives were superior to karanj cake when this effect was measured in terms of grain production of rice. Similar result were also obtained by Yadav and Srivastava (1987) in sandy loam red soil.

Patel (1991) tested the inhibitory effect of nitrapyrin (NP), neem oil (NO), acetone extract of neem oil (ANO), acetone extract of karanj oil

(AKO), ether extract of neem oil (ENO), petroleum extract of neem oil (PNO), ether extract of karanj oil (EKO) and petroleum extract of karanj oil (PKO) on the growth of pure culture of *Nitrosomonas* and *Nitrobacter* in ammonium calcium carbonate medium and soil incubation study. The effect of these retarders (0.25 ml ml⁻¹ of the medium) on periodical concentration of NH₄⁺-N from 7th day to 90th day generally followed the following decreasing trend :

$$NP > NO > KO \approx ANO \geq AKO \approx ENO \approx PNO \approx EKO \geq PKO \geq \text{Control}$$

Thus in pure culture medium nitrification inhibition was maximum when treated with nitrapyrin followed by neem oil and karanj oil.

In the soil incubation study, the nitrification rate was found to be in the following order :

$$PKO \geq PNO \approx EKO \geq ENO \geq KC \geq NC \approx AKO \approx ANO \geq KO \approx NO$$

Thus in soil incubation study nitrification inhibition was maximum when treated with karanj and neem oils followed by their acetone extracts.

2.6.1.1.3 Mahua : Seed cake and extracts of mahua (*Bassica latifolia* Roxb., *Madhuca indica* G mel., *Madhuca latifolia* L.) are known to possess nitrification inhibition property (Bains *et al.*, 1971; Gohl, 1981; Naphade and Wasnik, 1974-75; Muthuswamy *et al.*, 1977; Turker *et al.*, 1989; Mago and Totawat, 1989a; Kurup, 1994). Mahua seed cake and extracts contain saponin, which is responsible for nitrification inhibition. In an incubation study with clay loam soil Muthuswamy *et al.* (1977) found that the inhibitory effect of the mahua cake extract did not persist more than 20 days. Mago and Totawat (1989b) in an laboratory incubation study observed that among the test chemicals nitrification inhibition property follows the order :

$$\text{Neem cake} > \text{mahua cake} > \text{sulphar} > \text{gypsum} > \text{coal tar}$$

when cakes were applied at 300 g kg⁻¹ of urea.

2.6.1.1.4 *Citrullus colosynthis* : *Citrullus colosynthis* Schrad. (local name Ghurumba or Thumbi, Cucurbitaceae) oil cake has been tested as a

nitrification inhibitor under laboratory conditions (Jain *et al.* 1980). Nearly 66-87% of the applied nitrogen was found to be transferred to NO_3^- -N within 21 days in case of urea alone but application of urea in conjunction with 5% cake (by wt of urea) resulted in an appreciable drop in NO_3^- formation (59-60%) and a considerable rise in NH_4^+ -N content. An increase in the proportion of cake (10 or 20%) did not further enhance NH_4^+ -N conservation instead high dose of cake (20%) caused accumulation of NO_2^- to the extent of 9.6 ppm which is beyond the toxic limit. This study suggested the possibility of use of this cake as nitrification inhibitor at low concentrations (5-10%).

2.6.1.1.5 *Shorea robusta* : Sal (*Shorea robusta*) cake is reported to possess some nitrification inhibition property. Turker *et al.* (1989) reported that the maximum yield of wheat grain and straw were given by karanj and sal cake coated urea as compared to neem, coal tar and nitrohumic acid coated urea. Sal cake coated urea produced maximum protein N in grain and straw followed by karanj cake.

2.6.1.1.6 Miscellaneous : Prasad *et al.*, (1989) applied urea at a dose 50 kg ha^{-1} treated with *Azadirachta indica*, *Madhuca indica*, *Ricinus communis* and *Onosma hispidum* oils. Highest nitrification inhibition (65.3%) was observed with *O. hispidum* followed by *P. pinnata*.

Patra and Sahu (1983) observed that among isobutylidene diurea, lac coated urea and karanj coated urea, karanj cake coated urea gave the highest grain yield of 5.1 ton ha^{-1} .

In a field experiment on the relative efficiency of nitrification inhibition by different nonedible oils (applied at 10% by weight of fertilizer) namely neem, karanj, mahua, undi (*Ricinus communis* L.) and ratanjyoti (a nonedible oil plant found in Maharastra) and their effects on the yield and nitrogen uptake by wheat, Prasad *et al.*, (1986) observed the highest nitrification inhibition (73.9 %) with undi followed by ratanjyoti (67.3%) and

karanj (64.2 %) oils at tillering stage. All these treatments increased the yield and N-uptake of wheat. The effect of different nonedible oils plus 50 kg N ha⁻¹ on the grain yield and nitrogen uptake followed the order :

Ratanjyoty oil = karanj oil > neem extract = undi oil > neem oil

Mathew (1986) tested several organic sources (neem cake, neem leaf cake, *Eucalyptus globosus* leaf, *Calotropis gigantea* leaf, turmeric powder, tea waste and cashew shell powder) and observed that these materials showed varying degrees of nitrification inhibition properties.

Geethalakshmi *et al.* (1998) reported that the rate of nitrification was reduced in treatments receiving urea coated with indigenous nitrification inhibitors such as neem cake (5 : 1); cashew shell powder (5 : 2) and turmeric powder (100 : 1).

In green house studies conducted on alluvial soil of IARI farm 6 nonedible oilseed cakes viz. amaltash (*Cassia fistula*), ashok (*Surea sp*), Jamun (*Syzium cumini*) karanj, nagchampa (*Calophyllum inophyllum*) and neem in combination with urea were evaluated for their nitrification regulating capacity on direct seeded rice (Basmati 2) and wheat (Kalyan sona) grown in sequence. The highest dry matter yields of both direct and residual wheat were recorded under neem cake treatment (IARI, 1999).

2.6.1.2 Biological sources of nitrification inhibitors other than nonedible oil seeds

Several plant products such as vegetable tannins and waste tea have been reported to retard nitrification in soils (Basaraba, 1964). Waste tea, which is not marketed, and discarded from tea factories, contains a huge proportion of oxidised polyphenols with tannin like properties. The tea and the polyphenols are reported to possess nitrification inhibition property, urease activity and also releases nitrogen slowly during its decomposition in soil. When added @ 8 % w/w of soil the waste tea completely checked nitrate formation for 16 days (Fernando and Roberts, 1976; Krishnapillai, 1979; Sahrawat, 1980a).

Bhagat and Verma (1989) reported that cutch obtained from the bark of *Acacia catechu* possessed good nitrification inhibition property. Based on a comparison of cutch with nitrapyrin, it was suggested that it could be used as a substitute for nitrapyrin as an inhibitor of nitrification.

Lignosulfonate, a waste product of pulp and paper industry, is reported to have urease and nitrification inhibition property. Xie *et al.* (1993) reported that after incubation for 24 hour, 80% urea remained in solution with simultaneous addition of jack bean urease and lignosulfonate whereas 20% in solution without lignosulfonate. When soil was incubated without urea, NO_3^- -N content with addition of lignosulfonate was 0.5% and it was 10.1% without it.

Ram *et al.* (1993) studied the effect of mint (*Mentha arvensis*) distillation waste, pyrethrum (*Tanacetum cinerarifolium*) flowers and neem cake on urea, in sandy loam soil of pH 8.5 (Entisol). Amended samples had less NO_3^- than controls and pyrethrum and mint wastes possessed higher nitrification inhibitory effect than neem cake. Addition of pyrethrum and DCD gave 23% and 30% more yield than prilled urea alone (Ram *et al.*, 1996).

Suresh and Ramanathan (1994) studied the effect of marigold (*Tagetes erecta*) leaves, perwinkle (*Catharanthus roseus*) shoots, neem cake and *G. maculata* leaves on *Nitrosomonas* population count and found that *G. maculata* showed the highest effect and *C. roseus* did not have any marked effect.

Coating of mustard cake on urea also reduced the potential nitrogen losses. Beside increased grain yield of rice (cv. Pusa 33) it also increased the apparent nitrogen recovery in Inceptisol (Purakayastha *et al.*, 1997a). Mustard cake retarded urea hydrolysis and consequently reduced ammonia volatilization loss (Purakayastha, 1997b).

2.6.2 Nonbiological natural sources as nitrification inhibitors

Coal acids have been evaluated as slow release nitrogenous fertilizers. Conversion of coal acid by nitric acid oxidation to water soluble

polycarboxylic acids followed by ammoniation have led to the development of ammonium polycarboxylate (AMP) and composite mixtures of ammonium polycarboxylate, ammonium nitrohumate, ammonium nitrate and some mineral matter derived from coal. Researches carried out at Central Fuel Research Institute, Dhanbad, also showed that coal acids can be effectively utilised as nitrification inhibitors (Mazumder, 1980). Application with 10% coal acids to ammonium sulphate and 15-20% in urea gave maximum NUE and resulted in 15-30% yield increase in rice and wheat.

Urea-gypsum adduct have been found to provide slow release and nitrification inhibition properties (Thomas and Prasad, 1982; Ali and Singh, 1994; Tripathi *et al.*, 1999).

Pyrites are known to possess nitrification inhibition property. Though their activity is much less than the commercial inhibitors, these can be used with advantage in those areas where these are easily available because of its low cost, ease of application and the presence of sulphur (Blaise and Prasad, 1993). Pyrite reduced ammonia loss from soil surface applied urea (Blaise and Prasad, 1995). Blaise and Prasad (1996) found pyrite blended urea did not give promising result under flooded condition.

Shcherbakov *et al.*, (1995) reported that local application of zeolite with or without urea reduced the intensity of the nitrification process in soil. The use of zeolite also avoids soil acidification when N fertilizers are applied. On the basis of their experiment on a leached Chernozem soil in Russia they concluded that local application of urea in combination with zeolite as a natural nitrification inhibitor is very promising.

2.7 Polymer

Use of hydrophilic polymers as carriers of plant nutrients has recently gained much interest in scientific community. Hydrophilic polymers are a class of polymers which dissolve in or are swollen in water. These polymers are generally classified as – natural polymers derived from

polysaccharides (viz. starch, guar gum, gum arabic, agar, and alginates), semisynthetic polymers (primarily cellulose derivatives) and synthetic polymers (viz. crosslinked polyacrylamide, hydroxy ethyl methacrylate, and polyvinyl alcohol).

Many authors have reported the use of hydrophilic polymer based urea products as a slow release nitrogenous fertilizer (Malhi and Nyborg, 1983; Salman *et al.*, 1990; Brar *et al.*, 1990; Verma and Saxena, 1990; Peacock and Dipaola, 1992; Abraham and Pillai, 1995; Takahashi and Ono, 1996; Blaise *et al.*, 1996; Nyborg *et al.*, 1999).

When aqueous nutrient containing solutions are used to hydrate a polymer, a considerable amount of nutrient enters into the polymer structure during its expansion. The subsequent release is then based on the diffusive properties of the polymer, its decomposition rate and the nature of the nutrient salt (Mikkelsen, 1994). The release rate of polymer coated urea can be controlled by changing the amount of the coating material. Release of urea from PCU follows a two stage first order mechanism and dissolution of urea in water decreases with increasing coating percentage (Salman *et al.*, 1990).

In an attempt to control nitrogen retention and release, many different polymers have been tried with success such as starch graft copolymer of potassium polyacrylate and polyacrylamide (Henderson and Hensley, 1985); hydrophilic polymers based on polyacrylate, vinyl alcohol and starch (Smith and Harrison, 1991); vinyl alcohol acrylic acid copolymer (Magalhaes, *et al.*, 1987); N, N-methylenebisacrylamide (NNMBA) crosslinked acrylamide (Gandeja *et al.*, 1991), guar based polymer (Mikkelsen *et al.*, 1993), low density polyethylene (3 and 6%) (Blaise and Prasad, 1996), melamine formaldehyde resin (El-Leboudi *et al.*, 1997). Ethylene vinyl acetate, natural rubber, polyvinyl chloride, polystyrene etc are used as sealant for coating fertilizer urea with polymer (Abraham and Pillai, 1995).

2.8 Application techniques of nitrification inhibitors:Coating

The recommended dosage of the inhibitors ranging from 0.5 to 1 kg *a.i.* ha⁻¹ has to be uniformly dispersed throughout the soil surface, which is an onerous task, further complicated by their tenacious sorption capacity towards clays and organic matter. Application of nitrification inhibitors depends upon the physicochemical properties of the compound itself. Nitrapyrin being soluble in organic solvent can be blended with urea whereas AM and ST has to be mixed directly with the fertilizers. Better results were obtained with the application of the fertilizers precoated with inhibitors than with the simple physically blended mixtures. An ideal situation would, therefore, be to put both of them on the same microsite in close proximity. Efficient coating techniques as well as chemical impregnation within the fertilizer matrix might be useful as demonstrated by studies with neem cake (Thomas and Prasad, 1982). It is economical to restrict their application only to the oxidised layer rather than the total soil volume (Sahrawat, 1980b).

The most widely used method for coating of urea is coating of prills (mostly done to improve physical property) with formaldehyde based proprietary formulation after prilling (Suri, 1990). The injection of formaldehyde into urea solution, though not real coating, is also reported. There are several procedures available for coating urea, among them coating with the help of spray nozzles on falling urea from belt conveyor is most common. Proper coating is related to following key factors :

- ❖ Correlation between belt conveyor speed and falling condition of urea.
- ❖ Pattern of spray of the spray nozzle.
- ❖ Mixing condition after the spray coating.

The standard system of coating in the fertilizer plant consists of a coating drum followed by a mixer (80-85% coating efficiency). This system

is alright for new plants but for a existing plant it may prove costly. Spraying of coating substances on urea curtain is another system (60-70% coating efficiency) suitable for existing plants as it does not involve any changes in the system of conveying of prills to storage and bagging sites.

The important point is to see that these materials or compounds are intimately mixed with fertilizers before application to soil. In this regard mobility of fertilizers and inhibitors with the soil solution should also be considered. Dissimilar mobility of the fertilizers and nitrification inhibitors from a coated material or mixture of the fertilizer and inhibitor in a system may result ineffectiveness of these materials (Sahrawat, 1980b).

Coating of urea by lac was indigenously developed based on kirilac- a by-product of the lac industry having practically no value (Bhowmik, 1980). The product contained urea (73%), lac (16%), double boiled linseed oil (3.65%), soapstone (4.38%), wax (2.9%) and microbiocide (0.14%). In this technology urea is heated at 80°C in a specially designed tumbling pan and double boiled linseed oil is added. After an hour when linseed oil becomes tacky kirilac powder is added in 10 minutes. Temperature is maintained at 80°C for 30 minutes. To improve the physical properties, soapstone powder is added subsequently.

Shirley (1989) developed a method for coating sulfur on urea in which a bond is formed between the sulfur coating and the fertilizer granule by providing a special bonding layer between the inner core of fertilizer and the external sulfur coating. In this method fertilizer granules are sprayed with molten sulfur and molten fertilizer simultaneously either as separate sprays or mixed spray. Then a coating of sulfur is applied.

Sulfur coated urea is prepared by spraying molten sulfur on to heated urea pellets, which in turn is sprayed with molten wax and subsequently coated with a conditioner which is added to prevent caking

of the fertilizers. Both the wax and sulfur are necessary to produce a retardant coating (Gould *et al.*, 1986).

Ayyer (1989) reported coating of phosphogypsum on urea. In this two step process a binder (0.5 %) is incorporated in the coating to ensure long term stability. This binding agent is an aqueous solution of chemicals, which polymerise on contact with gypsum, thereby converting the gypsum coating in a hard shell. Optimum quantity of gypsum required was found to be 20% and this product mainly reduced NH_3 volatilization upto 40%.

A neem extract-Nimin containing neem triterpenes responsible for nitrification inhibition in soil has been developed by Godrej Soap Industries (Vyas, 1991). A concentrated neem extract, which contains as much as 5% neem bitters is prepared by removing major part of triglycerides and free fatty acids by successive treatment. The resultant fraction was subsequently solvent extracted to concentrate the desired meliacins. The Nimin thus obtained requires no additional adhesives and on coating 1% (w/w) gives golden yellow colour to prill and the coating can be done by the farmers themselves.

In both the sulphur and bitumen coated urea formaldehyde, the mineralization and nitrification rates are slower. The sulphur coating of SCU (sulphur coated urea) and the bond formation between urea and formaldehyde and also bitumen coating of urea formaldehyde act as strong moisture barriers, which restrict the release of nitrogen. Sawdust urea formaldehyde coating also showed slow mineralization (Chakraborty *et al.*, 1992).

Freney *et al.* (1992) experimented with wax coated calcium carbide to ensure slow release of acetylene to inhibit nitrification and denitrification for 75 days and result in 46% higher recovery of applied nitrogen in plant-soil system at harvest. Similar results were reported by other researchers (Banerjee and Mosier, 1989; Rochester *et al.*, 1996; Keerthisinghe *et al.*, 1996; Chaiwanakupt *et al.*, 1996)

A simple method of coating urea with neem cake has been developed at IARI (Parmar and Singh, 1993). In this method urea is coated with 20% neem cake in a rotating drum using coal tar 1% as adhesive in kerosine oil as a solvent.

LUXURIANCE a new type of compound fertilizer with slow nitrogen release characteristics has been developed in China (Xhang *et al.*, 1991). It has a nucleus of prilled urea or ammonium nitrate which is coated with acidic and organic binders. Potash, chelated micronutrient and nitrification inhibitors can be incorporated in the coating. A novel method of preparing controlled release fertilizers has been developed by Vlnaty and Elizer (1991). In this method water soluble nutrient element like nitrogen is first coated with a colloid formed by the acidification of preconditioned calcium metasilicate with citric acid and then mixed with cement.

Development of commercially viable process to convert neem oil into an emulsion for coating urea has been reported by Suri (1995). Urea prills were coated by spraying the coating medium (5-10% neem oil in water emulsion) on the urea curtain at a point on the conveyor belt nearest to the prilling tower. With this process it is possible to increase neem oil content to a maximum of 1.0 kg of neem oil ton^{-1} of urea in the plant.

Linseed oil has also been tried for coating urea. For linseed oil coating, urea and linseed oil mixed in proportion of 1 : 2 were kept in contact with each other for 45 days at $26.2 \pm 1.8^\circ\text{C}$. The coated urea thus obtained was deep brown, somewhat lumpy and sticky containing 35.36% N. This coated fertilizer gave 116.5% increase in yield of dry matter of maize over control (no treatment) whereas full recommended dose gave 50.6% increase over control (Suri and Datta, 1995).

Coating of urea with polymers has been reported to increase NUE of applied urea. Zhang *et al.* (1998) tested some polymer coated urea and found that among them Shertritt G, Polyon 4% and Sulfurkote released

most of their N within the growing season while others Esso-T-90, Meister 7 released half of their nitrogen. The growth and colour of turf grass were more uniform in some controlled release fertilizer treatments (viz. Esso-T-90, Meister-7, Polyon 4% and Sulfurkote) than in noncoated urea and NH_4NO_3 treatments. Chisso Asahi Co. Tokyo has developed two fertilizers Meister and Lp by coating resin (Gandeja, 1991). The resin coating is primarily a blend of polyolefin type resin, ethylene vinyl acetate copolymer and polyvinylidene chloride type resin as major component. Polyolefin coated fertilizers belonging to thermoplastic resin group showed most controlled release (Shoji and Kanno, 1994).

2.9 Mode of action of nitrification inhibitors

Within the last two decades, substantial progress has been made towards understanding the modes of action of specific inhibitors of nitrification. It has been observed that NH_3 monooxygenase (AMO) has a broad substrate range for catalytic oxidation and the inhibitory effects of many compounds are due to competition for active sites. Acetylene groups of compounds like 2-ethynylpyridine and phenylacetylene are oxidized by normal catalytic cycle of AMO to highly reactive products which covalently bind enzyme causing irreversible inhibition (Hyman *et al.*, 1988; McCarty and Bremner, 1991). AMO contains copper in its active site and large number of compounds containing thiono 'S' (viz, 2-mercaptobenzothiazole, 3-mercapto-1,2,4-triazole etc.) inhibit AMO activity by binding with copper within the active site. Very little information is available regarding mode of action of heterocyclic nitrogenous compounds but it has been suggested that inhibitory influence is closely related to the presence of ring nitrogen (McCarty, 1999).

Most nitrification inhibitors inhibits nitrification by retarding the oxidation of $\text{NH}_4^+\text{-N}$ to $\text{NO}_2^-\text{-N}$ by *Nitrosomonas* sp. Various NIs showed remarkable differences in sensitivity to *Nitrosomonas*. Research with different strains of *Nitrosomonas* showed remarkable differences in sensitivity to

nitrapyrin (Laskowski and Bidlack, 1977; Belser and Schmidt, 1981). It has been concluded that nitrapyrin does not affect the activity of entire population of *Nitrosomonas*.

Zacherl and Amberger (1990) tested the effect of DCD, nitrapyrin and thiourea on growth and metabolism of pure culture of *Nitrosomonas europaea* under laboratory conditions. Nitrapyrin (NS, 10 ppm) and thiourea (TU, 0.5 ppm) completely suppressed the growth of pure culture of *N. europaea* and 300 ppm DCD inhibited growth by 83 per cent. Hydroxylamine oxidoreductase was not affected by high concentrations of inhibitors (200 ppm DCD, 100 ppm NS, 100 ppm TU). Cytochrome oxidase activity was increased by 10% with 200 ppm DCD, not affected by 100 ppm TU and inhibited by 52% with 100 ppm nitrapyrin. Thus mode of action differed among the tested inhibitors.

2.10 Nitrification inhibitors, NUE and crop yield

Nitrification inhibitors have been successfully tried to increase NUE and yield in variety of crops which include rice (*Oryza sativa*), corn (*Zea mays*), wheat (*Triticum aestivum*), grain sorghum (*sorghum bicolor*), sugarcane (*Saccharum officinarum*), bell pepper (*Capsicum annuum*), potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*), cotton (*Gossypium hirsutum*), barley (*Hordeum vulgare*), oat (*Avena fatua*), sugarbeet (*Beta vulgaris*), spinach (*Spinacea oleracea*), lettuce (*Lactuca sativa*), radish (*Raphanus sativus*), cucumber (*Cucumis sativus*), cabbage (*Brassica oleracea* convar *capitata*), turnip (*Brassica rapa*) and several grasses (Slangen and Kerkhoff, 1984; Prasad and Power, 1995).

Rice is cultivated under moist agroclimatic conditions. Because of high percolation rate and preference of rice for NH_4^+ suggests that the nitrification inhibitors can be employed for increasing rice yield and improving the NUE. Various workers have reported that the use of nitrification inhibitors like DCD, neem cake, nitrapyrin, karanj cake etc increase the yield of rice and

NUE in rice field (Bains *et al.*, 1971; Rajale and Prasad, 1972; Sharma, 1978; Prasad and Prasad, 1980; Sudhakara and Prasad, 1986; Prasad *et al.*, 1989; Singh *et al.*, 1990; Joseph, 1991; Budhar *et al.*, 1991; Vimala and Subramanian, 1994; Deshmukh and Tiwari, 1996; Singh *et al.*, 1997; Shukla and Chauhan, 1998, Kumar *et al.*, 1999; Tripathi *et al.*, 1999). Nitrification inhibitors are also reported to increase the yield of wheat (Sharma and Kumar, 1998; Shirale and Nayeem, 1999; Goos *et al.*, 1999), sugarcane (Kumar and Hunsagi, 1997; Mahadevaswamy, 1997), corn (Randell and Schmidt, 1999), vegetables (Basu *et al.*, 1996, Kurup *et al.*, 1997).

2.11 *In vitro* evaluation of nitrification inhibitors

Sahrawat and Mukherjee (1977a) and Kuzvinzwa (1984) evaluated the nitrification inhibition property of karanjin and furfural derivatives respectively, by incubating 200 g soil in 500 ml beakers. The compounds were added in solution before adding fertilizer. Abraham and Pillai (1995) has studied the slow release property of their polymer coated urea products in a soil column placed in PVC tubes (90 mm diameter and 30 cm length). McCarty and Bremner (1991) studied the effect of nitrification inhibition of gaseous hydrocarbon in 10 g soil sample in 250 ml narrow mouth, screw cap bottle. Simillar studies were reported by Kpombrekou-A and Killorn with XDE-384 in 1995. Patel (1991) studied the nitrification inhibition properties with karanj and neem products with pure culture of nitrifying bacteria in liquid ammonium calcium carbonate solution. Study with pure culture of *N. europaea* is also reported (Zacherl and Amberger, 1990).

2.12 Determination of inorganic nitrogen in soil extracts

Various methods viz. potentiometric, colorimetric, ion chromatography and distillation have been described for determination of inorganic forms of nitrogen in soil extracts. Expense, rapidity, versatility and precision of the individual method are the deciding factors in deciding the choice of a method to be used in a particular laboratory.

1M and 2M potassium chloride (KCl) solutions are reported to be the most effective NH_4^+ extractants, whilst NO_2^- and NO_3^- recovery does not appear to be greatly affected by this extractant. A further advantage of KCl extract is that it may be stored for a number of days before analysis as no changes in NH_4^+ , NO_2^- and NO_3^- levels have been observed. Water (Ghosh *et al.*, 1983, Maynard and Kalra, 1993) and CaSO_4 solution (Singh *et al.*, 1999) are also commonly used as extractants for NO_3^- in those situations where chloride ions interfere with the estimation.

As distillation procedures are time consuming and potentiometric methods (notably the nitrate electrode) are prone to Cl^- ion interference, a number of colorimetric methods suitable for a large number of samples have been reported for laboratories not equipped with an auto analyser. For nitrate determination chromotropic acid, Szechrome NAS reagent, copperised cadmium reduction analysis, automatic analysis and phenol disulphonic acid methods have been reported (Sims and Jackson, 1971; Stock, 1983; Ghosh *et al.* 1983; Singh *et al.* 1999). Nitrite concentration is commonly estimated by the modified Greiss-Ilosvay method of diazotization with sulphanilamide and then coupled with N-(1-naphthyl) ethylenediamine to form an azo dye. Ammonium is determined by a manual indo-phenol blue method (IPB, Berthelot's reaction) which is extremely sensitive but requires stringent control to maintain reproducibility when applied as manual method. The basic method consists of reacting NH_4^+ with phenol (actually phenate) and hypochlorite (oxidizing agent) at high pH (11.5 – 12.0) in the presence of nitroprusside (catalyst) to form a blue colour (Dorich and Nelson, 1983; Searle, 1984). Stock (1983) evaluated these methods and compared with autoanalyzer and found that the results obtained from manual and automatic procedures are in close agreement for both nitrate and ammonium determinations.

2.13 General discussion on the review of literature

From the above review it becomes eminent that there is an urgent need to improve the efficiency of N-fertilizer use to achieve higher food production and minimize fertilizer related pollution of the environment. Various approaches have been suggested for increasing NUE out of which the use of the nitrification inhibitors is prominent. Vast array of compounds has been reported to inhibit nitrification in soil. Of these only eight (NS, AM, DCD, ST, TU, Etridiazole, MBT and ethylene) have been widely tested. Despite a great interest in nitrification inhibitors to date, only a few compounds have been adopted for agricultural use. The main problems are the high cost involved in the development and subsequent registration of effective nitrification inhibitors and the economics of their use in field conditions. There is a need to continue efforts to develop nitrification inhibitors that are inexpensive, readily available locally, and effective at reasonable rates of application.

In India several synthetic compounds and plant products have been reported to inhibit nitrification. Among the natural sources neem and karanj seed kernels extracts are the prominent ones. Though neem extract based products are already commercialized but commercial exploitation of karanj based products have not been done. Literature showed that karanjin, a furanoflavonoid in karanj seeds is mainly responsible for nitrification inhibition, and has outperformed neem based products in many experiment. Its commercial exploitation has not been attempted because the required developmental and standardization work has not been carried out. Nitrification inhibition property of karanjin has been well established but no effort has been made to evaluate karanjin based coated product. No information is available on application methodology of karanjin or karanj extract coated products. Karanj plant is commonly distributed throughout the country and its seeds are available cheaply. So the available knowledge on these materials needs to be pursued further in order to develop usable technologies.

Among the synthetic compounds furan based derivatives has shown excellent nitrification inhibition activity in laboratory incubation studies. These compounds were synthesized from furfural which is a industrially available cheap raw material. So it is reasonable to assume that any simple synthetic product developed from furfural, even if endowed with moderate activity can be economically used as an adjuvant in urea formulations. Though number of compounds has been tested but still more compounds are needed to be synthesized and tested to arrive at an economically viable and environmentally acceptable compound. Thus there is a need to further exploit indigenously available materials and products as sources of nitrification inhibitors.

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MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagents

Synthesis : Furfural, ethyl bromide, *iso*-propyl bromide, *n*-butyl bromide, *n*-hexyl bromide, allyl bromide, potassium carbonate, pyridine, hydroxylamine hydrochloride, acetophenone, 4-hydroxyacetophenone, 4-chloroaniline, 3,4-dichloroaniline, 3-nitroaniline, aniline.

Nitric acid inhibition studies : Potassium chloride, calcium sulphate dihydrate, charcoal, sulfanilamide, N-(1-naphthyl)-ethylenediamine hydrochloride, sodium nitrite (AR), sodium nitroprusside, sodium monohydrogen phosphate, sodium hypochlorite (4%), ethylenediaminetetraacetic acid, ammonium sulfate (AR), phenol (AR), sulphuric acid (AR), liquor ammonia (0.88 specific gravity), potassium nitrate (AR).

General : Hydrochloric acid, sodium hydroxide, 2,4-dinitrophenyl hydrazine, iodine.

Solvents : Acetone, hexane, ethanol, methanol, dichloromethane, diethyl ether.

All the chemicals and reagent were purchased from local market (S. D. Fine Chem Ltd. and Qualigens Fine Chemical) and used without further purification. Furfural was freshly distilled everytime before use. The organic solvents used in the study were of commercial grade and were purified before use.

3.1.2 Test compounds

♦ Furfural oxime ethers : Five ethers namely N-O-ethyl furfural oxime (EOE), N-O-*iso*-propyl furfural oxime (POE), N-O-*n*-butyl furfural oxime (BOE), N-O-*n*-hexyl furfural oxime (HOE) and N-O-allyl furfural oxime (AOE) were synthesized in the laboratory.

♦ Furfural chalcones : Three chalcones namely, 4-(furan-1-yl)-but-3-en-2-one (furfurylideneacetone, ACC), 3-(furan-1-yl)-1-phenyl-prop-2-en-1-one (APC) and 3-(furan-1-yl)-1-(4-hydroxyphenyl)-prop-2-en-1-one (HAPC) were synthesized in the laboratory.

♦ Furfural Schiff bases : Four furfural Schiff bases namely, furfurylideneaniline (AS), furfurylidene-4-chloroaniline (CAS), furfurylidene-3,4-dichloroaniline (DCAS) and furfurylidene-3-nitroaniline (NAS) were synthesized in the laboratory.

♦ Karanj, alcohol extract of karanj seed kernels, alcohol extract of neem seed kernels, karanj oil, coal tar. All these materials except coal tar were extracted from the respective seed kernels in the laboratory. Karanj (*Pongamia glabra* Vent.) seed kernels were purchased from Neem Mission, Pune and Neem (*Azadirachta indica* A. Juss) seeds were obtained from local market. Coal tar was obtained from local market.

♦ Nitrapyrin (NS) : Nitrapyrin (90% purity was obtained from Dow Elanco, Indianapolis, USA).

3.1.3 Test fertilizer (N source)

Urea (46.6% N) prills were purchased locally.

3.1.4 Soil

Soil used in the study was collected from the institute farm.

3.1.5 Instrumentation

Thin layer and column chromatography : Thin layer chromatography was performed on the thick silica gel G plates containing 13 per cent gypsum as binder and pre-activated at 100°C. Silica gel 60-120 mesh was used for column chromatography using hexane and hexane-acetone gradient.

Spectroscopy : The proton magnetic resonance (PMR) spectra were recorded on a Varian EM-360L, 60 MHz instrument. Samples were

dissolved in CDCl_3 /deuterated acetone and tetramethylsilane (TMS) was used as an internal standard ($\delta 0.0$). Chemical shifts were reported in δ values relative to TMS.

Infrared (IR) spectra were scanned on a Nicolet Fourier Transform Infrared spectrophotometer, Model Impact 400 (FTIR) in either nujol mull or KBr disc.

Colorimetric estimation was done in a Varian, Series 634, UV-VIS double beam spectrophotometer.

High performance liquid chromatography (HPLC) was carried out on Thermoseparation Product P-2000 HPLC system fitted with an UV detector.

Melting points : All melting points were recorded in a sulphuric acid bath in open capillary method and were uncorrected and reported in degree Celsius.

3.2 Methods

3.2.1 Synthesis of furfural oxime ether

Five furfural oxime ethers were synthesized in the laboratory following two step reaction (Scheme 1a and 1b, Chart 3.1). In the first step, furfural oxime was synthesized by reacting furfural with hydroxylamine hydrochloride. In the second step, furfural oxime was treated with the corresponding alkyl bromide to obtain furfural oxime ethers.

3.2.1.1 Synthesis of furfural oxime

Freshly distilled furfural (0.2 mol, 19.2 g) and hydroxylamine hydrochloride (0.1 mol, 13.9 g) were refluxed in ethanol (100 ml) in the presence of pyridine (20 ml) on a water bath for one hour. Completion of the reaction was checked by TLC. After the end of the reaction ethanol was distilled off and ice cooled water (100 ml) was added. Furfural oxime precipitated out which was filtered, dried and recrystallized from ethanol as

fluffy, colourless, needles. mp, 86-87°C, ^1H NMR (CDCl_3) : δ 6.6 (m, H_b), 7.6 (m, 2H, H_a , H_c), 8.2 (s, 1H, $-\text{CH}=\text{N}-$), 10.0 (s, 1H, broad, D_2O exchangeable -OH), yield : 19 g, 86%.

3.2.1.2 Synthesis of furfural oxime ethers

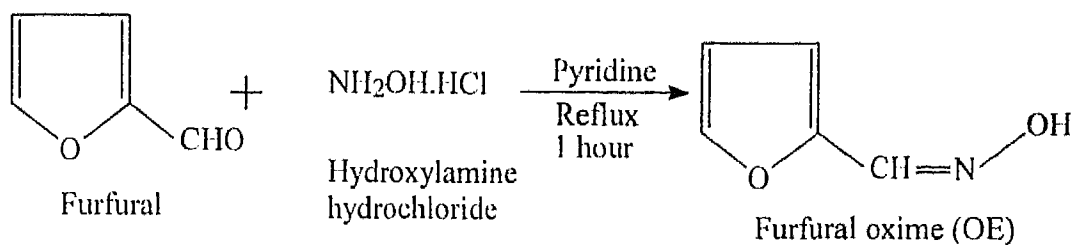
A solution of furfural oxime (0.03 mol) and alkyl bromide (0.04 mol) in dry acetone (200 ml) were refluxed in the presence of dry potassium carbonate (10 g) for 24 hours on a water bath. Completion of the reaction was checked by TLC using hexane-acetone (3 : 1) as solvent system. The spots were visualised by spraying with a solution of 2,4-dinitrophenyl hydrazine (2,4 DNP). On completion of the reaction two pink coloured spots appeared on the plate indicating the formation of two isomers (*syn* and *anti*). One isomer was more polar than the parent furfural oxime (lower R_f than furfural oxime) and the other isomer is less polar than it (more R_f than furfural oxime). After completion of the reaction, the solid potassium carbonate was filtered out, and the solvent was distilled off under reduced pressure. Then 100 ml ice cooled water was added and the mixture was extracted with diethyl ether (50 $\times$ 3 ml) and the organic phase was dried over anhydrous sodium sulphate. The solvent was then distilled off to obtain yellowish viscous liquid, which was subjected to column chromatography over activated silica gel (60-120 mesh) using hexane-acetone mixture as the eluant to yield yellowish viscous furfural oxime ethers. Polarity of the solvent mixture was adjusted to separate the two oxime ether isomers and the unreacted oxime.

3.2.1.2.1 N-O-Ethyl furfural oxime (EOE) : A solution of furfural oxime (0.03 mol, 3.3g) in acetone (200 ml) containing potassium carbonate (10 g) was refluxed with ethyl bromide (0.04 mol, 4.4 g) for 24 hours. After completion of the reaction, it was worked up and purified as mentioned earlier

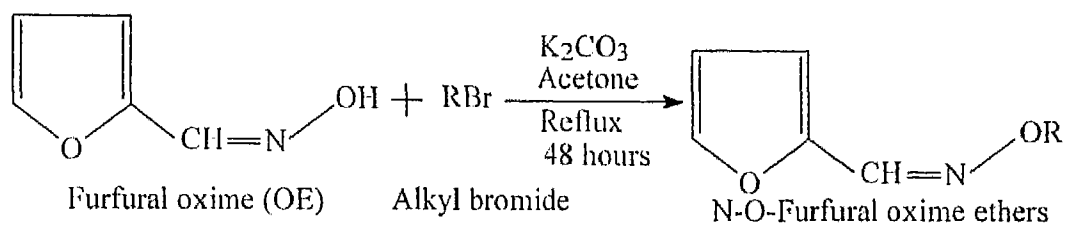
(Section 3.2.1.2) to give light yellowish N-O-ethyl furfural oxime. During column chromatography one isomer was eluted with 95 : 5 hexane-acetone mixture while the second was eluted with 90 : 10 hexane-acetone mixture. R_f : 0.35, 0.78 (hexane-acetone, 75 : 25); ^1H NMR (CDCl_3) : δ 1.6 (t, 3H, $-\text{CH}_2\text{CH}_3$, $J = 7\text{Hz}$), 4.1 (q, 2H, $-\text{CH}_2\text{CH}_3$), 6.8 (dd, 1H, H_b , *ortho* coupled and *meta* coupled, $J = 4\text{ Hz}$), 7.7 (d, 1H, H_a , *ortho* coupled, $J = 4\text{Hz}$), 7.9 (s, 1H, $-\text{CH}=\text{N}-$), 8.0 (d, 1H, H_c , *ortho* coupled, $J = 3\text{Hz}$), yield obtained 3.1 g (75%).

3.2.1.2.2 N-O-Isopropyl furfural oxime (POE) : A solution of furfural oxime (0.03 mol, 3.3 g) and isopropyl bromide (0.04 mol, 4.9 g) in dry acetone (200 ml) containing potassium carbonate (10 g) was refluxed on a water bath for 24 hours. After completion of the reaction, it was worked up and purified as mentioned earlier (Section 3.2.1.2) to give a light yellowish viscous liquid of N-O-isopropyl furfural oxime. During column chromatography, one isomer was eluted with 97 : 3, hexane-acetone mixture and the second with isomer with 92 : 8, hexane-acetone mixture. R_f : 0.38, 0.80 (hexane -acetone, 75 : 25); ^1H NMR (CDCl_3) : δ 1.6 (d, 6H, $-\text{CH}-(\text{CH}_3)_2$, $J = 6\text{ Hz}$), 4.4 [heptet, 1H, $-\text{CH}-(\text{CH}_3)_2$], 6.8 (dd, 1H, H_b , *ortho* coupled and *meta* coupled, $J = 4\text{ Hz}$), 7.8 (d, 1H, H_a , *ortho* coupled, $J = 4\text{ Hz}$), 7.9 (s, 1H, $-\text{CH}=\text{N}$), 8.0 (d, 1H, H_c , *ortho* coupled, $J = 3\text{Hz}$), yield obtained 3.4 g (75%).

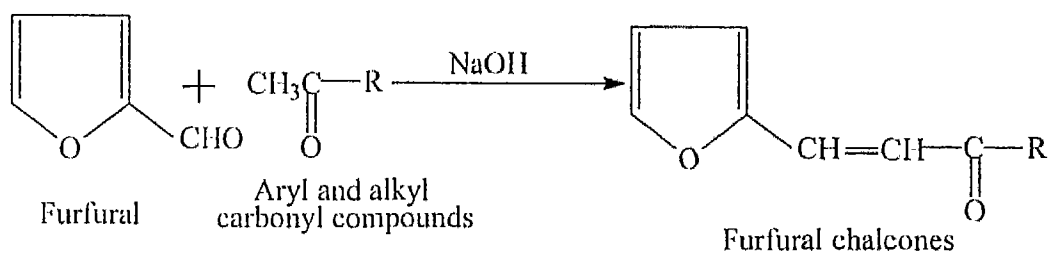
3.2.1.2.3 N-O-Butyl furfural oxime (BOE) : A solution of furfural oxime (0.03 mol, 3.3 g) and butyl bromide (0.04 mol, 5.5g) in dry acetone (200 ml) containing potassium carbonate (10 g) was refluxed on a water bath for 24 hours. After completion of the reaction, it was worked up and purified as mentioned earlier (Section 3.2.1.2) to give light yellowish viscous liquid of N-O-butyl furfural oxime. On column chromatography one isomer was eluted with 98 : 2, hexane-acetone mixture and the second with 95 : 5 hexane-acetone mixture. R_f : 0.38, 0.83 (hexane-acetone, 75 : 25); ^1H NMR (CDCl_3) :



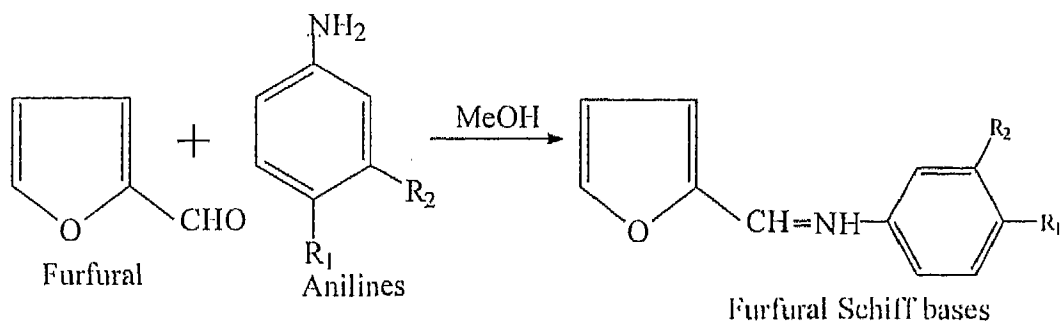
Scheme - 1a



Scheme - 1b



Scheme - 2



Scheme - 3

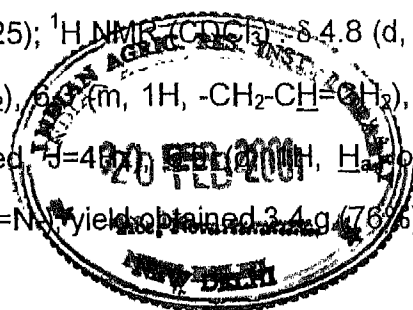
Chart 3.1 Schemes followed for synthesis of various furfural derivatives

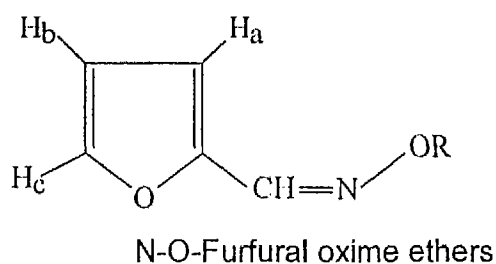
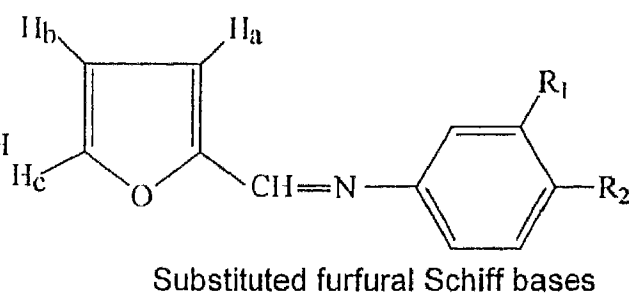
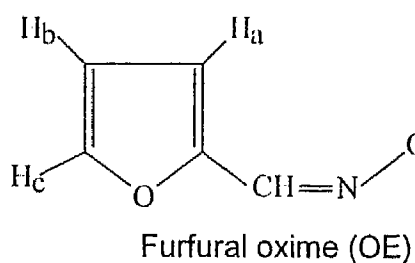
δ 1.2 [m, 3H, $-\text{CH}_2-(\text{CH}_2)_2\text{CH}_3$], 1.6 (m, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 2.0 (m, 2H, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 4.0 [t, 2H, $-\text{O}-\text{CH}_2-(\text{CH}_2)_2\text{CH}_3$, $J = 6\text{ Hz}$], 6.7 (dd, 1H, H_b , *ortho* coupled and *meta* coupled, 1Hz), 7.7 (d, 1H, H_a , *ortho* coupled, $J = 4\text{ Hz}$), 7.8 (s, 1H, $-\text{CH}=\text{N}-$), 7.9 (d, 1 H, H_c *ortho* coupled, $J = 3\text{ Hz}$), yield obtained 3.8 g (76%).

3.2.1.2.4 N-O-Hexyl furfural oxime (HOE) : A solution of furfural oxime (0.03 mol, 3.3 g) and hexyl bromide (0.04 mol, 6.6 g) in dry acetone (200 ml) containing potassium carbonate (10 g) was refluxed on a water bath for 24 hours. After completion of the reaction it was worked up and purified as mentioned earlier (Section 3.2.1.2) to give light yellowish viscous liquid of N-O-hexyl furfural oxime. During column chromatography one isomer was eluted with 99 :1, hexane-acetone mixture and the second with 95 : 5, hexane-acetone mixture. R_f : 0.39, 0.84 (hexane-acetone, 75 : 25); $^1\text{H NMR}$ (CDCl_3) : δ 1.0 [m, 3H, $\text{CH}_2-(\text{CH}_2)_4-\text{CH}_3$], 1.4 [m, 8H, $-\text{CH}_2-(\text{CH}_2)_4\text{CH}_3$], 4.2 [m, 2H, $-\text{CH}_2-(\text{CH}_2)_4\text{CH}_3$], 6.7 (dd, 1H, H_b), 7.4(d, 1H, H_a , *ortho* coupled, $J = 4\text{ Hz}$), 7.9-7.7 (m, 2H, H_c , $-\text{CH}=\text{N}-$), yield obtained 4.3 g (74%).

3.2.1.2.5 N-O-Allyl furfural oxime (AOE) : A solution of furfural oxime (0.03 mol, 3.3 g) and allyl bromide (0.04 mol, 4.8 g) in dry acetone (200 ml) containing potassium carbonate (10 g) was refluxed on a water bath for 24 hours. After completion of the reaction it was worked up and purified as mentioned earlier (Section 3.2.1.2) to give light yellowish viscous liquid of N-O-allyl furfural oxime. During column chromatography one isomer was eluted with 99 : 1 hexane-acetone mixture and the second with 99 : 5 hexane-acetone mixture. R_f : 0.25, 0.76 (hexane-acetone, 75 : 25); $^1\text{H NMR}$ (CDCl_3) : δ 4.8 (d, 2H, $-\text{OCH}_2-$, $J = 4\text{ Hz}$), 5.5 (m, 2H, $-\text{CH}_2-\text{CH}=\text{CH}_2$), 6.3 (m, 1H, $-\text{CH}_2-\text{CH}=\text{CH}_2$), 6.8 (dd, 1H, H_b , *ortho* coupled and *meta* coupled, $J = 4\text{ Hz}$), 7.4 (d, 1H, H_a , *ortho* coupled, 4 Hz), 7.8 (d, 1H, H_c), 7.9 (s, 1H, $-\text{CH}=\text{N}-$), yield obtained 3.4 g (76%).

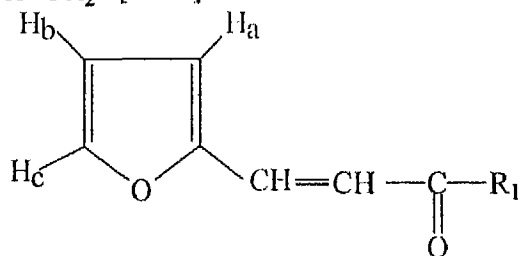
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$R_1 = H$	$R_2 = H$ [AS]
$R_1 = H$	$R_2 = Cl$ [CAS]
$R_1 = Cl$	$R_2 = Cl$ [DCAS]
$R_1 = NO_2$	$R_2 = H$ [NAS]

$R = CH_2CH_3$	[EOE]
$R = CH(CH_3)_2$	[POE]
$R = CH_2(CH_2)_2CH_3$	[BOE]
$R = CH_2(CH_2)_4CH_3$	[HOE]
$R = CH_2-CH=CH_2$	[AOE]



Substituted furfural chalcones

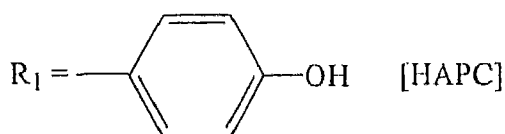
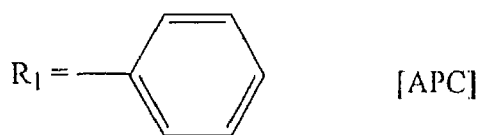


Chart 3.2 Synthesized furfural derivatives

3.2.2 Synthesis of furfural chalcone derivatives

Aromatic aldehyde condenses with aliphatic or mixed alkyl aryl ketones in the presence of aqueous alkali (Scheme 2, Chart 2.1) to form α,β unsaturated ketones or chalcones (Claisen-Schmidt reaction). Three furfural chalcones namely, 4-(furan-1-yl)-but-3-en-2-one (furfurylideneacetone), 3-(furan-1-yl)-1-phenyl-prop-2-en-1-one, and 3-(furan-1-yl)-1-(4-hydroxyphenyl)-prop-2-en-1-one were synthesized.

3.2.2.1 4-(Furan-1-yl)-but-3-en-2-one (furfurylideneacetone, ACC) : In a 250 ml conical flask equipped with a magnetic stirrer, freshly distilled furfural (0.1 mol, 9.6 g), water (100 ml) and acetone (0.26 mol, 15 g) were taken. The mixture was cooled to 10°C, stirred and 10 ml, 20% sodium hydroxide solution was added with intervals. Stirring was continued for 4 more hours. After completion of the reaction (TLC), 10% sulfuric acid was added until the mixture became acidic to litmus, whereupon milkiness disappeared and the liquid separated out into layers. The lower organic layer was separated, dried over anhydrous sodium sulfate and distilled in a rotavapor under reduced pressure. Furfurylideneacetone was collected which solidified on cooling (mp, 39°C) and yielded 8.2 g (60%), R_f : 0.61 (hexane-acetone, 75 : 25); ^1H NMR (CDCl_3) : δ 2.5 (s, 3H, $-\text{CO}-\text{CH}_3$), 6.6 (dd, 1H, H_b , *ortho* coupled and *meta* coupled, $J = 4$ Hz), 6.9 (d, 1H, $\text{CH}=\text{CHCO}$, $J = 4$ Hz), 7.4 (s, 1H, H_a), 7.5 – 8.0 (m, 2H, $-\text{CH}=\text{CHCO}$, H_c).

3.2.2.2 3-(Furan-1-yl)-1-phenyl-prop-2-en-1-one (APC) : In a conical flask equipped with a magnetic stirrer, furfural (0.1 mol, 9.6 g), ethanol (100 ml) and acetophenone (0.1 mol, 12 g) were taken and cooled in a bath of crushed ice. Twenty five ml, 10% NaOH solution was added at intervals and the mixture was stirred. The temperature of the mixture was maintained at 35°C and stirred until the solution became thick. After stirring, the mixture was

poured into ice cold water and neutralized with dilute sulfuric acid until it became neutral to litmus. A solid mass was obtained which was then filtered and dried. The crude chalcone recrystallized from methanol as pale yellow crystal, mp, 45-46°C. The chalcone after drying in air weighed 15.8 g (80% yield), R_f : 0.65 (hexane-acetone, 75 : 25); $^1\text{H NMR}$ (CDCl_3) : δ 6.6 (dd, 1H, H_b , $J = 4$ Hz), 6.9 (d, 1H, $-\text{CH}=\text{CH}-\text{CO}-$, 4 Hz), 7.7 (m, 6H, 3H_y , H_a , H_c , $-\text{CH}=\text{CH}-\text{CO}$), 8.3 (m, 2H, 2H_x).

3.2.2.3 3-(Furan-1-yl)-1-(4-hydroxyphenyl)-prop-2-en-1-one (HAPC) : In a stoppered conical flask equipped with a magnetic stirrer, furfural (0.1 mol, 9.6 g), ethanol (100 ml) and *p*-hydroxy acetophenone (0.1 mol, 13.6 g) were taken and cooled in a bath of crushed ice. Twenty five ml of 20% NaOH solution was added at intervals. Rest of the method was followed as referred in section 3.2.2.2, mp, 160°C, R_f : 0.32 (hexane – acetone, 75 : 25); $^1\text{H NMR}$: ($\text{CDCl}_3/\text{acetone } \text{D}_6$) : δ 6.6 (dd, 1H, H_b , $J = 4\text{Hz}$), 6.8 (d, 1H, $-\text{CH}=\text{CH}-\text{CO}$, 4 Hz), 7.1 (m, 2H, H_y , *ortho* coupled and *meta* coupled, $J = 8$ Hz), 7.5 (s, 1H, H_a), 7.7 (m, 2H, $\text{CH}=\text{CH}-\text{CO}$, H_c), 8.1(m, 2H, H_x), 9.0 (s, 1H, OH , broad, D_2O exchangeable), yield, 16.6 g (77%).

3.2.3 Synthesis of furfural-Schiff bases/anils

Furfural easily condenses with anilines to produce compounds known as anils or Schiff bases (Scheme 3, Chart 2.1). A mixture of furfural (0.1 mol, 9.6 g), substituted anilines (0.1 mole) and methanol (50 ml) were taken in a beaker and heated gently on a water bath. The solution was then cooled by placing it on ice bath. The separated solid was filtered and recrystallized from methanol. Four Schiff bases namely, furfurylideneaniline, furfurylidene-4-chloroaniline, furfurylidene-3,4-dichloroaniline and furfurylidene-3-nitroaniline were synthesized.

3.2.3.1 Furfurylideneaniline (AS) : A mixture of furfural (0.1 mol, 9.6 g), aniline (0.1 mole, 9.3 g) and methanol (50 ml) were taken in a beaker, heated and processed as described earlier (Section 3.2.3). mp, 57°C; R_f : 0.56 (hexane – acetone, 75 : 25); ^1H NMR (CDCl_3) : δ 6.8 (dd, 1H, $\underline{H}_b$, *ortho* coupled and *meta* coupled, $J = 4$ Hz.), 7.2 (d, 2H, $2\underline{H}_x$, *ortho* coupled, $J = 8$ Hz), 7.4-7.8 (m, 4H, $3\underline{H}_y$, $\underline{H}_a$), 7.9 (s, 1H, $\underline{H}_c$), 8.5 (s, 1H, $-\underline{\text{CH}}=\text{N}-$); yield 13.6 g (79.53%).

3.2.3.2 Furfurylidene-4-chloroaniline (CAS) : A mixture of furfural (0.1 mol, 9.6 g), 4-chloroaniline (0.1 mole, 12.8 g) and methanol (50 ml) were taken in a beaker, heated and processed as described earlier (Section 3.2.3). mp, 58°C; R_f : 0.57 (hexane-acetone, 75 : 25); ^1H NMR (CDCl_3) : δ 6.8 (dd, 1H, $\underline{H}_b$, *ortho* coupled and *meta* coupled, $J = 4$ Hz), 7.2 - 7.8 (m, 5H, 4 Ar- $\underline{H}$, $\underline{H}_a$), 7.9 (s, 1H, $\underline{H}_c$), 8.5 (s, 1H, $-\underline{\text{CH}}=\text{N}-$), yield 16.4 g (79.84%).

3.2.3.3 Furfurylidene-3-4-dichloroaniline (DCAS) : A mixture of furfural (0.1 mol, 9.6 g), 3,4-dichloroaniline (0.1 mole, 16.2 g) and methanol (50 ml) were taken in a beaker, heated and processed as described earlier (Section 3.2.3). mp, 43°C; R_f : 0.57 (hexane-acetone, 75 : 25); ^1H NMR (CDCl_3) : δ 6.8 (dd, 1H, $\underline{H}_b$, *ortho* coupled and *meta* coupled, $J = 4$ Hz), 7.3 (m, 1H, $\underline{H}_x$, *ortho* coupled and *meta* coupled), 7.5 (m, 2H, $\underline{H}_z$, $\underline{H}_a$), 7.9 (m, 2H, $\underline{H}_y$, $\underline{H}_c$), 8.5 (s, 1H, $-\underline{\text{CH}}=\text{N}-$), yield 18.96 g (79 %).

3.2.3.4 Furfurylidene-3-nitroaniline (NAS) : A mixture of furfural (0.1 mol, 9.6 g), 3-nitroaniline (0.1 mol, 13.8 g) and methanol (50 ml) were taken in a beaker, heated and processed as described earlier (Section 3.2.3). mp, 56°C, R_f : 0.45 (hexane-acetone, 75 : 25); ^1H NMR (CDCl_3) : δ 6.8 (dd, 1H, $\underline{H}_b$, $J = 4\text{Hz}$, *ortho*

coupled and *meta* coupled), 7.3 (d, H_a , $J = 3$ Hz, *ortho* coupled), 7.8 – 6.5 (m, 5H, 4Ar- H , H_c), 8.6 (s, 1H, $-CH=N-$), yield 17.32 g (80.18%).

3.2.4 Extraction of karanjin from karanj seed kernels

Finely ground karanj seed kernels were defatted in a Soxhlet apparatus with *n*-hexane (bp, 65-70°C). Five hundred gram of the dried and finely powdered seed material was taken in a thimble and extracted with 2 L *n*-hexane for 24 hours. At the end of the extraction, the extract at the bottom of the extraction chamber became colourless. It left no oily spot on the filter paper when it's drop was placed on it. The greenish extract was filtered through cotton wads and concentrated to half of its volume using a rotavapor at 45°C. The concentrated extract was kept in a freezer at approximated –5°C for 72 hours. After 72 hours, the concentrated hexane extract deposited karanjin, which was collected by filtration. Crude karanjin was washed with *n*-hexane and crystallized from ethanol. It was recrystallized from ethanol until a sharp and constant melting point was observed. The purified crystals were white, needle shaped, mp, 156-157°C (literature mp, 158°C, Manjunath *et al.*, 1939). It was found to be homogeneous on TLC (hexane-acetone, 9 : 1, $R_f = 0.5$). Karanjin gave blue colour with concentrated sulfuric acid and orange yellow colour on reduction with magnesium and hydrochloric acids. Similar colour reaction was reported by Sahrawat, 1973. $\lambda_{\max}^{\text{MeOH}} : 268, 308$ (literature $\lambda_{\max}^{\text{MeOH}} : 268, 308$, Sahrawat, 1973), $\nu_{\max}^{\text{nujol}} : 1640$ (C=O). It's purity was further checked by HPLC. Purified karanjin gave a big sharp peak (R_t , 7.98 minute) on HPLC. HPLC parameters were as follows :

Apparatus : Thermoseparation product, P 2000

Column : C-18

Solvent system : Methanol : water = 80 : 20

Detector : UV-detector, wavelength, 250 nm

Flowrate : 1 ml min⁻¹

Yield of karanjin obtained was 0.93% of the karanj seed kernels used. Singh (1968) obtained 0.97% crude deposit of karanjin from petroleum ether extract of karanj seed kernels. The present results corroborate the same.

3.2.5 Extraction of karanj oil

After deposition of karanjin, the hexane extract was decanted off, filtered using a cotton pad and distilled using a rotavapor at 45°C to remove hexane completely. Karanj oil thus obtained was stored in an amber coloured bottle. The karanj oil obtained was light brown liquid, which solidified on freezing. 39% oil was obtained from karanj seed kernels.

3.2.6 Alcohol extraction of defatted karanj seed kernels

Five hundred gram of the defatted seed kernel was extracted with 2 L of ethyl alcohol in a Soxhlet apparatus for 48 hours or till colourless extract was obtained. The ethanol extract was distilled under vacuum using a rotavapor at 45°C. After removing the solvent completely, it was stored in an amber coloured bottle in a refrigerator.

The alcohol extract of defatted karanj seed kernels were found to be highly viscous with a little flowing capacity. Extractives were blackish brown in colour and had a soft and sticky consistency forming a semisolid mass. The extractives could be taken on a glass rod in sufficiently large quantity as to show their viscous nature. The extractives were found to contain 1.70% N. Yield obtained was 11.32% of defatted karanj seed kernel used.

3.2.7 Alcohol extraction of defatted neem seed kernels

Neem seed kernels were procured from the local market and crushed to fine powder. Five hundred gram sample was extracted in distilled *n*-hexane for 24 hours employing a mechanical stirrer to aid crushing cum extraction. After 24 hours, the extract was filtered through a buchner funnel under reduced pressure. The residue was further stirred for two more times,

as above, to remove the oil completely. After defatting, the seed residue was similarly extracted with 2 L ethanol for 24 hours. The alcohol extract was then filtered through a buchner funnel under reduced pressure. The residue was reextracted twice. All the ethanol extracts were pooled and ethanol was removed under reduced pressure employing a rotavapor. The extract thus obtained was highly viscous and brown in colour. It was stored in an amber coloured bottle in a refrigerator.

3.2.8 Evaluation of nitrification inhibition property

3.2.8.1 Collection of soil for the incubation experiments : The soil used for the *in vitro* incubation experiments was collected from the farm of the institute. Composite soil samples were collected in bulk from the cultivated fields of known history from a depth of 0-15 cm following the standard sampling procedure.

3.2.8.2 Preparation of soil : The soil was air dried at room temperature, ground and passed through 2 mm sieve. It was thoroughly mixed before use.

3.2.8.3 Characterization of the soil : The physical and chemical characteristics of the soil used in incubation studies are mentioned in Table 3.1.

3.2.8.4 Application of nitrification inhibitor : Test chemicals were applied in the soil in two forms, solution form and coated form.

3.2.8.4.1 Solution form : In this method of application, the test chemicals were dissolved in suitable solvent (acetone/dichloromethane) and calculated amount of the solution was added in the soil. Solvent was selected on the basis of the solubility of the compounds.

3.2.8.4.2 Coated form : Urea prills were coated by spraying the prills with the help of specially designed graduated glass sprayer with a fine nozzle. The test chemicals were first dissolved in a suitable solvent (hexane/acetone/ethanol/dichloromethane) and then sprayed slowly upon the prills. Solvents

Table 3.1 : Physico-chemical properties of the test soil

Particulars	Value	Methods
<u>Mechanical analysis</u>		
Sand (%)	60.8	Hydrometer method (Bouyoucos, 1962)
Silt (%)	18.7	
Clay (%)	20.5	
<u>Chemical analysis</u>		
Organic carbon (%)	0.50	Walkley and Black method (Jackson, 1967)
Available N (kg ha ⁻¹)	55.72	Modified Kjeldahl's method (Jackson, 1967)
Ammoniacal nitrogen (mg kg ⁻¹)	3.20	Indophenol blue method (Keeney and Nelson, 1989)
Nitrate nitrogen (mg kg ⁻¹)	8.54	Phenoldisulphonic acid method (Ghosh <i>et al.</i> , 1983)
Nitrite nitrogen (mg kg ⁻¹)	traces	Modified Greiss Illosvay method, (Keeney and Nelson, 1989)
pH (soil : water = 1 : 2.5)	7.9	Beckman's pH meter (Jackson, 1967)
EC at 25°C (dSm ⁻¹)	0.35	Electrical conductance method (Richards, 1954)
<u>Physical characteristics</u>		
Water holding capacity	35.5	Keen-Rackzowski box method (Piper, 1966)
Permanent wilting point (%)	7.81	Pressure membrane method (Richards, 1954)
Bulk density	1.51	Core sampling method (Piper, 1966)

were chosen on the basis of the solubility of the test chemicals. Coal tar was dissolved in hexane, karanjin in dichloromethane whereas alcohol extracts in ethanol. Special care was taken during spraying of ethanol extract, as urea is soluble in ethanol.

3.2.8.5 Method of experiment (Incubation studies)

Nitrification inhibition study was conducted in laboratory by incubating the treated soils in a BOD incubator. The soil was incubated in beakers. In all following six experiments were designed:

- Experiment 1. Evaluation of furfural derivatives as nitrification inhibitors.
- Experiment 2. Evaluation of nitrification inhibitory effect of karanj extractives based coated products.
 - 2a. Effect of coating agents on nitrification inhibition.
 - 2b. Effect of karanjin coated urea on nitrification inhibition.
- Experiment 3. Comparison of coating vs. solution application of furfural derivatives on nitrification inhibition.
- Experiment 4. Comparison of coating vs. solution application of karanjin on nitrification inhibition.
- Experiment 5. Effect of mixtures of karanjin and synthetic furfural derivatives on nitrification inhibition.
- Experiment 6. Effect of mixtures of synthetic furfural derivatives and alcohol extract of karanj seed kernels on nitrification inhibition.

All the experiments were laid following randomised complete block design (RCBD) with three replicates. In every treatment, soils were incubated with 200 mg kg⁻¹ urea-N. In each experiment, nitrapyrin (N-Serve) was used as a standard at 5% dose of applied urea-N. For each set of treatments, a control (treated with only 200 mg kg⁻¹ urea-N without the test chemicals) was taken.

Table 3.2 : Treatments

Treatment	Symbol	Dose (% urea-N basis)
N-O-Ethyl furfural oxime	EOE	5, 10, 15, 20
N-O-Isopropyl furfural oxime	POE	5, 10, 15, 20
N-O-Butyl furfural oxime	BOE	5, 10, 15, 20
N-O-Hexyl furfural oxime	HOE	5, 10, 15, 20
N-O-Allyl furfural oxime	AOE	5, 10, 15, 20
Furfural oxime	OE	5, 10, 15, 20
4-(Furan-1-yl)-but-3-en-2-one	ACC	5, 10, 15, 20
3-(Furan-1-yl)-1-(4-hydroxyphenyl)-prop-2-en-1-one	HAPC	5, 10, 15, 20
3-(Furan-1-yl)-1-phenyl-prop-2-en-1-one	APC	5, 10, 15, 20
Furfurylideneaniline	AS	5, 10, 15, 20
Furfurylidene-4-chloroaniline	CAS	5, 10, 15, 20
Furfurylidene-3,4-dichloroaniline	DCAS	5, 10, 15, 20
Furfurylidene-3-nitroaniline	NAS	5, 10, 15, 20
Coal tar	C	2
Alcohol extract of karanj seed kernels	KA	1, 2.5, 5
Alcohol extract of neem seed kernels	NA	1, 2.5, 5
Coal tar 2 % + KA	CKA	1, 2.5, 5
Karanj oil	KO	1, 2.5, 5
Karanjin	KNx	1, 2.5, 5, 7.5, 10
Coal tar 2 % + karanjin	CKNx	1, 2.5, 5, 7.5, 10

Continued.....

Treatment	Symbol	Dose (% urea-N basis)
KA 5 % + karanjin	KA5KNx	1, 2.5, 5, 7.5, 10
NA 5% + karanjin	NA5KNx	1, 2.5, 5, 7.5, 10
Coal tar 2% + KA 5% + karanjin	KA5CKNx	1, 2.5, 5, 7.5, 10
Karanj oil 2.5% + karanjin	KO2.5KNx	1, 2.5, 5, 7.5, 10
Karanjin (Solution)	KS	5, 7.5
EOE coated	CEOEx	2.5, 5, 10
CAS coated	CCASx	2.5, 5, 7.5
EOE 5% + KNx + C 2%	EOE5KNx	2.5, 5, 7.5
CAS 5% + KN 2.5% + C 2%	CAS5KN2.5	
CAS 2.5% + KN 5% + C 2%	CAS2.5KN5	
KA 5% + EOEx	KA5EOEx	2.5,5
KA 5% + CASx	KA5CASx	2.5, 5
Nitrapyrin	NS	5 %

X = Represents the dose of the compounds/substance immediately preceding it and mentioned in column three of the corresponding rows.

3.2.8.5.1 Evaluation of furfural derivatives as nitrification inhibitors

3.2.8.5.1.1 Treatments : Twelve synthesized furfural derivatives and one intermediate furfural oxime, were tested for nitrification inhibitory effect in laboratory incubation studies. All the furfural derivatives (five furfural oxime ethers, three chalcones, four furfural Schiff bases and furfural oxime) were tested at five doses [5, 10, 15, and 20% of applied urea-N along with control (urea alone)]. Nitrapyrin (N-Serve) was taken as the reference inhibitor and was used @ 5% of the applied urea-N. All the treatments are shown in Table 3.2.

3.2.8.5.1.2 Incubation : Incubation was done in 500 ml capacity beakers. Two hundred gram soil was weighed per beaker. It was first mixed thoroughly with the calculated amount of the test chemical (2, 4, 6 and 8 mg for 5, 10, 15 and 20% dose of applied urea-N, respectively) in acetone in a large beaker and mixed thoroughly. In all the treatments including control, same volume of acetone was maintained. After thorough mixing, 40 mg urea-N (200 mg urea-N per kg of soil) in aqueous solution was added and mixed thoroughly. Distilled water was added to bring the soil moisture level to one third of water holding capacity of the soil. The treated soil was transferred to beakers and covered with muslin cloth with the help of a rubber band. The controls were similarly processed with urea alone at 200 mg kg⁻¹ urea-N level without adding any furfural derivative. For nitrapyrin, the soil was prepared in the similar way and only one dose of 5 % of urea-N was applied. All the beakers were accurately weighed, labeled and kept in a BOD incubator at 28°C, and 98% RH. Soil moisture was maintained by adding distilled water every alternate day after taking the difference of weight.

3.2.8.5.2 Evaluation of nitrification inhibitory effect of karanj extractives based coated products

3.2.8.5.2.1 Effect of coating agents on nitrification inhibition

For coating karanjin on urea prills, a binder or coating aid is

required. Coal tar, a commonly used binder or coating aid had been tried for this purpose. Beside coal tar, the highly viscous alcohol extracts of neem and karanj seed kernels and karanj oil were also tried. These substances are known to have some nitrification inhibitory effect of their own. So an experiment was conducted to ascertain the nitrification inhibitory effect of these materials.

3.2.8.5.2.1.1 Treatments : Karanj oil (KO), alcohol extract of karanj seed kernels (KA) and alcohol extract of neem seed kernels (NA) were tested for inhibition of nitrification at three doses 1, 2.5 and 5% of applied urea-N. Coal tar (C) was used at only one dose, 2% of applied urea-N. In one set of treatments, coal tar 2% + KA at three different doses viz., 1, 2.5, and 5% were tested.

3.2.8.5.2.1.2 Coating : Urea prills were coated following the procedure as mentioned in 3.2.8.4.2. Karanj oil and coal tar were sprayed as solutions in hexane. The alcohol extracts were sprayed very slowly, at time intervals, as ethanolic solutions. When both the coal tar and the alcohol extracts were to be coated, first coal tar was sprayed, followed by the alcohol extracts.

3.2.8.5.2.1.3 Incubation : Soil incubation study was carried out in 50 ml capacity polythene beakers. One kg of air dried soil was taken for each treatment and thoroughly mixed with the calculated amount of coated urea products in a large beaker. Distilled water was added to bring the soil moisture to one third of the soil water holding capacity. Treated soil (equivalent to 25 g of dry soil) was taken in 12, 50 ml capacity beakers. Estimations of NH_4^+ , NO_2^- and NO_3^- were done on the 15th, 30th, 45th and 60th day. For each treatment, three replications were taken. Hence there were 12 beakers for each treatment, along with three beakers for control. After filling, the beakers were covered with muslin cloth and tied with rubber

band. These beakers were incubated in a BOD incubator at 28°C, RH 98% (saturated with moisture).

3.2.8.5.2.2 Effect of karanjin coated urea on nitrification inhibition

3.2.8.5.2.2.1 Treatments : Experiment conducted with coated urea products revealed that the alcohol extracts of both neem and karanj seed kernels performed well at 5% dose of applied urea-N. For coating with karanjin, six sets of treatments were designed. In each set different coating agent was used. These treatments were : no coating agent + karanjin (KNx), coal tar 2% + karanjin (CKNx), alcohol extract of karanj seed kernels 5% + karanjin (KA5KNx), alcohol extract of neem seed kernels 5% + karanjin (NA5KNx), coal tar 2% + alcohol extract of karanj seed kernels 5% + karanjin (KA5CKNx) and karanj oil 2.5% + karanjin (KO2.5KNx). In each treatment set, karanjin was applied @1, 2.5, 5, 7.5 and 10% of applied urea-N. All the treatments are shown in Table 3.2. Control (no treatment) was taken in each set. Nitapyrin was taken as standard and applied as 5% of urea-N dose. The experiment was laid following RCBD with three replicates.

3.2.8.5.2.2.2 Coating : At first, urea prills were coated with the binder/coating agent following the procedure mentioned in Section 3.2.8.5.2.1.2. Coal tar was coated at 2% of applied urea-N dose whereas alcohol extracts and karanj oil were added at 5 and 2.5% of applied urea-N dose, respectively. After spraying of the binder, the calculated amount of karanjin was sprayed in dichloromethane.

3.2.8.5.2.2.3 Incubation : Method of incubation was same as mentioned in Section 3.2.8.5.2.1.3.

3.2.8.5.3 Comparison of coating vs solution application of karanjin on nitrification inhibition

3.2.8.5.3.1 Treatments : To study the added advantage of coating of urea

prills with karanjin over solution application, an experiment was conducted in which karanjin in solution and coated form (Section 3.2.8.4) were applied in two doses 5% and 7.5%. Urea was coated with karanjin by spraying in dichloromethane. No binder was used for coating the karanjin.

3.2.8.5.3.2 Incubation : For coating treatments, incubation methodology as mentioned in Section 3.2.8.5.2.1.3 was followed. For application in the form of solution, one kg air dried soil was taken and mixed with the calculated amount of the test chemical in acetone. After thorough mixing, 200 mg urea-N prills were added and mixed thoroughly followed by addition of distilled water to bring the soil moisture to one third of water holding capacity of soil. From the treated soil, soil equivalent to 25 g of dry soil was taken in each of 12 beakers, and incubated in a BOD incubator at 28°C after covering each beaker with a muslin cloth.

3.2.8.5.4 Comparison of coating vs. solution application of furfural derivatives on nitrification inhibition

3.2.8.5.4.1 Treatments : An experiment was conducted to see whether there is any effect of application techniques on nitrification rate. Two synthesized furfural derivatives EOE and CAS were used in this study. They were applied in two ways – in solution form and in coated form (Section 3.2.8.4). EOE was tested at 5 and 10% of applied urea-N, whereas CAS was tested at 2.5, 5 and 7.5% of applied urea-N. Urea was first coated with 2% coal tar and then calculated amount of EOE and CAS was coated.

3.2.8.5.4.2 Incubation : Incubation was done in 50 ml beakers. For coating treatments, the methodology as mentioned in Section 3.2.8.5.2.1.3 was followed. For application in solution form, the methodology as mentioned in Section 3.2.8.5.3.2 was followed.

3.2.8.5.5 Effect of mixtures of karanjin and synthetic furfural derivatives on nitrification inhibition

The results with synthetic furfural derivatives showed that some of the compounds possessed good activity. Karanjin also showed considerable nitrification inhibitory activity. The mode of action of these two classes of compounds are not known. However, a synergistic or antagonistic activity could be expected based on their modes of action. An experiment was therefore planned to evaluate the effect of their mixtures on the nitrification inhibition. Studies on the synthetic furfural derivatives revealed that EOE was the most effective among oxime ether series of compound and CAS was the most effective among the synthesized Schiff bases. In this experiment, mixtures of these two compounds with karanjin were evaluated.

3.2.8.5.5.1 Treatments : Five treatments were evaluated, three with EOE and two with CAS. In case of EOE, the prills were coated with 5% EOE and 2.5, 5 and 7.5% karanjin, respectively. For CAS, the prills were coated with CAS 2.5% + karanjin 5% and CAS 5% + karanjin 2.5%. All the doses were on per cent urea-N basis. In all the cases, the prills were first coated with coal tar and then mixture of calculated amount of karanjin and synthetic furfural derivatives were coated.

3.2.8.5.5.2 Incubation : Method of incubation is described in Section 3.2.8.5.2.1.3

3.2.8.5.6 Effect of mixtures of synthetic furfural derivatives and alcohol extract of karanj seed kernels on nitrification inhibition

The alcohol extract of karanj seed kernels are cheaper than karanjin because of higher yields and simplicity of extraction. So the mixtures of alcohol extract with furfural derivatives were explored to improve the economics of the candidate nitrification inhibitors.

3.2.8.5.6.1 Treatments : Four treatments were tested. Those were : KA 5% + EOE 2.5%, KA5% + EOE 5%, KA 5% + CAS 2.5 % and KA 5% + CAS 5%.

3.2.8.5.6.2 Coating : Coating of the urea prills were done following the same procedure as mentioned earlier (Section 3.2.8.4.2). The prills were first coated with alcohol extract followed by synthetic compounds.

3.2.8.5.6.3 Incubation: Method of incubation is described in Section 3.2.8.5.2.1.3.

3.2.8.6 Sampling

3.2.8.6.1 Sampling from five hundred ml capacity beakers : From five hundred ml capacity beakers samples were drawn after 15, 30, 45 and 60 days of incubation. Before sampling, distilled water was added to make up for the loss in weight due to evaporation of water and mixed thoroughly. For estimation of nitrate, soil sample equivalent to 20 g (Ghosh *et al.*, 1983) of dry soil was withdrawn and for estimation of nitrite and ammonium, soil sample equivalent to 5 g (Keeney and Nelson, 1989) of dry soil was withdrawn. Same beaker was used for further sampling.

3.2.8.6.2 Sampling from fifty ml capacity beakers : In case of 50 ml capacity beakers on each sampling day, three beakers (three replications) were taken out for each treatment. From each beaker soil equivalent to 20 g of dry soil was taken for estimation of nitrate and soil equivalent to 5 g of dry soil was taken for estimation of nitrite and ammonium. Before sampling, distilled water was added to make up for the loss in weight due to evaporation of water and mixed thoroughly. For each replication one beaker was used.

3.2.8.7 Extraction of ammonium, nitrite and nitrate from the soil samples : Nitrate was extracted in 50 ml distilled water following the procedure of Ghosh *et al.*, (1983). Soil sample and distilled water were shaken in a screw capped plastic bottle in a mechanical shaker for an hour. At the end of the shaking, a

pinch of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) was added and the sample shaken further for 5 minutes for easy filtration.

Nitrite and ammonium were extracted in 50 ml 2 M KCl aqueous solution. A pinch of Darco G-60 (made free from ammonium and nitrite) was added for obtaining a clean extract. The soil with the extractant was shaken for half an hour in a mechanical shaker and the extractant was filtered through Whatman No. 1 filter paper and taken in a plastic bottle. The bottles were capped and stored in a refrigerator, before analysis.

3.2.8.8 Estimation of ammonium, nitrite and nitrate : Ammonium was estimated following Indophenol blue method and nitrite by the modified Greiss-Ilosvay method (Keeney and Nelson, 1989). Nitrate was estimated by the phenol disulphonic acid method (Ghosh *et al.*, 1983).

3.2.8.9 Calculation of nitrification inhibition percentage : The contents of ammonium, nitrate and nitrite were obtained from the standard curves and expressed in mg kg^{-1} . The nitrification rate was calculated as follows (Sahrawat, 1980) :

$$\frac{(\text{NO}_3^- + \text{NO}_2^-)\text{-N}}{(\text{NH}_4^+ + \text{NO}_3^- + \text{NO}_2^-)\text{-N}} \times 100$$

Per cent nitrification inhibition was calculated as follows (Sahrawat, 1980) :

$$\frac{\text{Nitrification rate in control} - \text{nitrification rate in sample}}{\text{Nitrification rate in control}} \times 100$$

3.2.8.10 Statistical treatment of the data : The nitrification inhibition percentage data from all the experiment were statistically analysed following the procedure laid out by Gomez and Gomez (1984). The analysis of variance

was computed and treatment means were compared by Duncan's Multiple Range Test (DMRT) at 5% level. Statistical analysis was carried out with arc sine transformed data.

3.2.9 Evaluation of physicochemical properties of the coated urea products

3.2.9.1 Flowability : All the coated urea products were visually evaluated for flowability.

3.2.9.2 Total N content : It was estimated by Kjeldahl digestion method (Tripathi, 1995).

3.2.9.3 Size distribution : Particle size of the coated products were evaluated using 2.8 mm and 1 mm IS sieves.

3.2.9.4 Moisture content : Coated urea products were first ground in a porcelain pestle and mortar to pass IS Sieve No. 40. Grinding was done as quickly as possible to avoid loss or gain of moisture. Five gram of the ground sample was accurately weighed in a shallow porcelain dish and dried for 24 hour in a vacuum desiccator over sulfuric acid to a constant weight. Moisture percentage by weight was calculated by the formula :

$$\frac{W_1}{W_2} \times 100$$

W_1 = Loss in weight in g on drying and W_2 = Weight in g of the prepared sample taken for the test.

3.2.10 Evaluation of the effect of furfural derivatives on germination of chickpea seeds

In order to see the effect of synthetic furfural derivatives on germination of seeds, a germination test was carried out with chickpea (*Cicer arietinum*) seeds. Two oxime ethers (EOE and POE) and two furfural Schiff bases (CAS and NAS) were tested at 10 and 40 ppm levels (soil basis,

equivalent to 5 and 20 per cent of applied urea-N). In 500 ml beaker, 200 g soil was taken and treated with calculated amount of the test chemical(s) and mixed thoroughly. Water was added to bring the soil moisture condition to two third of water holding capacity. After preparation of the soil, 20 chickpea seeds were sown in each beaker. Each treatment was replicated five times. A control was taken in which no test chemical was added. The beakers were kept at room temperature and moistened regularly. After ten days, the number of germinated seeds and vigour of seedlings were evaluated visually.

RESULTS AND DISCUSSION

- 4.1 Synthesis of furfural derivatives
 - 4.1.1 Synthesis of furfural oxime ether
 - 4.1.2 Synthesis of furfural chalcones
 - 4.1.3 Synthesis of furfural Schiff bases
- 4.2 Nitrification inhibitory effect of the test chemicals
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 - 4.2.1.1 Furfural oxime ethers
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- 4.3 Effect of test chemicals on the accumulation of ammonium, nitrite and nitrate in incubated soil.
 - 4.3.1 Accumulation of ammonium
 - 4.3.2 Accumulation on nitrite
 - 4.3.3 Accumulation of nitrate
- 4.4 Physicochemical properties of the coated urea products
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 - 4.4.2 Total N content
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RESULTS AND DISCUSSION

The present investigation was carried out with the objective to develop suitable chemicals from indigenous sources which may be advantageously utilized for increasing the efficiency of nitrogenous fertilizers by regulating the rate of nitrification and thereby, minimizing the loss of nitrogen by leaching and denitrification. Two leads have been explored in this study. Firstly, twelve synthetic furfural derivatives have been tested for their possible inhibitory effect on nitrification in *in vitro* soil incubation studies. Secondly, efforts have been made to develop technology for making of karanjin based coated urea products. Beside these two major thrusts some associated studies namely, effect of mixture of karanj seed kernel extractives and synthetic derivatives on nitrification, effect of coating verses solution application of synthetic derivatives on nitrification and effect of synthetic furfural derivatives on germination of chickpea (*Cicer arietinum*) seeds have also been carried out. Physico-chemical properties of the new products have been also evaluated.

4.1 Synthesis of furfural derivatives

Kuzvinzwa (1974) and Sahrawat (1977) reported the structure-activity relationships between various furan derivatives and their nitrification inhibition property. Based on the information available in literature, synthesis of twelve furfural based products were designed.

4.1.1 Synthesis of furfural oxime ether : Kuzvinzwa (1974) reported that furfural oxime possess excellent nitrification inhibition property. In this study, five derivatives of furfural oxime, having different N-O-alkyl groups were synthesized. Synthesis of the nitro derivatives of furfural oxime were not attempted because of the earlier report (Kuzvinzwa, 1974) that the nitro substitution in the furan ring impart general bactericidal property instead of

selective activity against *Nitrosomonas*. Such compounds generally lead to the accumulation of the toxic quantities of nitrites.

The oxime ethers were synthesized, following the reaction of furfural oxime with alkyl halides using anhydrous potassium carbonate and acetone. The inorganic base ionizes the oximes to form anion, which then reacts with alkyl bromide to form corresponding oxime ethers by SN_2 reaction. All precautions were taken during the reaction in order to ensure moisture free conditions for higher yields. Around 75% yield were obtained in most reactions. Loss of compound during column chromatography was mainly responsible for poor yield of the product. Two isomers, *syn* and *anti* were produced for each oxime ether. Testing was carried out with the mixture of these two isomers. The formation of the oxime ethers was confirmed by disappearance of $-OH$ peak of furfural oxime and appearance of peaks for alkyl protons in the NMR spectrum. The characteristic peak of $-CH=N$ (δ 7.9-8) was found distinct for each oxime ether. The spots for oxime ethers took time to appear in the TLC plates after spraying of 2,4-DNP whereas furfural oxime gave spots immediately. Two isomers gave two spots, one above the furfural oxime and the other below it.

4.1.2 Synthesis of furfural based chalcones : Three chalcones were synthesized following Claisen-Schmidt reaction. Formation of furfural based chalcones were confirmed by the disappearance of aldehyde proton of furfural (δ 9.9) and two α proton from acetyl group (CH_3-CO) of the carbonyl compounds and appearance of peaks for $-CH=CH-$ protons. The chalcones gave pink coloured spots on TLC plates when sprayed with 2,4 DNP.

4.1.3 Synthesis of furfural Schiff bases : Furfural and anilines both are known for their nitrification inhibition property (Sahrawat, 1977; Thompson and Corke, 1969). Their condensation products are therefore expected to show greater nitrification inhibition property. To exploit this idea four Schiff bases or anils were synthesized and tested as nitrification inhibitors.

Literature scans revealed that chloro substitution on the phenyl ring increases activity of anilines while nitro substitution has a general bactericidal effect. Two chloro substituted furfurylideneanilines and one nitro substituted furfurylideneaniline were synthesized and their activity compared with the unsubstituted furfurylideneaniline to evaluate the effect of such substitution. The structures of the Schiff bases were confirmed by the disappearance of $-\text{NH}_2$ peak at δ 3.8-3.5 and appearance of $-\text{CH}=\text{N}-$ peak at δ 8.4 in their PMR spectra.

4.2 Nitrification inhibitory effect of the test chemicals

Nitrification inhibitory effect was studied in the laboratory with soil incubation studies. Nitrification inhibition percentage (NI%) as proposed by Sahrawat (1980) was taken as criteria for evaluating the performances of the test chemicals. The amount of ammonium, nitrite and nitrate present in soil in sampling days, do indicate the nitrification inhibition property of the test chemicals and their combination but they are not effective for comparison specially for large numbers of treatments. Bundy and Bremner (1973) evaluated the performance of their product by per cent inhibition of nitrification which was calculated from : $(C-S) / C \times 100$, where S = amount of $(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$ produced in the soil sample treated with the test compound and C = amount of $(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$ produced in the control (no test compound added). But such a criteria is only suitable for soils containing insignificant amount of ammonium. In soils containing significant quantity of ammonium, the criteria as proposed by Sahrawat (1980) mentioned in Section (3.2.8.9) is more justifiable and hence used in this study.

4.2.1 Evaluation of furfural derivatives as nitrification inhibitors

Results obtained in *in vitro* soil incubation studies with furfural derivatives are reported in Table 4.1 (NI%) and Table 4.8 (ammonium, nitrite and nitrate contents) and are graphically depicted in Figs. 4.1 to 4.5.

Treatment means in terms of NI% were compared by Duncan's Multiple Range Test at 5% level and presented in Table 4.1. Treatments followed by a common letter indicated nonsignificant difference between the treatments. Statistical analysis for each sampling day showed that the differences between the treatment means were highly significant even at 1% level [F value for 15th, 30th, 45th and 60th day being 69 (63), 123 (116), 168 (162) and 122 (123), respectively; the values in parentheses having been obtained from arc sine transformation data]. The treatment $\times$ day interaction has also been found to be highly significant [F value = 20.85 (18.07)].

4.2.1.1 Furfural oxime ethers : Furfural oxime ethers in general have been found to be effective nitrification inhibitors. Among the various oxime ethers, N-O-ethyl furfural oxime (EOE) was most active. At 5% and 20% doses, it showed 67.04 and 79.67% NI (nitrification inhibition), respectively on 15th day. The corresponding values for 45th day were 53.58 and 69.20% NI, respectively. The standard nitrapyrin at 5% dose gave 83.14 and 72.81% NI on the 15th and 45th day, respectively. So on the 15th day 20% dose of EOE did not differ substantially with nitrapyrin but with the passage of time the difference increased. When the dose of EOE was increased from 5 to 20%, the inhibitory effect on nitrification did not increased proportionately, however, activity of each dose of EOE differed significantly from the other. This trend was observed for all the oxime ethers on all the days. In some isolated cases there were no significant difference between two successive doses for example, the NI activity of the 15 and 20% doses of N-O-allyl furfural oxime (AOE) on 15th as well as on 45th day did not differed significantly. Nitrification inhibition activity of EOE fell gradually with time upto 45th day. On 15th day at 20% dose, 79.67% inhibition was observed whereas on the 30th and 45th day NI activity was found to be 74.54 and 69.20%, respectively. After the 45th day, the activity fell sharply and reached 41.80% on the 60th day. All the other doses of EOE as well as all the doses of POE and BOE behaved similarly.

Table 4.1: Effect of furfural derivatives on nitrification inhibition

Compound*	Dose (% urea-N)	Nitrification inhibition (%)**			
		15 th Day	30 th Day	45 th Day	60 th Day
EOE	5	67.04 ^{mno}	60.64 ^{lmno}	53.58 ^l	27.78 ^{lmno}
	10	72.48 ^{ghijk}	68.88 ^{cdef}	59.15 ^{jk}	33.07 ^{hi}
	15	76.05 ^{cdef}	70.79 ^c	65.76 ^{def}	37.27 ^{fg}
	20	79.67 ^b	74.54 ^b	69.20 ^{bc}	41.80 ^d
POE	5	66.90 ^{mno}	59.23 ^{mnpq}	51.45 ^{lm}	23.75 ^{qrs}
	10	69.85 ^{klm}	64.44 ^{ij}	58.02 ^k	32.12 ^{hij}
	15	74.97 ^{defgh}	69.32 ^{cdef}	62.88 ^{fgh}	36.51 ^{fg}
	20	79.16 ^b	71.47 ^c	66.41 ^{cde}	39.24 ^{def}
BOE	5	65.08 ^{op}	58.18 ^{nopq}	49.41 ^{mn}	24.40 ^{pqrs}
	10	69.28 ^{lmn}	65.02 ^{hi}	54.00 ^l	29.49 ^{klm}
	15	73.80 ^{fghi}	67.29 ^{fgh}	59.59 ^{ijk}	33.32 ^{hi}
	20	76.99 ^{bcd}	70.18 ^{cde}	62.41 ^{ghi}	36.57 ^{fg}
HOE	5	53.06 ^{tu}	48.83 ^{wx}	37.21 ^t	20.91 ^{tuv}
	10	59.55 ^{rs}	52.04 ^{tuv}	41.11 ^{qrs}	26.11 ^{opqr}
	15	64.43 ^{opq}	57.19 ^{pqr}	50.86 ^{lm}	32.18 ^{hij}
	20	69.15 ^{lmn}	65.82 ^{ghi}	59.00 ^k	34.70 ^{gh}
AOE	5	67.18 ^{mno}	60.65 ^{lmno}	38.37 ^{rst}	18.83 ^{vw}
	10	72.83 ^{ghijk}	64.19 ^{ij}	40.85 ^{qrs}	20.78 ^{uv}
	15	75.47 ^{defg}	68.93 ^{cdef}	47.66 ^{no}	22.62 ^{stu}
	20	77.18 ^{bcd}	71.00 ^c	49.88 ^{mn}	27.13 ^{mnp}
OE	5	61.74 ^{qr}	49.44 ^{vw}	32.61 ^{uv}	18.13 ^w
	10	65.31 ^{op}	55.06 ^{rs}	37.92 st	20.76 ^{uv}
	15	71.61 ^{ijkl}	60.04 ^{lmnop}	42.71 ^{pq}	25.60 ^{opqr}
	20	73.76 ^{fghi}	62.17 ^{ijkl}	44.74 ^{op}	26.42 ^{nopq}
APC	5	65.27 ^{op}	47.00 ^x	30.42 ^{vw}	15.62 ^{xy}
	10	70.41 ^{ijkl}	50.49 ^{uvw}	33.68 ^u	22.21 ^{stu}
	15	74.99 ^{defgh}	58.90 ^{nopq}	41.28 ^{qr}	29.03 ^{klmn}
	20	77.64 ^{bcd}	62.02 ^{kilm}	47.71 ^{no}	32.80 ^{hi}
HAPC	5	50.12 ^u	36.91 ^z	26.83 ^x	9.73 ^z
	10	54.20 ^t	41.59 ^y	33.45 ^{uv}	13.73 ^y
	15	59.68 ^{rs}	46.83 ^x	39.48 ^{qrst}	17.41 ^{wx}
	20	62.27 ^{pqr}	52.65 ^{stu}	41.64 ^{qr}	26.09 ^{opqr}
ACC	5	67.52 ^s	43.09 ^y	28.73 ^{wx}	13.59 ^y
	10	63.27 ^{pq}	50.30 ^{uvw}	32.68 ^{uv}	19.41 ^{vw}
	15	66.50 ^{no}	58.11 ^{opq}	39.49 ^{qrst}	24.20 ^{qrs}
	20	70.91 ^{ijkl}	61.11 ^{klmn}	45.22 ^{op}	30.52 ^{ijkl}

Continued.....

Compound*	Dose (% urea-N)	Nitrification inhibition (%)**			
		15 th Day	30 th Day	45 th Day	60 th Day
CAS	5	72.80 ^{ghijk}	67.74 ^{defg}	59.37 ^{ijk}	32.06 ^{hij}
	10	78.62 ^{bc}	70.38 ^{cd}	63.36 ^{efgh}	37.03 ^{fg}
	15	82.89 ^a	75.52 ^{ab}	66.95 ^{cd}	47.87 ^{bc}
	20	84.59 ^a	77.36 ^a	70.04 ^b	49.22 ^b
DCAS	5	70.03 ^{klm}	60.38 ^{lmno}	51.21 ^{lm}	32.36 ^{hij}
	10	73.19 ^{ghij}	63.68 ^{ijk}	60.35 ^{hijk}	38.62 ^{ef}
	15	77.21 ^{bcd}	68.89 ^{cdef}	62.25 ^{ghij}	45.10 ^c
	20	79.18 ^b	71.17 ^c	66.38 ^{cde}	47.09 ^{bc}
NAS	5	72.00 ^{hijkl}	53.83 st	31.55 ^{uvw}	23.50 ^{rst}
	10	73.39 ^{ghij}	58.44 ^{nopq}	38.59 ^{rst}	30.69 ^{ijk}
	15	74.81 ^{defgh}	68.76 ^{lcdef}	57.17 ^k	39.62 ^{def}
	20	79.28 ^b	70.84 ^c	65.02 ^{defg}	41.39 ^{de}
AS	5	69.22 ^{lmn}	51.66 ^{tuvw}	30.61 ^{vw}	22.01 ^{stu}
	10	70.97 ^{ijkl}	56.92 ^{qr}	38.53 ^{rst}	28.89 ^{klmn}
	15	74.03 ^{efghi}	67.56 ^{efgh}	52.13 ^{lm}	36.97 ^{fg}
	20	76.01 ^{cdef}	69.33 ^{cdef}	64.57 ^{defg}	39.45 ^{def}
NS	5	83.14 ^a	76.55 ^{ab}	72.81 ^a	54.55 ^a

* EOE (N-O-ethyl furfural oxime), POE (N-O-isopropyl furfural oxime), BOE (N-O-butyl furfural oxime), HOE (N-O-hexyl furfural oxime), AOE (N-O-allyl furfural oxime), OE (furfural oxime), ACC [4-(furan-1-yl)-1-but-3-en-2-one], APC [3-(furan-1-yl)-1-phenyl-prop-2-en-1-one], HAPC [3-(furan-1-yl)-1-(4-hydroxyphenyl)-prop-2-en-1-one], CAS (furfurylidene-4-chloroaniline), NAS (furfurylidene-3-nitroaniline), DCAS (furfurylidene-3,4-dichloroaniline), AS (furfurylideneaniline), NS (nitrapyrin).

** Treatment means are average of three replicates. Treatment means are compared by Duncan's Multiple Range Test (DMRT) at 5% level after arc sine conversion of percentage data shown in the Table. Those followed by a common letter in a column show nonsignificant difference.

Activity of all the four doses of EOE decreased at the same rate. The reduction in the activity of these chemicals with passage of time was attributed to their losses, probably due to their metabolism by soil microorganisms. The gradual reduction in activity of these compounds with time may also be associated with the reestablishment of the population of the nitrifying bacteria.

The activity pattern of N-O-isopropyl furfural oxime (POE) was similar to EOE. At 5 and 20% dose of POE, it showed 66.90 and 79.16% NI activity, respectively on the 15th day, whereas on the 45th day the values were 51.45 and 66.41%, respectively. Like EOE, it also showed comparable activity with nitrapyrin at higher dose (20%) but at lower dose (5%) difference was quite substantial.

In case of N-O-butyl furfural oxime (BOE) similar trend of activity as described earlier was observed with a difference that it was less active as compared to the earlier ones. BOE at 5% dose showed 65.08% and at 20% dose 76.99% NI activity on the 15th day.

NI activity of N-O-hexyl furfural oxime (HOE) was the worst among the five oxime ethers. At lower doses (5 and 10%) the nitrification inhibitory effect started falling after the 30th day whereas at the higher doses (15 and 20%), the activity fell rapidly after the 45th day. Thus, the activity of HOE lasted for longer time at higher doses (15 and 20%). The activity of HOE was not only low but also did not persist for longer period as compared to the earlier compounds.

N-O-allyl furfural oxime (AOE) showed considerable NI activity initially (15th day) but the activity decreased rapidly after the 30th day for all the four doses. On 15th, 30th and 45th days, the NI activity of the compound at 5% dose were 67.18, 60.65 and 38.37%, respectively. The corresponding values for EOE were 67.04, 60.64 and 53.58%, respectively. Thus, initially though there was no statistically significant difference between AOE and EOE at the

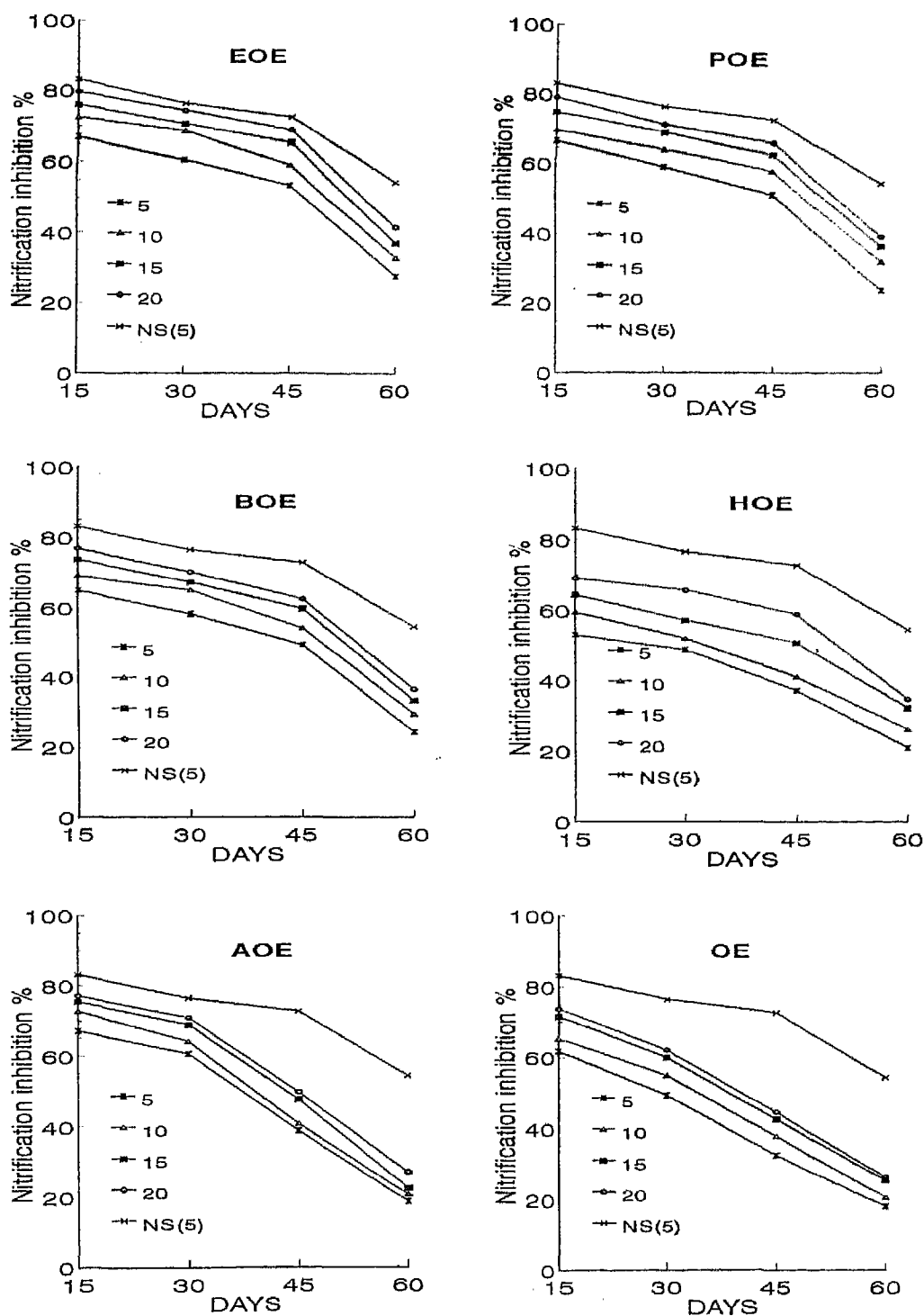


Fig.4.1 Effect of various furfural oxime ethers and nitrapyrin on nitrification inhibition (doses are expressed as percentage of applied urea-N)

15th and 30th day but on the 45th day the difference was pronounced. So, AOE did not show persistent activity and this can be attributed to allyl group which is probably more susceptible to degradation.

The intermediate furfural oxime possesses considerable activity initially but the activity fell rapidly from the 15th day at all the doses of application.

Amongst the various oxime ethers, EOE was found to be the most effective though statistically at different doses and time it was at par with POE. Apparently, an increase in the number of carbon atoms in the alkyl chain, reduced the NI activity of the compounds. EOE 5% did not differ significantly from POE 5 and 10%, BOE 5 and 10%, HOE 15% and AOE 5%. Between POE and BOE though the NI data showed an apparent superiority of POE but there was no statistically significant difference between them on the 15th and 30th day. However, on the 45th day POE showed significantly superior activity than BOE at 5, 10 and 15% doses. Thus POE can be adjudged better than BOE. Those compounds that are active at low doses also showed a long lasting effect. Those, which are not active at low dose, showed long-lasting effect only at higher doses.

As compared to the intermediate furfural oxime, the activity of oxime ethers like EOE, POE and BOE were significantly higher at all the doses. Initially the difference was less pronounced but after the 15th day the difference became more pronounced. On the 45th day, at 5% dose the NI of EOE and OE were 53.58% and 32.61%, respectively and at 20% dose the values were 69.20% and 44.74%, respectively. Thus, the greatest advantage of oxime ethers as compared to the parent oxime were increased persistence of activity in soil. Upto the 30th day HOE performed poorly than OE at lower doses (5 and 10%) of application but after 30th day the same doses of HOE outperformed OE.

The NI activity of higher doses (15 and 20%) of EOE, POE and

The NI activity of higher doses (15 and 20%) of EOE, POE and BOE were comparable to the standard nitrification inhibitor nitrapyrin. However, unlike these test chemicals, the activity of nitrapyrin persisted even after the 45th day. Lower doses (5 and 10%) of these compounds performed poorly as compared to 5% of nitrapyrin.

From this study it can be concluded that furfural oxime ethers as a class show considerable nitrification inhibitory property. Activity decreases with increase in chain length as well as introduction of double bond on the alkyl group. On the basis of the study, five test compounds can be arranged in the following order of activity :

$$\text{EOE} \geq \text{POE} > \text{BOE} > \text{HOE} \approx \text{AOE}$$

4.2.1.2 Furfural chalcones : Among the three chalcones tested 3-(furan-1-yl)-1-phenyl-prop-2-en-1-one (APC) performed the best. On the 15th day APC showed 65.27% NI activity at 5% dose and 77.64% at 20% dose. The corresponding figures for the 45th day were 30.42% and 47.71%, respectively. Thus APC showed good activity initially which could not be sustained for longer period. The activity started decreasing at a faster rate from the beginning giving almost a straight line (Fig. 4.2). All the doses exhibited the same trend. Higher doses showed greater activity but did not give a long lasting effect. All the four doses showed statistically significant difference in activity. At the highest dose (20%), APC showed less activity (77.64%) as compared to NS (83.14%) and the difference widened with passage of time.

The remaining two chalcones namely, 4-(furan-1-yl)-but-3-en-2-one (ACC) and 3-(furan-1-yl)-1-(4-hydroxyphenyl)-prop-2-en-1-one (HAPC) were less active than APC. Like APC, the activity of ACC and HAPC also decreased quickly with time. On the 15th day HAPC showed 50.12% NI activity at 5% dose and 62.27% at 20% dose whereas the corresponding values for the 45th day were 26.83 and 41.64%. So, starting from 15th day, the activity of the compound showed

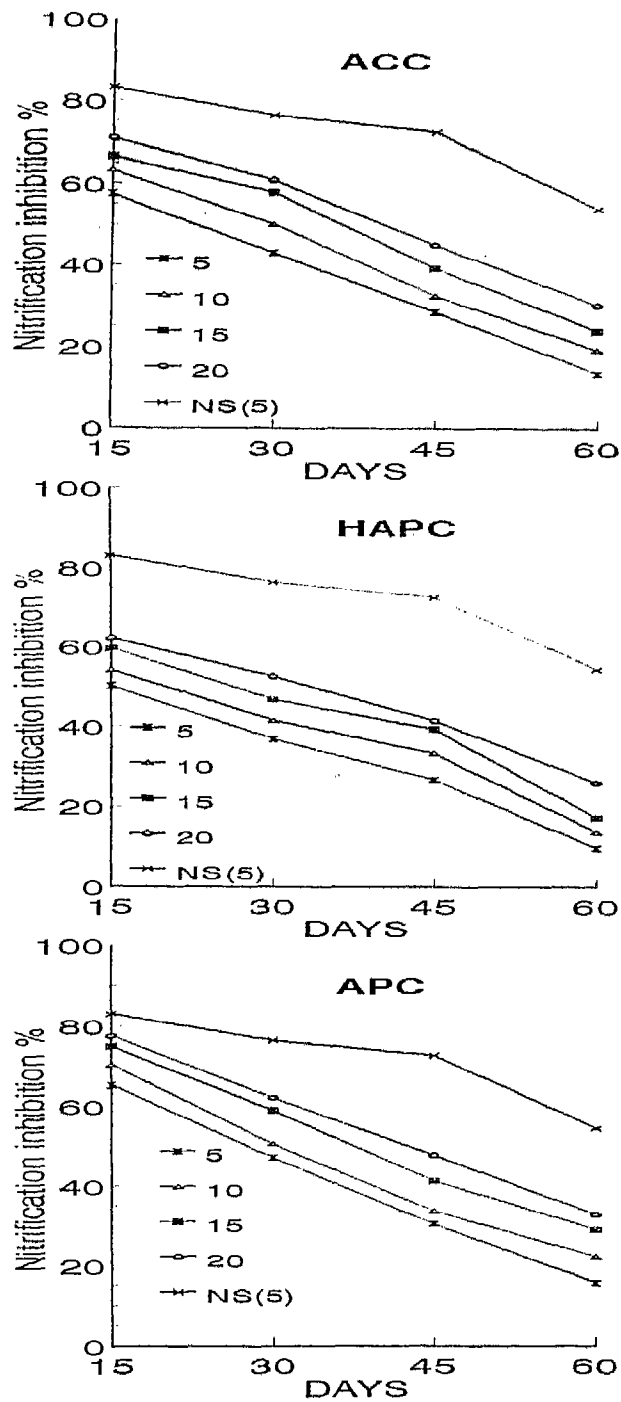


Fig.4.2 Effect of furfural chalcones and nitrapyrin on nitrification inhibition (doses are expressed as percentage of applied urea-N)

substantial difference with nitrapyrin even at highest doses (20%) of application.

The activity pattern of ACC was similar to APC. Like, HAPC, the highest dose (20%) was not comparable with nitrapyrin (5%) on the 15th day. On the 15th day, the 5 and 20% dose showed 57.52 and 70.91% NI activity, respectively.

Among these three chalcones, APC performed the best followed by ACC and HAPC. The same dose of these three chalcones showed significant difference in activity on the 15th, 30th and 60th days, whereas the difference in activity on the 45th day was not significant. Introduction of hydroxy group in the phenyl ring significantly reduced the activity of these compounds. The chalcones as a class failed to show significant NI property and cannot be considered as potential class of compound for such activity.

4.2.1.3 Furfural Schiff bases : Among the furfural Schiff bases (furfurylideneanilines), CAS (furfurylidene-4-chloroaniline) outperformed all the other Schiff bases. When applied at 5 and 20% doses, it showed 72.80 and 84.59% NI activity, respectively on the 15th day. The activity at higher doses (15 and 20%) did not differ significantly from NS (5%) on the 15th and 30th days and was comparable even upto the 60th day. The lower doses (5 and 10%) showed comparable activity with nitrapyrin upto 30th day. The lower doses (5 and 10%) of CAS inhibited 60% nitrification as compared to control upto 45th day. Like nitrapyrin, the activity of CAS decreased gradually upto 45th day and rapidly after 45 days. All the doses of CAS followed this pattern of activity. The 5% dose of CAS was significantly inferior in performance as compared to the 10% dose. The 15 and 20% doses did not differ significantly from each other on the 15th, 30th and 60th day.

DCAS (furfurylidene-3,4-dichloroaniline) at higher doses (15 and 20%) showed excellent NI activity. On the 15th day, DCAS showed 70.03% NI activity at 5% dose and 79.18% at 20% dose. The corresponding figures for the 45th day were 51.21 and 66.38%. Thus even at the 5% dose, this compound inhibited more than 50% nitrification upto 45th day. The

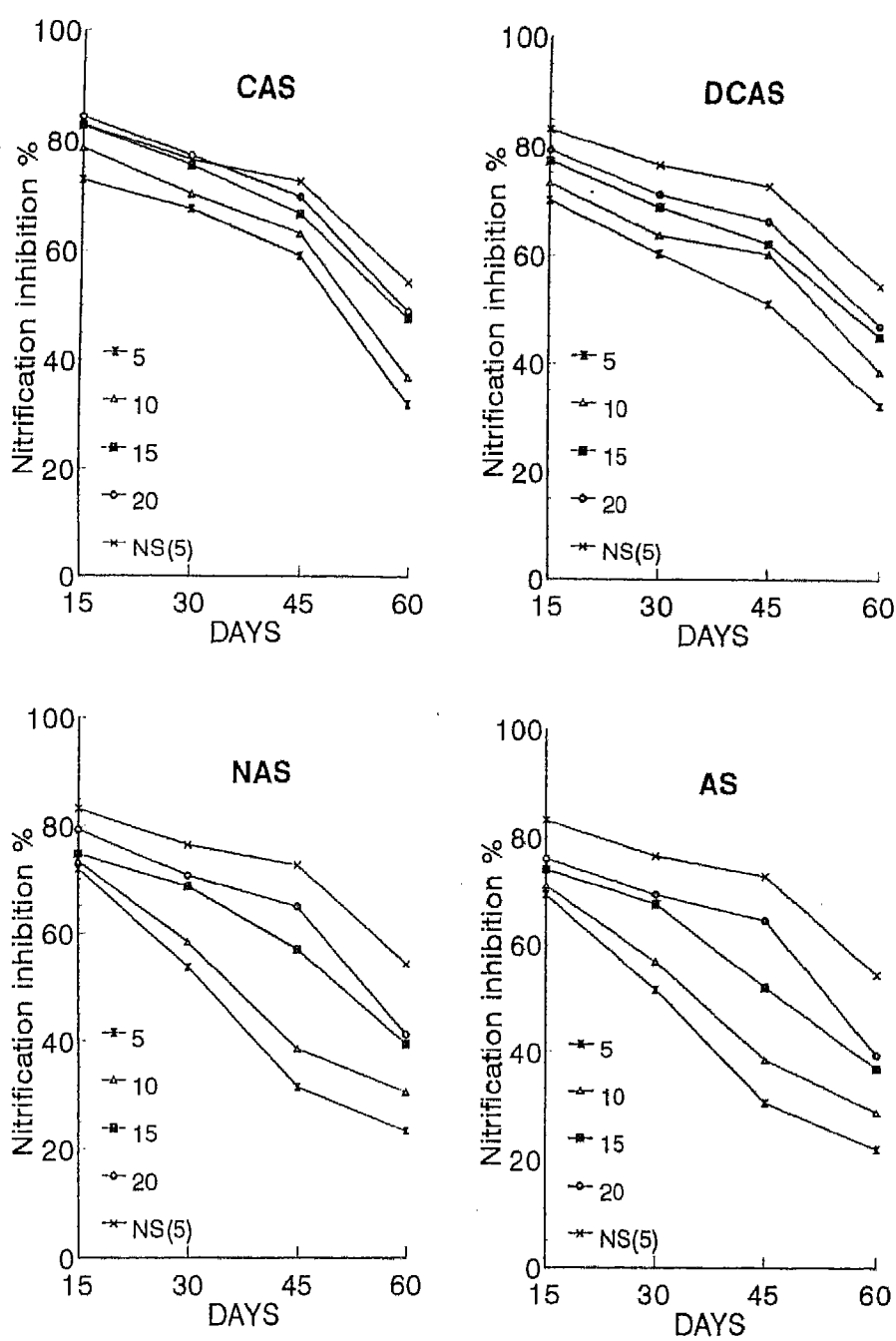


Fig.4.3 Effect of furfural Schiff bases and nitrpyrin on nitrification inhibition (doses are expressed as percentage of applied urea-N)

higher doses (15 and 20%) of DCAS showed marginally inferior activity as compared to nitrapyrin. Like CAS, this compound also did not showed significant difference between 15 and 20% dose, except slightly higher activity on the 45th day. Thus the 15% dose may be considered maximum for both CAS and DCAS.

NAS (furfurylidene-3-nitroaniline) initially (upto 30th day) showed considerable nitrification inhibition property, which with the passage of time decreased considerably. The NI activity at the 5 and 20% doses of NAS on the 15th day were 72.00 and 79.28% and on 45th day were 31.55 and 65.02%. Initially on the 15th day the difference of activity between NAS and NS was not substantial but with the passage of time the difference widened.

AS (furfurylideneaniline) also showed considerable NI property initially. In case of AS on the 15th day, NI activity at 5 and 20% doses were 69.22 and 76.01%, respectively whereas on the 45th day the values were 30.61 and 64.57%, respectively. Thus it can be concluded that higher doses only increased the persistence of activity upto the 45th day but did not necessarily increased the activity to a considerable extent. In case of both NAS and AS higher doses (15 and 20%) showed long lasting effect (Fig 4.3). The 20% dose of both the compounds showed greater persistence of activity (upto 45th day) followed by the 15% dose. In case of 5 and 10% dose the activity started diminishing rapidly from the 15th day onwards.

From the above observation it is evident that introduction of chlorine atom in the phenyl ring increased persistence as well as activity. However, introduction of more than one chlorine atom reduced the activity as in case of DCAS as compared to the monochlorinated derivative (CAS). The activity of DCAS was significantly inferior to CAS in most doses and reported that activity increased with the number of chlorine substituents in the aniline ring sampling days except on the 60th day. Thomson and Corke (1969) reported that the activity increased with the number of chlorine substituents in the aniline ring. Introduction

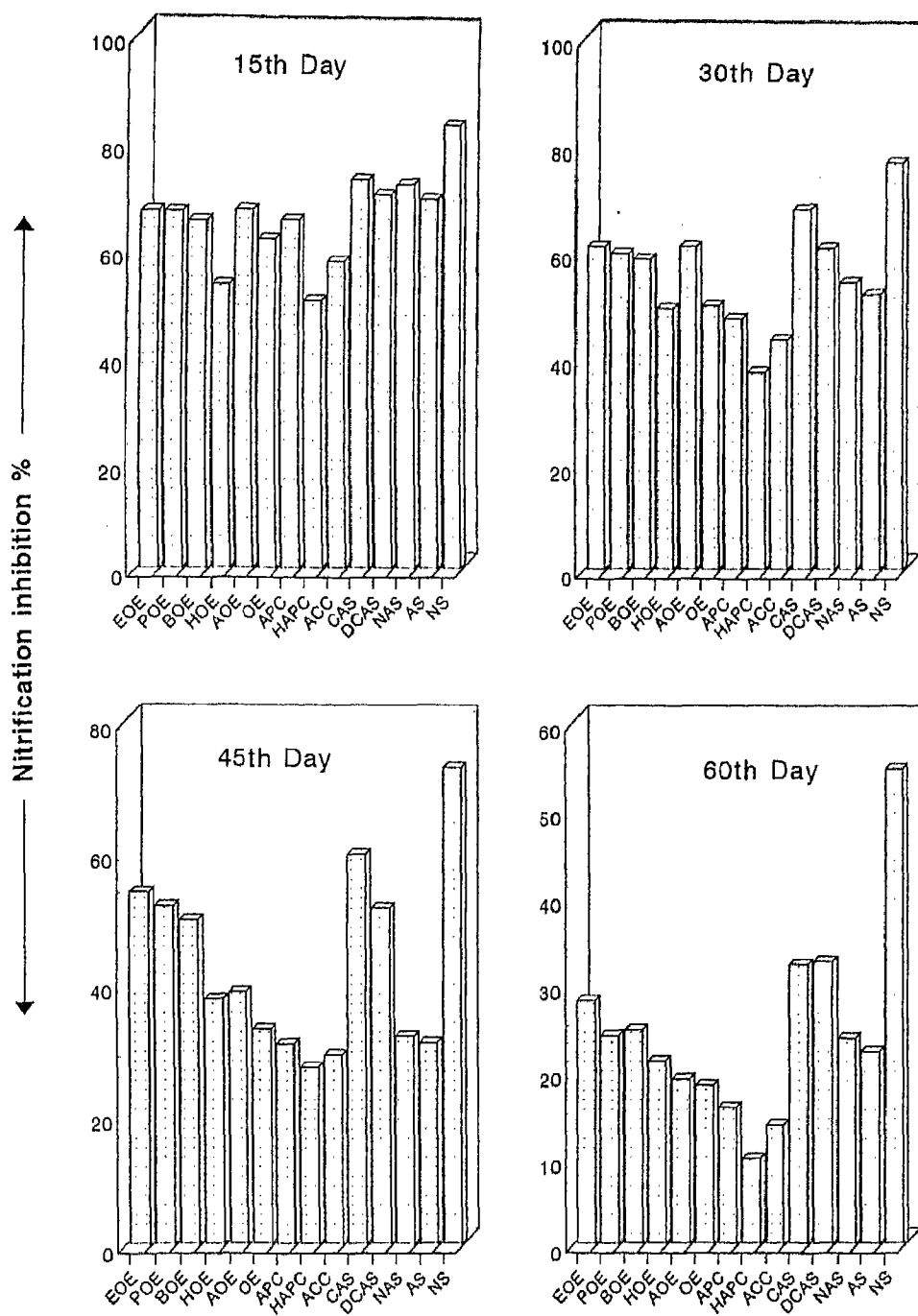
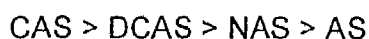


Fig.4.4 Comparative effect of furfural derivatives on nitrification inhibition at 5% of urea-N application

of nitro group in the meta position also failed to improve the activity. The decreased activity of 3,4-dichloro and 3-nitro derivatives indicated that substitution in the meta position may be the reason for reduction in activity. More derivatives are however needed to be synthesized and tested before arriving at such conclusion.

There was no significant difference in activity between NAS and AS at most of the doses and periods though in some cases (such as 20% dose on the 15th day) significantly better activity was recorded with NAS. The nitro group failed to improve the activity of the substituted Schiff base. The NI activity of nonchlorinated Schiff base though initially higher, did not persist for longer period.

Initially all the Schiff bases showed similar activity at a given dose and time but with time the difference in activity between the two chlorinated Schiff bases and the nonchlorinated Schiff bases widened. On the basis of the results obtained the four derivatives can be arranged according to their activity as follows :



4.2.1.4 Comparison of activity of synthetic furfural derivatives : An appraisal of the NI values indicated that at 5% dose, all the four Schiff bases, oxime ethers (except HOE) and one chalcone (APC) have more or less similar activity (Fig. 4.4 and Fig. 4.5) on the 15th day. In general, the Schiff bases performed slightly better than the oxime ethers. HAPC, ACC and HOE performed poorly. On the 15th day, the Schiff bases were undoubtedly the best, followed closely by the oxime ethers. The chalcones, except APC were the poor third.

With the passage of time at 5% dose difference in activity between chalcones to Schiff bases and oxime ethers widened. The %NI data on the 45th day clearly indicated that at this dose, 3 oxime ethers [EOE (53.58%), POE (51.45%) and BOE (49.41%)] and two Schiff bases [CAS

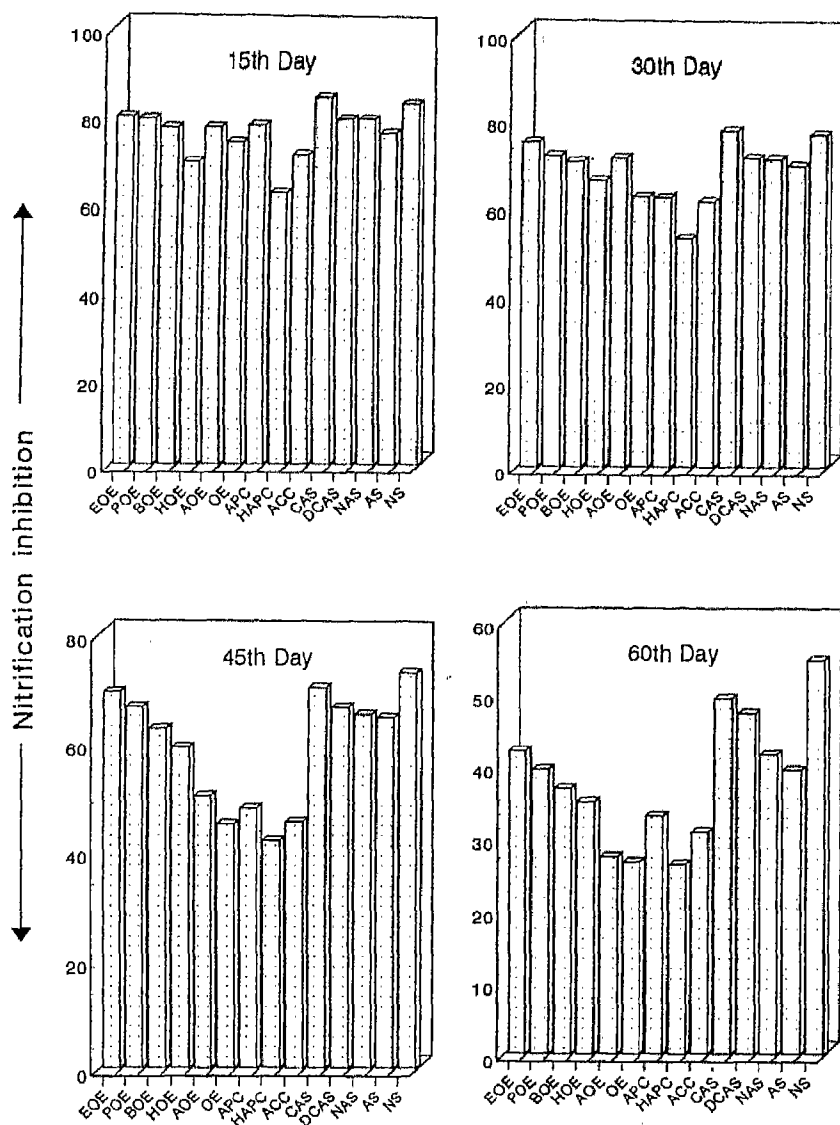


Fig.4.5 Comparative effect of furfural derivatives on nitrification inhibition at 20% of urea-N application

(59.37%) and DCAS (51.21%)] were quite comparable and performed much better than the other compounds. It can be concluded that CAS and DCAS are the best among the synthesized compounds followed by EOE, POE and BOE.

When NI activity at 20% dose was compared (Fig. 4.5) it was observed that except HOE, ACC and HAPC, the others performed distinctly better and were comparable on the 15th day. On the 45th day, difference in %NI values of the compounds revealed that the chalcones did not have long-lasting effect whereas the Schiff bases, as a group showed quiet persistent activity (Fig. 4.5). The difference between the Schiff bases and EOE started widening only from 60th day but upto 45th day they were comparable.

4.2.2 Evaluation of nitrification inhibitory effect of karanj extractives based coated products

Application of nitrification inhibitors offers the greatest problem in practical situations. The large volume of available literature clearly brings out that numerous compounds capable of retarding nitrification in soil under controlled conditions in laboratory are available. However, these often show poor performance in the field. Number of reasons have been put forward for such inconsistency and poor application techniques is one of them. The nitrification inhibitors need to be intimately mixed with the fertilizer(s) so that each fertilizer particle is thoroughly covered before its application to the soil. It is assumed that the coating of fertilizers with the nitrification inhibitors may be more effective than their physical mixing.

Coating of karanjin on urea surface requires a binder. Beside coal tar, a well known binder three more substances, alcohol extract of karanj seed kernels (KA), alcohol extract of neem seed kernels (NA) and karanj oil (KO) have been tried. As alcohol extracts are viscous and sticky in nature they can be effectively used as binder. In addition to the binding effect, these materials also possess nitrification inhibition activity.

4.2.2.1 Effect of coating agents on nitrification inhibition : The nitrification inhibitory effect (NI%) and accumulation of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ are depicted in Table 4.2 and 4.9, respectively. The data have been graphically presented in Fig.4.6. Statistical analysis showed a significant difference between the treatment means on each sampling day and day $\times$ treatment interaction was also significant.

Among the binders tested, NA turned out to be the most effective in influencing NI activity. When applied at 5% dose it showed the highest activity on the 15th (34.36%), 30th (15.16%) and 45th (7.47%) day. On the 60th day, none of the treatments showed any activity. All the three doses differed significantly in activity in a similar way to the other coating agents. In all the treatments, the activity declined with time. When various treatments were compared among themselves, it was observed that NA at 5% dose was the best at all the three sampling days followed by CKA5 and KA5. CKA5 gave 28.10, 10.07 and 3.64% NI at the 15th, 30th and 45th day, respectively whereas the corresponding figures for KA5 were 23.45, 6.66 and 3.00%, respectively. In this experiment KA has performed poorly than NA though in literature superior activity of KA as compared to NA has been reported (Sahrawat 1982a). The activities of alcohol extract depend upon their processing. In this experiment poor activity of KA as compared to NA may be due to the exhaustive deoiling step in which most of the physiologically active compounds along with karanjin was removed. A higher test concentration may also have been needed. The NI activity of karanj seed kernel extract depends upon the karanjin content. KA with higher amount of karanjin is expected to perform better. The difference in preparation of alcohol extracts may be responsible for variation in results obtained by different researchers.

Karanj oil at 5% dose failed to show any significant NI activity (12.30% on the 15th day). This is in line with the finding of Patel (1991), who

Table 4.2 : Effect of coating aids/binders on nitrification inhibition

Coating aid*	Dose (% Urea N)	Nitrification inhibition (%)**		
		15 th Day	30 th Day	45 th Day
KA	1.0	6.02 ^h	2.46 ^{hi}	0.17 ^j
	2.5	11.92 ^f	4.20 ^e	1.31 ^{gh}
	5.0	23.45 ^d	6.66 ^d	3.00 ^{cd}
NA	1.0	9.24 ^g	4.45 ^e	-
	2.5	16.33 ^e	5.89 ^d	2.56 ^{dek}
	5.0	34.36 ^b	15.16 ^b	7.47 ^b
CKA	1.0	12.00 ^f	3.78 ^{ef}	1.28 ^{gh}
	2.5	12.45 ^f	6.54 ^d	2.08 ^{ef}
	5.0	28.10 ^c	10.07 ^c	3.64 ^c
C	2.0	11.83 ^f	2.81 ^{gh}	0.53 ⁱ
KO	1.0	4.53 ^l	0.54 ^j	0.16 ^j
	2.5	6.71 ^h	2.00 ⁱ	0.98 ^h
	5.0	12.30 ^f	3.33 ^g	1.71 ^{fg}
NS	5.0	79.59 ^a	71.83 ^a	67.03 ^a

* KA (ethanol extract of karanj seed kernels), NA (ethanol extract of neem seed kernels), C (coal tar), CKA (coal tar 2% + KA), KO (karanj oil), NS (nitrapyrin).

** Treatment means are average of three replicates. Treatment means are compared by Duncan's Multiple Range Test (DMRT) at 5% level after arc sine conversion of percentage data shown in the Table. Those followed by a common letter in a column show nonsignificant difference.

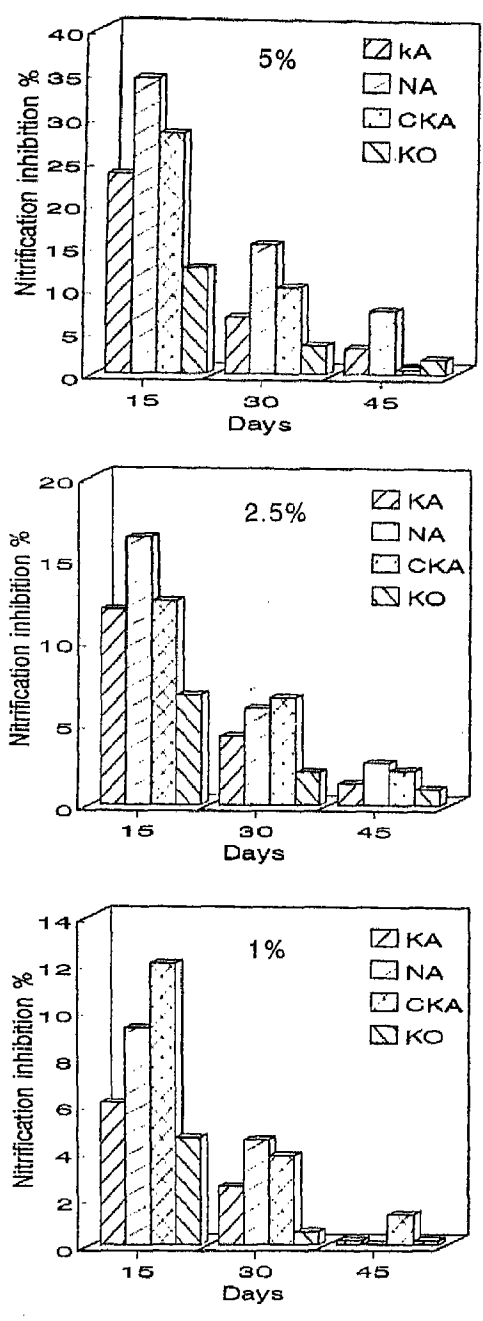


Fig.4.6 Effect of various coating aids/binders on nitrification inhibition.

reported that karanj oil possessed considerable nitrification inhibitory activity when tested on pure culture of nitrifiers but showed less activity in incubated soils. Coal tar also exhibited mild effect on nitrification. On the 15th day it inhibited 11.83% nitrification at 2% dose.

NA and KA coated urea prills showed good physical forms and were free flowing, At 5% dose, little stickiness was observed which is desirable for further coating of inhibitors. This 5% dose was therefore selected for further coating of karanjin on urea prills. In case of karanj oil, 2.5% dose was selected because at 5%, the oil imparted considerable stickiness to urea.

Based on this experiment, the 5% dose of the alcohol extracts of karanj and neem seed kernels and 2% coal tar were finalised for further coating of karanjin on urea.

4.2.2.2 Effect of karanjin coated urea on nitrification inhibition : Six coating techniques of karanjin were evaluated. The results in terms of NI% have been reported in Table. 4.3a and accumulation of ammonium, nitrite and nitrate-N presented in Table 4.10. The data have been graphically presented in Fig. 4.7 to Fig. 4.10 and in Fig.4.16. The analysis of variance showed that for each sampling day, the differences between treatment means were significant. F value for the 15th, 30th, 45th and 60th day were 667(616), 512(445), 662(580) and 227(193) respectively. The treatment $\times$ day interaction has been found to be highly significant [F value 25.29(18.07)].

Without binder

When karanjin was coated on urea prills without any binder, it showed 63.10% NI at 10% dose as compared to 13.09% at 1% dose. Even at 10% dose, the NI activity was lower than 5% nitrapyrin (82.59%). The doses showed significant differences in activity amongst themselves on all the four sampling days. Activity of karanjin fell rapidly with passage of time, showing

Table 4.3a : Effect of karanjin coated urea products (along with coating aids) on nitrification inhibition

Treatment		Nitrification inhibition (%)**			
Coating aid*	KNx %***	15 th Day	30 th Day	45 th Day	60 th Day
No Coating aid used	1.0	13.09 ^f	10.26 ^a	1.75 ^p	-
	2.5	17.73 ^a	12.81 ^{no}	4.25 ^o	0.79 ^{mn}
	5.0	32.20 ^m	20.15 ^j	11.15 ^l	3.15 ^k
	7.5	52.42 ^j	35.58 ^g	20.87 ^{hi}	5.76 ^{hi}
	10.0	63.10 ^f	45.20 ^o	30.22 ^f	12.06 ^f
C	1.0	16.60 ^a	10.82 ^{pq}	1.00 ^a	0.38 ⁿ
	2.5	30.04 ^{mno}	17.83 ^{kl}	5.04 ^o	1.24 ^{lm}
	5.0	51.08 ^j	31.01 ^h	16.37 ^j	3.95 ^{jk}
	7.5	60.14 ^g	44.30 ^o	25.68 ^g	9.04 ^g
	10.0	67.35 ^e	51.58 ^c	37.22 ^d	14.82 ^{de}
KA5KNx	1.0	27.96 ^o	12.36 ^{nop}	2.44 ^p	0.82 ^{mn}
	2.5	35.32 ⁱ	16.98 ^{lm}	6.25 ⁿ	1.34 ^{lm}
	5.0	58.26 ^{gh}	31.63 ^h	19.41 ⁱ	6.58 ^h
	7.5	72.04 ^d	47.97 ^d	30.08 ^f	14.23 ^{def}
	10.0	76.03 ^b	56.02 ^b	41.13 ^c	18.64 ^{bc}
NA5KNx	1.0	38.95 ^k	15.75 ^m	8.63 ^m	4.62 ^{ij}
	2.5	40.83 ^k	21.86 ^j	10.22 ^j	6.00 ^{hi}
	5.0	54.38 ⁱ	32.85 ^h	21.92 ^h	9.15 ^g
	7.5	71.06 ^d	51.39 ^c	34.30 ^e	15.42 ^d
	10.0	74.65 ^{bc}	57.11 ^b	44.01 ^b	19.08 ^b
KA5CKNx	1.0	28.57 ^{no}	13.85 ⁿ	8.77 ^m	1.71 ^l
	2.5	40.12 ^k	19.88 ^{ijk}	7.68 ^m	3.00 ^k
	5.0	60.14 ^g	33.67 ^{gh}	20.55 ^{hi}	7.01 ^h
	7.5	73.11 ^{cd}	50.64 ^c	31.76 ^f	16.15 ^{cd}
	10.0	76.91 ^b	56.89 ^b	41.43 ^c	20.17 ^b
KO2.5KNx	1.0	18.64 ^q	11.46 ^{opq}	4.37 ^o	0.81 ^{mn}
	2.5	22.32 ^p	18.05 ^{kl}	4.26 ^o	1.45 ^{lm}
	5.0	30.97 ^{mn}	21.72 ⁱ	13.85 ^k	2.91 ^k
	7.5	56.95 ^h	37.71 ^f	21.33 ^{hi}	9.37 ^g
	10.0	63.74 ^f	44.94 ^e	32.20 ^{ef}	12.45 ^{ef}
NS5		82.59 ^a	76.28 ^a	67.17 ^a	54.50 ^a

* C (coal tar 2%), KA5KNx (ethanol extract of karanj seed kernels 5% + Karanj), NA5KNx (ethanol extract of neem seed kernels 5% + karanjin), KA5CKN (coal tar 2% + KA5% + karanjin), KO2.5KNx (karanj oil 2.5% + karanjin), NS (nitrapyrin).

** Treatment means are average of three replicates. Treatment means are compared by Duncan's Multiple Range Test (DMRT) at 5% level after arc sine conversion of percentage data shown in the Table. Those followed by a common letter show insignificant difference.

*** Doses are mentioned in terms of per cent urea-N basis.

almost a straight line (Fig 4.7). On the 15th day, at 10% dose the NI was 63.10% and on the 45th day, 30.22%, losing almost 50% of its inhibitory effect. These results show wide variation with the results obtained by Sahrawat (1973) who applied karanjin in acetone solution form. Sahrawat obtained 58% and 47% NI with 5% karanjin on the 15th and 45th day whereas at 10% karanjin 60 and 58% inhibition were observed on the 15th and 45th day. In the present study, the lower dose of karanjin showed a poor performance and even at the higher dose, the activity did not persist for longer period.

Coating vs solution application

To solve this anomaly, an experiment was designed to compare the activity between solution and coated application of karanjin at 5 and 7.5% levels. No binder was employed for coating. The results obtained are presented in Table. 4.3b. The results showed no significant difference between these two application techniques. Hence the difference could be attributed to the different physicochemical and microbiological properties of soil.

With binder

When coal tar 2% was used as a binder to coat karanjin (Table 4.3a), NI was considerably increased on the 15th day, in all the doses as compared to the treatments where karanjin was coated without any binder. This increase was particularly marked at 2.5 and 5% doses (17.73 to 30.04% and 32.20 to 51.08%, respectively) but less significant at higher doses viz. at 10% karanjin, activity increased from 63.10% to 67.35%. On the 45th day at lower doses (1 and 2.5%) there was no sizeable improvement in activity but at higher dose (7.5 and 10%) difference was quite substantial. At 10% dose, on all the days, significant improvement was observed between urea coated with karanjin and urea coated with coal tar and karanjin. The improvement in activity with coating may be because of a close proximity between urea and

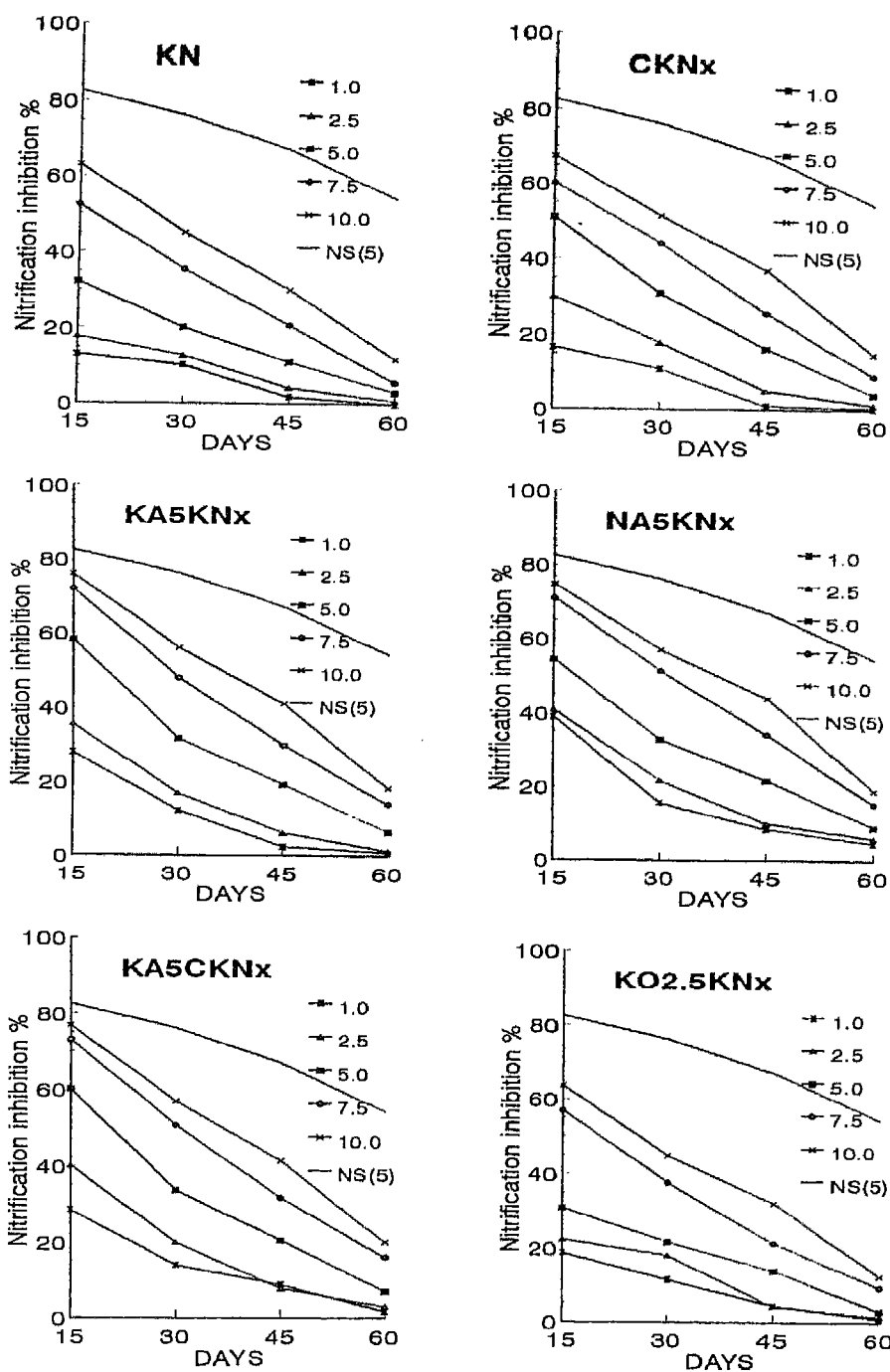


Fig.4.7 Effect of karanjin coated urea products (alongwith coating aids) and nitrpyrin on nitrification inhibition (doses are percentage of applied urea-N)

Table 4.3b : Effect of coating vs solution application of karanjin on nitrification inhibition

Dose (% urea-N)	Application method	Nitrification inhibition (%)*			
		15 th day	30 th day	45 th day	60 th day
5.0	Coating	32.24 ^a	20.02 ^a	11.06 ^a	3.30 ^a
	Solution	33.65 ^a	20.44 ^a	11.68 ^a	3.61 ^a
7.5	Coating	51.56 ^b	36.14 ^b	20.42 ^b	5.11 ^b
	Solution	53.00 ^b	36.79 ^b	21.33 ^b	5.68 ^b

* Treatment means are average of three replicates. Treatment means are compared by Duncan's Multiple Range Test (DMRT) at 5% level after arc sine conversion of percentage data shown in the Table. Those followed by a common letter show insignificant difference.

karanjin molecule. Due to coating, karanjin can move along with urea molecule and both remain adjacent to each other. Thus in the microsite, inhibitory activity is higher as compared to the bulk of the soil. In addition to this, nitrification inhibitory property of coal tar also provides an additional effect.

Karanj oil as a binder, failed to improve the activity over karanjin alone. For 1, 5 and 10% of doses of karanjin on the 15th day the NI for treatment KO2.5KN were 18.64, 30.97 and 63.74% whereas the corresponding data for CKN were 16.60, 51.08 and 67.35% respectively, and for KN (alone) were 13.09, 32.20 and 63.10%. Thus, karanj oil as a binder failed to improve the activity of karanjin. Moreover, the physical form of the product was also not quite satisfactory. It seems that the poor binding ability of oil was responsible for poor activity as compared to the coal tar coated products. During mixing of KO coated products, karanjin might have got separated from the surface of the prills, thus failing to reach the microsite where urea molecule was present. So it can be concluded that the karanj oil is not suitable as a binder for coating karanjin.

In treatments employing 5% KA, NA and CKA as binders, a considerable improvement in activity was observed. At 10% dose of karanjin, on 15th and 30th days, no statistically significant differences in activity were observed in all the three binder treatments. The treatments KA5KN10 and KACKN10 showed initially (15th day) higher NI (76.03% and 76.91%, respectively) as compared to NA coated urea at same dose and time (74.65%). With the passage of time, NA coated urea products showed significantly higher value (44.01% at 10% dose of karanjin on 45th day) as compared to KA5KN10 and KA5CKN10 (41.13% and 41.43%, respectively). This difference in activity pattern between the two alcohol extracts is supported by the earlier report that NA is inferior to KA initially but it has more long-lasting effect (Sahrawat, 1982a). So mixture of these two alcohol extracts along with karanjin may perform better than their individual application.

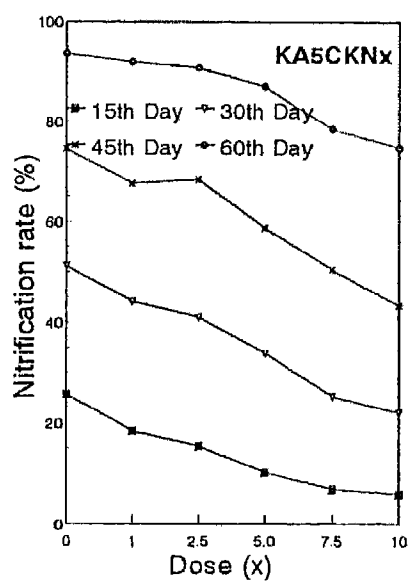
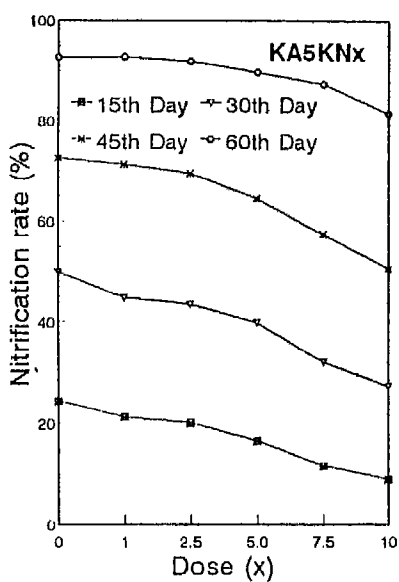
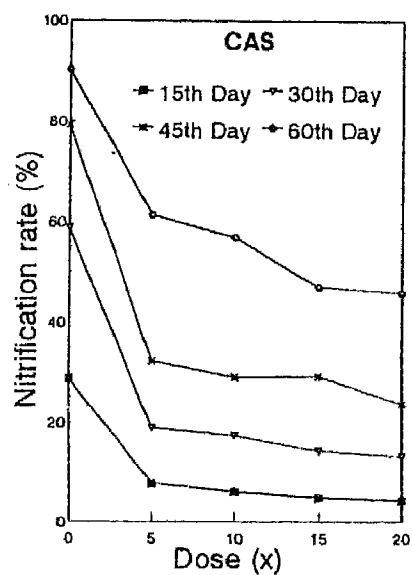
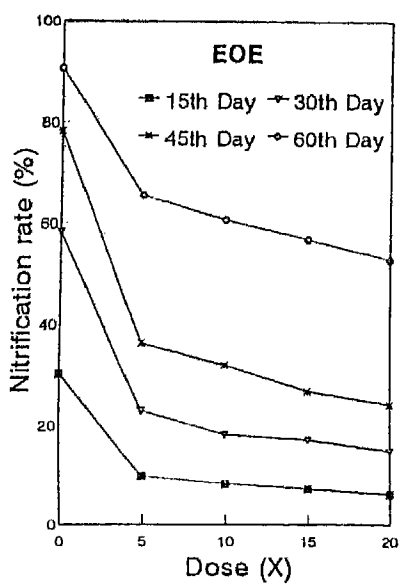


Fig.4.8 Effect of some treatments (EOE, CAS, KA5KNx and KA5CKNx) on nitrification rate

At lower doses of karanjin, NA coated urea products performed better than KA coated products. On the 15th day, 1.0 and 2.5% of karanjin gave 38.95 and 40.83% NI for NA coated products whereas the corresponding values for KA were 27.96 and 35.32%, respectively. Similarly, for CKA coated products the corresponding values were 28.57 and 40.12%, respectively. With increase in karanjin content, KA based products matched NA coated urea products. On the 15th and 30th days, KA5KN10 exhibited 76.03 and 56.02% NI, respectively whereas NA5KN10 showed 74.65% and 57.11% NI, respectively. When both coal tar and KA were used as a binder, trivial increase in activity was observed as compared to only KA treatment for most of the days and doses but activity was not significantly better.

In all the treatments, fall in activity with time was quite swift. The binders increased the persistence of NI activity but not substantially. Three best treatments NA5KN10, KA5KN10 and KA5CKN10 showed more than 40% NI on the 45th day. Though this figure was comparatively low as compared to nitrapyrin (67.17%) but nitrification inhibition by more than 40% as compared to control on the 45th day is by no means a small achievement. For higher doses of karanjin application (7.5 and 10%), the activity fell rapidly after 45th day (Fig 4.7). Thus it can be safely concluded that coated karanjin products (KA5KN, KA5CKN and NA5KN) remained active upto 45 days after application.

Increase in activity with an increase in dose of karanjin has been found to be highly significant in all the binder/binder combinations. For KA5CKNx treatments on the 15th day, the activity increased from 28.57% at 1% karanjin to 76.91% at 10% karanjin i.e. activity increased by nearly three times. Whereas on the 45th day the corresponding figures were 8.77 and 41.43% i.e. around 5 times. On the 15th day KA5KN1 showed 27.96% NI and KA5KN10 76.03% NI. So the increase in activity was by 2.7 times whereas on 45th day the corresponding values were 2.44% and 41.13% i.e. 16 times increase in activity.

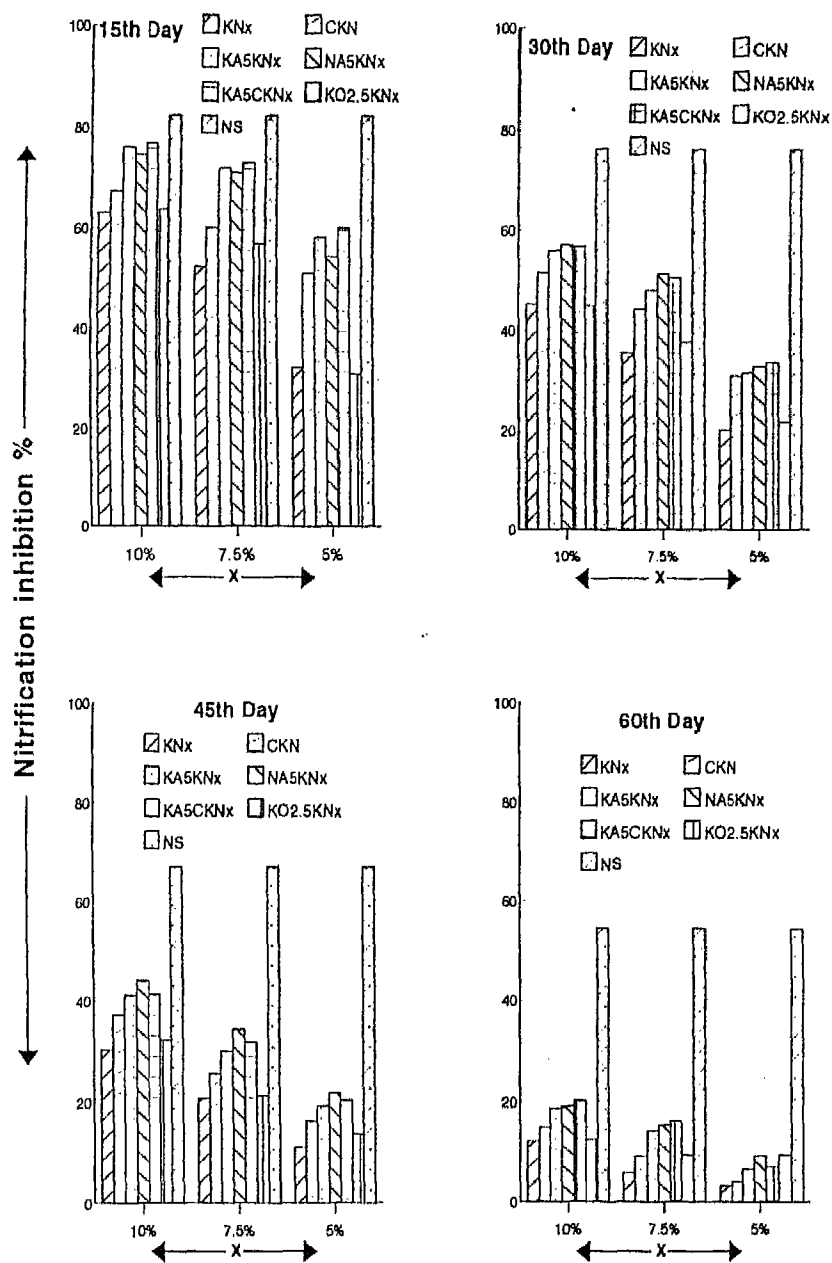


Fig.4.9 Comparative evaluation of various karanjin based treatments on nitrification

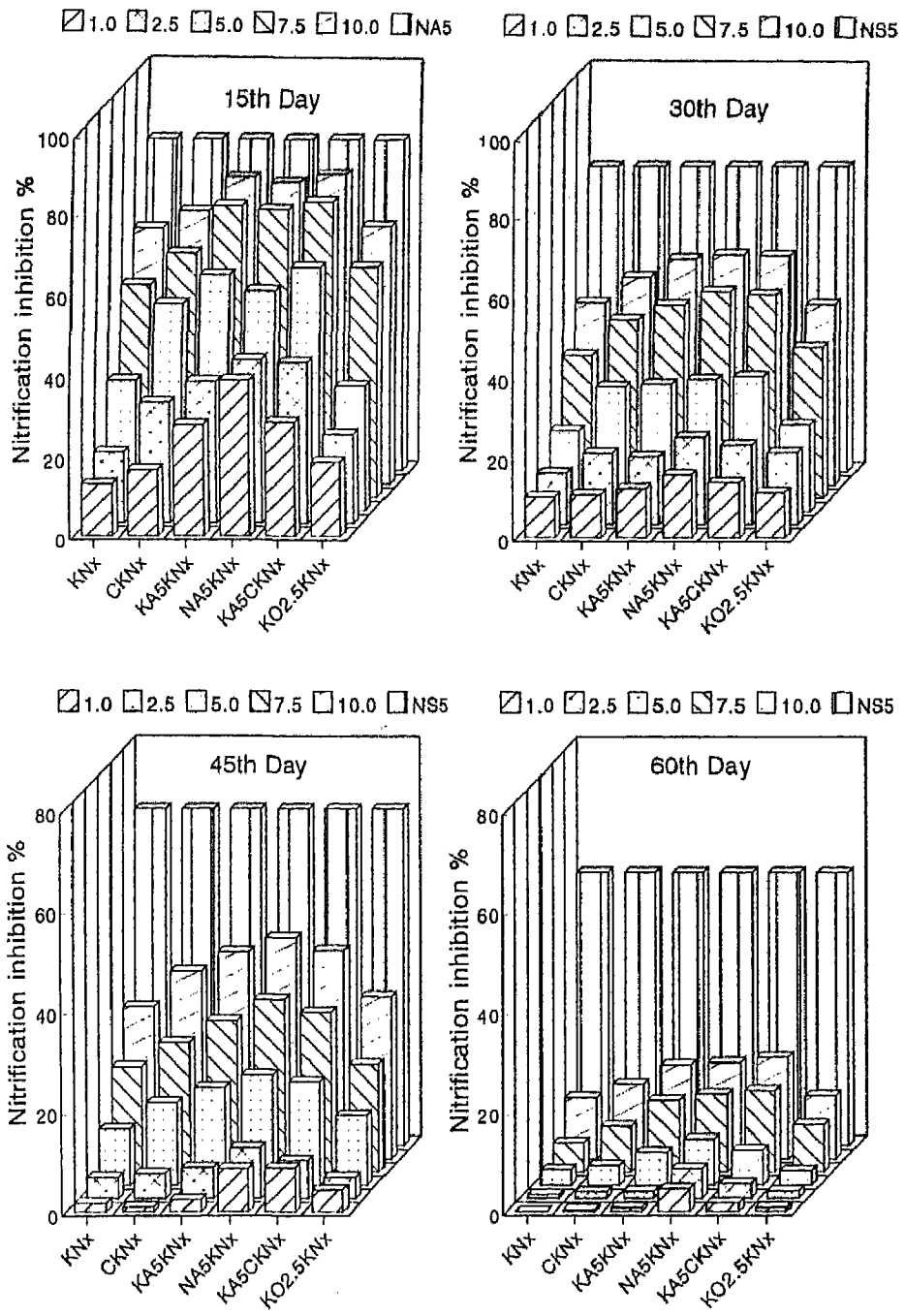


Fig.4.10 Daywise comparison of various karanjin based treatments on nitrification

Similar trend was shown by NA. Thus it can be concluded that the higher dose of karanjin increased the longevity of the activity.

The improved performance of alcohol extracts based coated urea products is due to their better binding ability and their own contribution in inhibiting the activity of *Nitrosomonas* species. The sticky consistency of the alcohol extracts helps to bind karanjin with greater efficiency. Among the coated treatments NA5KN10, KA5KN10 and KA5CKN10 was found to be the best followed by KA5CKN7.5, KA5KN7.5 and NA5KN7.5. These coated products apparently hold promise in increasing the NUE of urea.

4.2.3 Comparison of coating vs. solution application of furfural derivatives on nitrification inhibition

Among the synthetic compounds EOE and CAS have been found to be the most effective among oxime ethers and Schiff bases. These two compounds were coated on urea with the help of 2% coal tar and evaluated in comparison with the solution application (applied in solution form).

Upto 7.5% of CAS could be coated on urea without any adverse effect on its physical form. At concentrations in excess of 7.5%, CAS started segregating from the prill surface. Therefore, three doses viz. 2.5, 5.0 and 7.5% were taken for the study. EOE itself being a viscous liquid, could be coated upto 10% concentration by using 2% coal tar as a binder, with a limited deterioration in its physical properties. However, excellent products were obtained at 5% dose. Five and ten per cent doses of EOE were used in the study.

The nitrification inhibition data are reported in Table 4.4 and the values for ammonium, nitrate and nitrite are given in Table 4.11. A graphical comparison is made in Fig. 4.11. NI data showed that on the 15th day, the coated products in most of the cases showed better activity as compared to the similar dose of solution application but this difference was statistically insignificant. The NI value at the 5% dose of EOE on the 15th day for the coated and solution application were 67.90 and 65.13%, respectively and for

Table 4.4 : Effect of coating vs solution application of EOE and CAS on nitrification inhibition

Compound*	Dose (% urea-N)	Application method	Nitrification inhibition (%)**			
			15 th Day	30 th Day	45 th Day	60 th Day
EOE	5	Coating	67.90 ^d	61.24 ^c	56.51 ^c	29.16 ^d
		Solution	65.13 ^d	60.86 ^c	56.74 ^c	29.25 ^d
	10	Coating	74.05 ^{bc}	68.84 ^b	59.97 ^{bc}	36.16 ^b
		Solution	72.43 ^c	68.51 ^b	59.56 ^{bc}	34.97 ^{bc}
CAS	2.5	Coating	55.63 ^e	41.40 ^d	30.97 ^d	21.64 ^e
		Solution	53.22 ^e	39.03 ^d	31.34 ^d	21.85 ^e
	5	Coating	73.60 ^{bc}	65.78 ^b	60.35 ^{bc}	34.47 ^c
		Solution	72.43 ^c	67.78 ^b	59.77 ^{bc}	35.31 ^{bc}
	7.5	Coating	75.61 ^b	67.61 ^b	62.39 ^b	38.45 ^b
		Solution	76.43 ^b	68.98 ^b	62.13 ^b	37.45 ^{bc}
NS	5	Solution	82.13 ^a	75.59 ^a	67.55 ^a	53.37 ^a

* EOE (N-O-ethyl furfural oxime), CAS (furfurylidene-4-chloroaniline).

** Treatment means are average of three replicates. Treatment means are compared by Duncan's Multiple Range Test (DMRT) at 5% level after arc sine conversion of percentage data shown in the Table. Those followed by a common letter show insignificant difference.

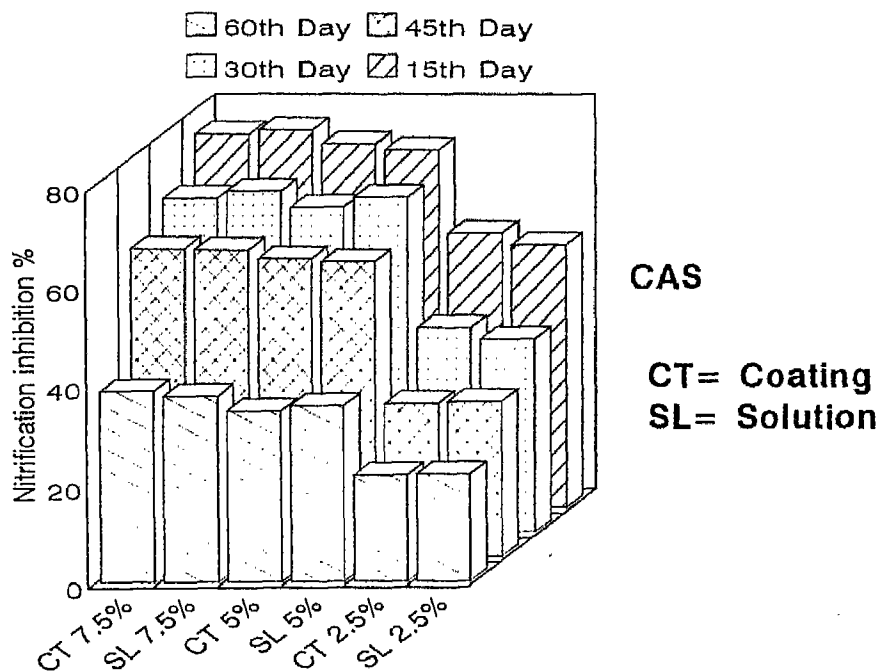
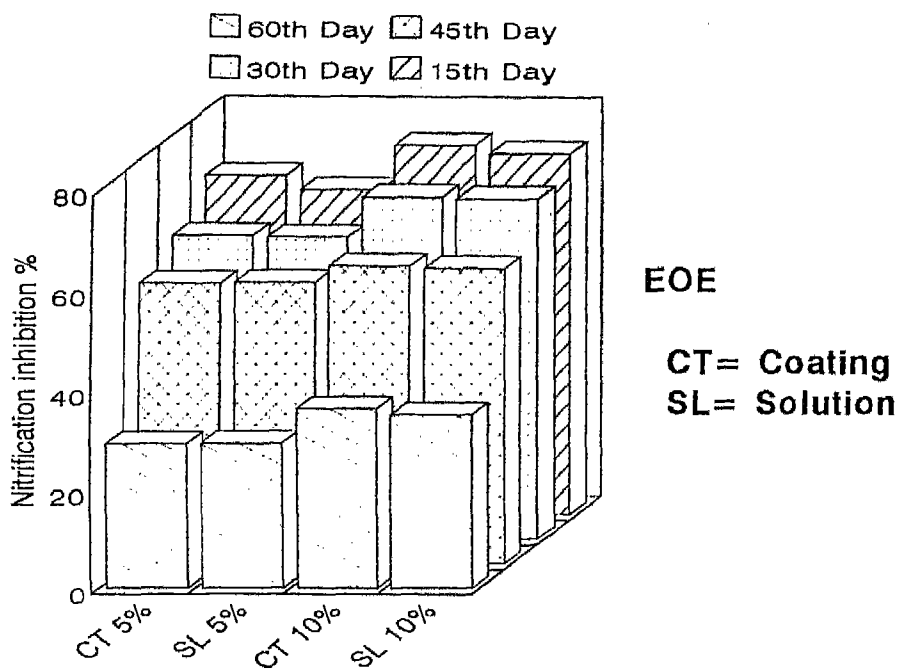


Fig.4.11 Comparative evaluation of coating and solution applications of EOE and CAS on nitrification Inhibition (doses are percentage of applied urea-N)

the 10% dose, these were 74.05 and 72.43%, respectively. The corresponding values for the CAS based applications at 2.5% dose were 55.63 and 53.22% and for the 7.5% dose, 75.61 and 76.43%, respectively. This difference in activity was, however, statistically insignificant. For the other sampling periods also, the difference between coated and solution applications were insignificant.

Thus coating with the synthetic derivatives failed to improve the performance of these products. Their application in the solution form appears to be quite efficient. By application in this form, through mixing of the chemicals was ensured. Further mixing of the components were ensured at the time of urea application also. However, such an application methodology can be practiced only in the laboratory. The, coated products would be more desirable for the field application.

Failure of the synthetic derivatives to register any improvement in activity when coated with coal tar as compared to karanjin, which showed improvement in inhibiting nitrification when applied after coating with coal tar, indicates that the high intensity of activity of the synthetics (high activity even at low doses) as compared to karanjin, is not improved further by the coating aids.

It is significant to note that 7.5% dose of CAS almost matched nitrapyrin (5%) upto 45th day in the nitrification inhibitory activity. After 45 days, the activity of the two differed widely.

4.2.4 Effect of mixtures of karanjin and synthetic furfural derivatives on nitrification inhibition

Among the synthetic compounds, EOE of furfural oxime ethers and CAS of furfural Schiff bases exhibited considerable nitrification inhibition property at 10-20% doses of application. At 5%, their performance was quite inferior as compared to the same dose of nitrapyrin. It would be desirable to improve their activity, at lower doses. Likewise karanjin at 10% dose exhibited significant activity but at doses below 10%, it did not performed well.

Table 4.5 : Effect of mixtures of karanjin and furfural derivatives on nitrification inhibition

Treatment (Coated)*	Nitrification inhibition (%)**			
	15 th Day	30 th Day	45 th Day	60 th Day
EOE5KN2.5	69.03 ^c	64.25 ^c	57.28 ^b	27.30 ^c
EOE5KN5.0	72.10 ^{bc}	68.62 ^b	60.43 ^b	29.15 ^c
EOE5KN7.5	74.33 ^b	68.66 ^b	64.77 ^a	34.38 ^b
CAS5KN2.5	75.25 ^b	66.17 ^c	60.35 ^b	36.37 ^b
CAS2.5KN5	64.85 ^d	57.67 ^d	48.73 ^c	19.01 ^d
NS5	82.13 ^a	75.59 ^a	67.55 ^a	53.37 ^a

* EOE (N-O-ethyl furfural oxime), CAS (furfurylidene-4-chloroaniline), KN (karanjin), NS (nitrapyrin).

** Treatment means are average of three replicates. Treatment means are compared by Duncan's Multiple Range Test (DMRT) at 5% level after arc sine conversion of percentage data shown in the Table. Those followed by a common letter show insignificant difference.

Table 4.6 : Effect of mixtures of alcohol extract of karanj seed kernels and furfural derivatives on nitrification inhibition

Treatment (Coated)*	Nitrification inhibition (%)**			
	15 th Day	30 th Day	45 th Day	60 th Day
KA5CAS2.5	63.01 ^d	59.57 ^d	33.79 ^d	24.56 ^c
KA5CAS5	74.41 ^b	69.62 ^b	60.27 ^b	32.23 ^b
KA5EOE2.5	61.33 ^a	57.80 ^e	31.88 ^e	24.46 ^c
KA5EOE5	69.77 ^c	67.31 ^c	55.24 ^c	24.07 ^c
NS5	80.30 ^a	75.34 ^a	68.47 ^a	47.56 ^a

* KA (ethanol extracts of karanj seed kernels), CAS (furfurylidene-4-chloroaniline) EOE (N-O-ethyl furfural oxime), NS (nitrapyrin).

** Treatment means are average of three replicates. Treatment means are compared by Duncan's Multiple Range Test (DMRT) at 5% level after arc sine conversion of percentage data shown in the Table. Those followed by a common letter show insignificant difference.

Therefore, it was considered of interest to explore a mixture of these compounds (synthetic derivatives and karanjin) at lower doses to see the change, if any, in the activity.

Urea prills coated with EOE5% + KN2.5% provided a resultant product with excellent physical properties. Karanjin could be coated upto 7.5% with EOE 5% without compromising with the free flowing character of the developed product. The results obtained with these developed products are reported in Table 4.5 and graphically presented in Fig. 4.12. A comparative evaluation of the nitrification inhibition percentage values of the mixtures with their components is presented in Table 4.7. and Fig 4.13.

In the treatment EOE5KN2.5 only 69.03% inhibition of nitrification was observed on the 15th day. On this day, urea prills coated with 2.5% karanjin and 2% coal tar gave 30.04% NI and urea coated with 5% EOE and 2% coal tar exhibited 67.90% inhibition. Thus additional 2.5% karanjin along with 5% EOE failed to bring about any sizable improvement in activity as compared to the same dose of EOE alone. In the treatment EOE5KN7.5, the NI value on the 15th day was 74.33 per cent. Thus higher dose of karanjin increased the activity but the effect was not pronounced. On the 15th, 45th and 60th days the treatment EOE5KN2.5 did not differ significantly from EOE5KN5. On the 15th and 30th days, the EOE5KN5 did not differ from EOE5KN7.5 but on the 45th and 60th days the difference in activity were significant. Thus higher doses of karanjin increased the persistence of the product. It seems that activity of the mixture was primarily because of EOE, though karanjin at higher doses increased the effect. There was no synergistic or antagonistic effect of the components of the mixture. Both the compounds probably have similar mode of action. At 45th day the NI% of EOE5KN7.5 decreased by only 10% (74.33% to 64.77%) which was less than that with nitrapyrin (82.13% to 67.55%). After the 45th day, the activity of all the treatments fell rapidly (Fig. 4.12). The effect

decreased after the 45th day, presumably due to the reestablishment of the population of nitrifying organisms.

CAS and karanjin being solid can be coated cumulatively upto 7.5%. Above this level, the materials started segregating from the surface of the urea prill employing coal tar 2% as a binder. Hence, two mixtures CAS5KN2.5 and CAS2.5KN5 were prepared and tested.

The NI% value for the treatments CAS5KN2.5 and CAS5% coated along with 2% coal tar were found to be 75.25 and 73.60% on the 15th day. On the other hand karanjin (2.5%) coated along with 2% coal tar gave 30.04% NI activity on the same day. The corresponding values on the 45th day were 60.35%, 60.35% and 5.04%. Thus at higher dose of CAS (5%), karanjin failed to bring about any improvement. The treatment CAS2.5KN5 gave 64.85% inhibition on the 15th day whereas the corresponding value of CKN5 and CCAS2.5 were 51.08 and 55.63%. So in this case higher dose of karanjin (5%) significantly increased the activity but not substantially. Same increase was observed on the 45th day, when the treatment CAS2.5KN5 showed 48.73% NI whereas CCAS2.5 showed 30.97% and CKN5 16.37% NI. So in this case slight synergism in activity was observed. Similarly on the 30th day increase in activity of the mixture as compared to its constituents was substantial. At lower dose of CAS (2.5%), 5% karanjin showed a long-lasting effect. It brought about significant improvement in activity but at higher doses of CAS in the mixture, the effect was more a function of CAS and karanjin failed to improve the effect. Thus it can be concluded that at lower dose of the synthetic derivatives, karanjin significantly improved the activity of the mixtures but at higher doses of synthetic derivatives, karanjin failed to do so.

On all the days and amongst all the treatments, nitrapyrin 5% performed significantly better though on the 45th day the treatment EOE5KN7.5 did not differ significantly with it.

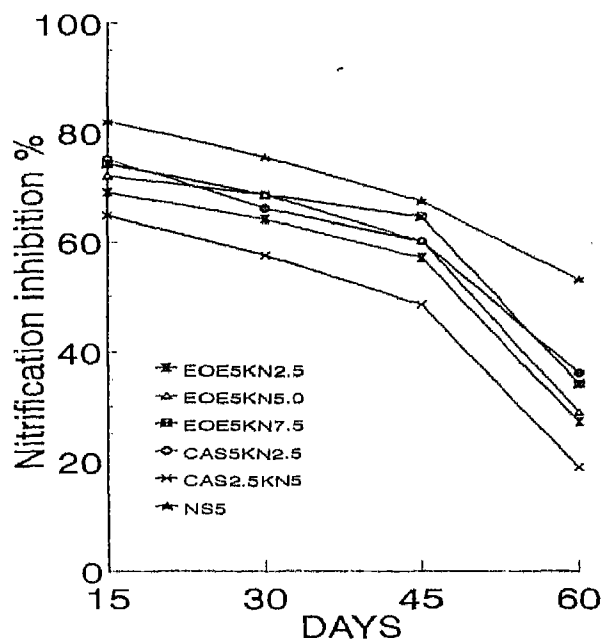
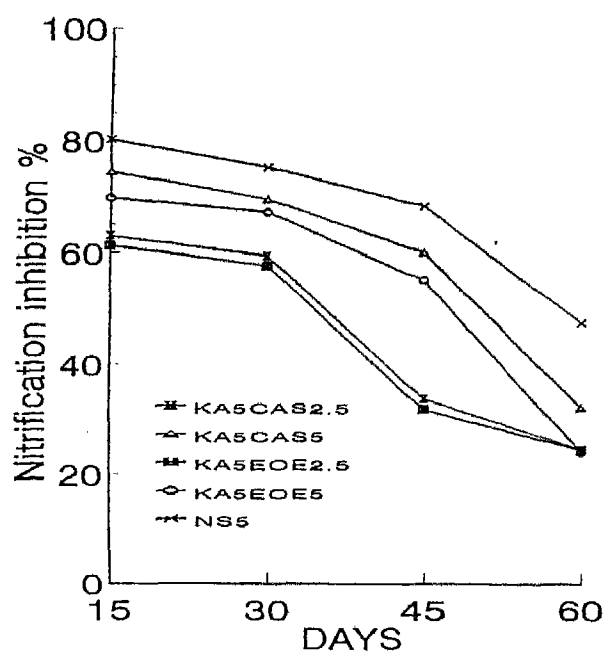


Fig.4.12 Effect of mixtures of furfural derivatives and karanj extractives on nitrification inhibition

4.2.5 Effect of mixtures of synthetic furfural derivatives and alcohol extract of karanj seed kernels on nitrification inhibition

Mixtures of alcohol extract of karanj seed kernels and the furfural derivatives have been tested to evaluate their joint effect on nitrification. Four treatments namely, KA5CAS2.5, KA5CAS5, KA5EOE2.5 and KA5EOE5 were tested. The results of this experiment are reported in Table 4.6 and graphically depicted in Fig. 4.12. The NI values of the mixtures have been compared with their constituents in Table 4.7.

In all the treatments, the physical properties of the products were satisfactory. In treatment KA5EOE5 these physical properties were slightly inferior as compared to other three treatments.

All the treatments showed significantly different activity from each other upto the 45th day. The graphical presentation (Fig. 4.12) of the data showed that when the synthetic furfural derivatives (EOE and CAS) were used at 2.5% dose, the activity decreased rapidly after 15 days and at 5% dose, after the 45th day. Among the four treatments KA5CAS5 was the best, followed by KA5EOE5, KA5CAS2.5 and KA5EOE2.5.

On the 15th day, KA5EOE2.5 showed 61.33% NI as compared to CEOE2.5 which showed 53.84% NI (Table 4.7). Corresponding data for 5% dose are 69.77% (KA5EOE5) and 67.90% (CEOE5). On the 30th day KA5EOE2.5 showed 57.80% inhibition as compared to CEOE2.5 which showed 47.25%. Corresponding data for the treatments KA5EOE5 and CEOE5 were 67.31 and 61.24%. Thus on the 15th and 30th day, coating with mixture increased the activity as compared to coating with coal tar and this improvement was more prominent at lower dose of EOE. At the higher dose of application of EOE, the NI activity became a function of EOE only. On the 45th day KA failed to impart significant improvement in the activity.

Table 4.7 : Comparative performance of mixtures and their constituents on nitrification inhibition

Treatment (Coated)*	Nitrification inhibition (%)**			
	15 th Day	30 th Day	45 th Day	60 th Day
CEOE2.5	53.84	47.25	31.49	22.09
CEOE5	67.90	61.24	56.51	29.16
CEOE10	74.05	68.84	59.97	36.16
CCAS2.5	55.63	41.40	30.97	21.64
CCAS5	73.60	65.78	60.35	34.47
CCAS7.5	75.61	67.61	62.39	38.45
KN2.5	17.73	12.81	4.25	0.79
KN5	32.20	20.15	11.15	3.15
KN7.5	52.42	35.58	20.87	5.76
CKN2.5	30.04	17.83	5.04	1.24
CKN5	51.08	31.01	16.37	3.95
CKN7.5	60.14	44.30	25.68	9.04
EOE5KN2.5	69.03	64.25	57.28	27.30
EOE5KN5	72.10	68.62	60.43	29.15
EOE5KN7.5	74.33	68.66	64.77	34.38
CAS5KN2.5	75.25	66.17	60.35	36.37
CAS2.5KN5	64.85	57.67	48.73	19.01
KA5EOE2.5	61.33	57.80	31.88	24.46
KA5EOE5	69.77	67.31	55.24	24.07
KA5CAS2.5	63.01	59.57	33.79	24.56
KA5CAS5.0	74.41	69.62	60.27	32.23

* CEOE (coal tar 2% + N-O-ethyl furfural oxime ether), CCAS (coal tar 2% + furfurylidene-4-chloroaniline), KN (karanjin), CKN (coal tar 2% + karanjin), KA (ethanol extract of karanj seed kernels), EOE5KN2.5 (C 2% + EOE 5% + KN 2.5%), CAS5KN2.5 (C 2% + CAS 5% + KN 2.5%), KA5EOE2.5 (KA 5% + EOE 2.5%), KA5CAS2.5 (KA 5% + CAS 2.5%).

** Treatment means are average of three replicates.

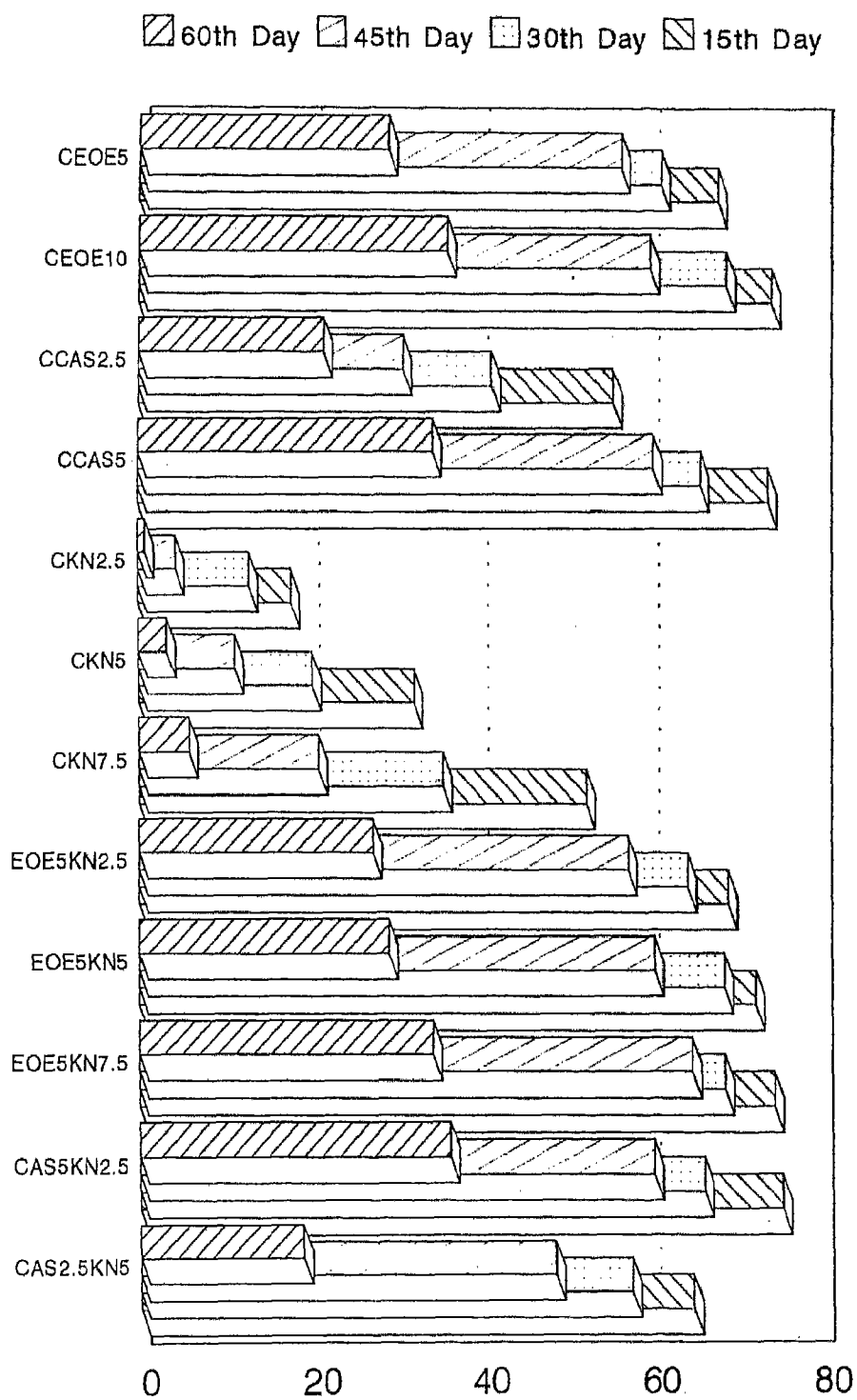


Fig.4.13 Comparative performance of mixtures and their constituents on nitrification inhibition

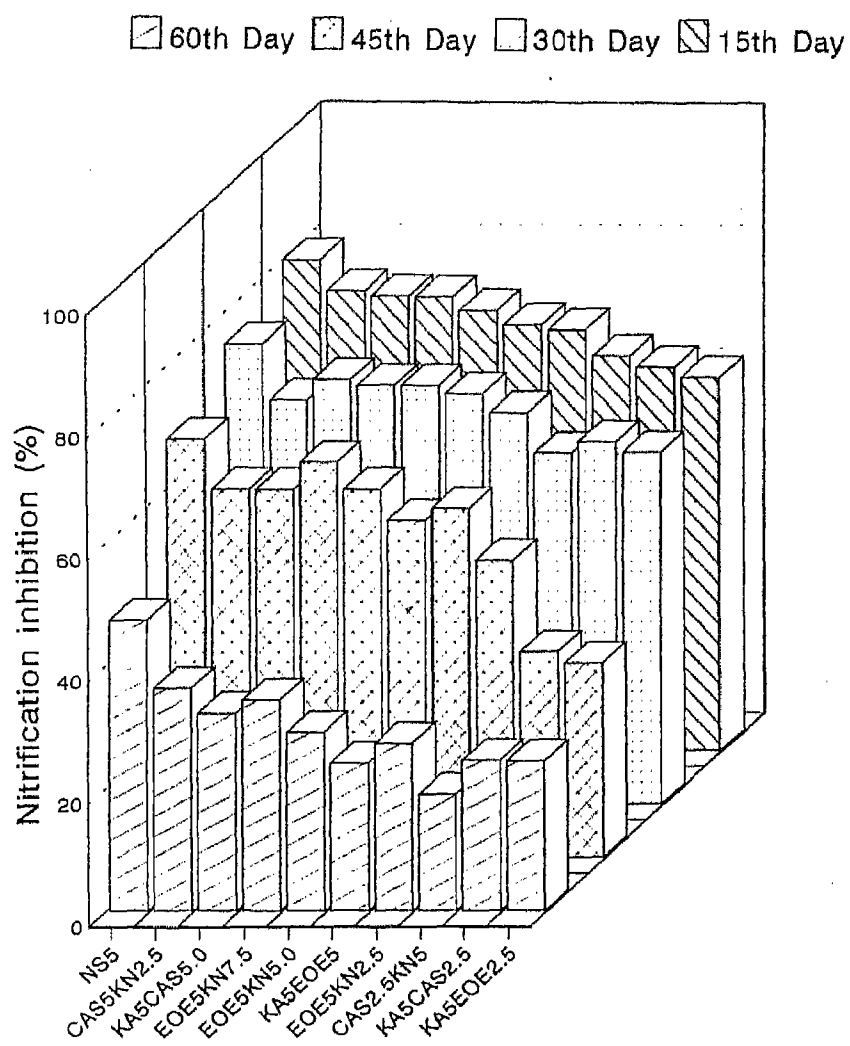


Fig.4.14 Daywise comparison of various mixtures on nitrification inhibition

Treatments based on CAS also revealed a similar trend in activity. Among the four treatments in reference (Table 4.6), KA5CAS5 performed the best, followed by KA5EOE5.

These four treatments along with the five other treatments with mixtures discussed in the preceding section (Table 4.5) have been compared in Fig. 4.14. Comparison between KA5CAS2.5 and CAS2.5KN5 showed almost similar activity except on the 45th day when CAS2.5KN5 performed significantly better (NI 48.73% against 33.79%), highlighting the effect of karanjin in increasing the persistence of activity.

When KA5CAS5 was compared with CAS5KN2.5 almost similar activity was observed. Thus a lower dose of karanjin (2.5%) did not have an added advantage over KA (Table 4.7) but its higher dose (5%) significantly increased the persistence of activity over KA. KA5EOE5 and EOE5KN2.5 showed the same trend in activity. A comparison between KA5EOE5 and EOE5KN5 showed that EOE5KN5 performed significantly better but the difference was not substantial.

4.3 Effect of test chemicals on the accumulation of ammonium, nitrite and nitrate in incubated soil

4.3.1 Accumulation of ammonium : Chemicals, which can temporarily inhibit the activity of *Nitrosomonas*, lead to the accumulation of ammonium in soil which is reflected by high NI% values. All the treatments showing high NI, led to an accumulation of considerable amount of $\text{NH}_4^+\text{-N}$. EOE at 5% dose showed $158.6 \text{ mg kg}^{-1} \text{ NH}_4^+\text{-N}$ on the 15th day and 60.2 mg kg^{-1} on the 60th day. Corresponding values for control were 114 and 15.9 mg kg^{-1} . So even at 5% dose 4 times more $\text{NH}_4^+\text{-N}$ was conserved on the 60th day as compared to control. In case of CAS at 5% dose, $66.4 \text{ mg kg}^{-1} \text{ NH}_4^+\text{-N}$ was present on the 60th day as compared to $16.3 \text{ mg kg}^{-1} \text{ NH}_4^+\text{-N}$ in control. Similarly with karanjin based treatment at 10% dose 3-4 times more $\text{NH}_4^+\text{-N}$ was accumulated on the 60th day. In karanjin coated products, the highest

Table 4.8 : Effect of furfural derivatives on the ammonium, nitrite and nitrate contents of the incubated soil

Compound	Dose*	NH ₄ ⁺ -N mg kg ⁻¹ **				NO ₂ ⁻ -N mg kg ⁻¹ **				NO ₃ ⁻ -N mg kg ⁻¹ **			
		15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day
EOE	0	114.0	70.7	36.4	15.9	1.0	0.8	0.5	-	48.2	98.1	130.2	155.6
	5	158.6	124.2	102.1	60.2	1.0	0.8	0.3	-	16.5	36.2	57.9	114.4
	10	164.7	137.1	116.7	68.3	0.8	0.3	0.3	-	14.1	30.1	54.5	105.6
	15	163.2	140.3	124.4	74.5	0.5	0.3	0.2	-	12.2	28.5	45.3	98.4
	20	165.4	145.1	128.9	81.6	0.3	0.2	0.1	-	10.5	25.1	40.8	91.3
POE	0	118.4	70.2	34.0	16.4	1.1	0.8	0.6	-	48.8	99.1	133.4	158.8
	5	155.3	124.5	97.3	53.3	0.8	0.7	0.3	-	16.1	38.5	61.2	119.3
	10	159.9	136.4	117.4	65.2	1.0	0.7	0.4	-	14.7	35.3	58.7	104.4
	15	162.2	134.2	120.8	74.2	0.9	0.8	0.5	-	12.1	28.7	50.3	100.7
	20	165.5	140.6	125.4	77.9	0.8	0.5	0.2	-	10.1	27.8	45.7	95.6
BOE	0	117.1	69.3	34.4	16.1	0.8	0.6	0.3	-	47.9	99.6	132.6	157.8
	5	156.6	122.1	97.2	54.1	0.7	0.4	0.2	-	17.2	39.7	65.1	118.2
	10	159.3	133.5	109.4	61.7	1.0	0.7	0.2	-	14.8	34.1	62.8	109.6
	15	159.5	134.3	117.2	68.8	0.6	0.3	-	-	12.7	31.9	55.4	105.4
	20	162.8	137.4	120.5	73.3	0.7	0.2	0.1	-	11.1	29.2	51.2	99.4
HOE	0	112.6	71.4	35.4	18.1	1.1	0.7	0.3	-	49.8	97.3	131.1	153.4
	5	148.4	117.7	84.7	50.9	0.9	0.6	0.3	-	24.5	48.9	82.6	123.1
	10	152.7	121.6	90.6	59.3	0.9	0.6	0.2	-	21.1	46.1	78.2	115.6
	15	153.4	127.3	103.7	67.7	0.5	0.3	0.1	-	18.6	41.6	65.4	104.4
	20	157.2	135.1	117.8	72.0	0.4	0.1	-	-	16.3	33.2	56.2	101.1
AOE	0	124.0	75.5	38.9	9.3	0.9	0.7	0.3	-	48.0	97.1	134.7	160.7
	5	154.4	135.2	89.4	41.1	1.0	0.9	0.3	-	14.8	37.7	81.7	135.5
	10	163.4	137.4	92.7	44.4	1.2	0.7	0.5	-	12.4	34.1	78.2	132.4
	15	165.0	140.6	101.4	47.1	1.4	0.7	0.3	0.1	10.9	29.2	69.1	128.2
	20	166.7	143.1	104.1	54.2	1.6	0.9	0.6	-	9.9	27.1	65.7	120.0
OE	0	123.4	74.4	39.6	16.7	1.1	0.7	0.8	0.1	46.4	96.1	130.1	154.4
	5	158.0	125.4	83.2	46.1	1.0	0.8	0.6	0.2	17.8	49.4	88.6	130.2
	10	158.4	127.4	89.6	50.4	1.2	1.0	0.5	0.4	15.7	42.4	81.1	126.1
	15	161.1	133.6	95.4	57.2	1.4	1.0	0.8	0.2	12.4	38.0	74.1	116.7
	20	162.7	138.2	99.6	58.5	1.6	1.1	0.7	0.1	11.2	36.5	72.7	115.5

Continued.....

Compound	Dose*	NH ₄ ⁺ -N mg kg ⁻¹ **			NO ₂ ⁻ -N mg kg ⁻¹ **			NO ₃ ⁻ -N mg kg ⁻¹ **					
		15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day
APC	0	118.4	69.1	35.6	17.4	1.0	0.8	0.4	-	47.3	97.5	131.9	155.9
	5	156.4	112.2	76.3	41.2	0.9	0.6	0.3	-	16.6	50.1	92.3	129.8
	10	158.9	116.6	79.4	51.6	0.7	0.4	0.3	-	14.2	47.4	86.6	120.3
	15	161.3	127.3	89.3	62.4	0.5	0.3	0.1	-	12.1	40.2	76.8	110.2
	20	164.6	131.7	98.9	68.5	0.4	0.2	-	-	11.0	37.6	69.3	104.7
HAPC	0	112.8	71.7	35.8	18.8	1.1	0.8	0.3	-	49.1	97.8	131.6	153.6
	5	139.4	103.4	71.4	34.2	0.7	0.4	0.2	-	24.6	59.1	96.6	140.5
	10	142.5	109.2	80.4	40.1	0.9	0.5	0.2	-	22.5	55.3	88.1	133.2
	15	146.7	116.7	88.4	46.2	0.6	0.3	0.1	-	20.2	51.6	80.2	128.7
	20	148.3	123.4	91.1	58.4	0.4	0.2	-	-	19.1	46.4	77.3	112.6
ACC	0	119.3	68.4	37.4	16.1	1.0	0.7	0.3	-	46.4	98.3	133.4	157.4
	5	153.6	109.6	74.3	37.1	0.8	0.5	0.2	-	20.3	55.1	93.2	134.6
	10	155.2	115.3	79.1	46.6	0.7	0.4	0.2	-	17.4	47.6	87.6	126.7
	15	154.8	124.8	86.4	55.1	0.4	0.4	0.1	-	15.9	40.7	77.4	121.3
	20	157.5	129.9	95.4	65.5	0.3	0.1	0.2	-	13.9	38.7	71.2	111.7
CAS	0	118.4	70.6	34.4	16.3	1.2	0.4	0.2	-	46.5	100.4	131.2	152.4
	5	159.3	138.4	117.7	66.4	0.4	0.4	0.1	-	13.1	32.0	55.8	105.5
	10	163.6	139.4	122.2	75.1	0.7	0.3	0.1	-	10.0	29.1	49.9	99.1
	15	166.4	144.5	126.5	90.1	1.0	0.2	0.2	-	7.6	24.1	44.7	80.2
	20	168.5	147.8	130.4	92.5	0.9	0.3	0.1	-	6.9	22.4	40.5	78.4
DCAS	0	118.4	70.8	36.1	18	1.5	0.9	0.4	-	49.4	98.4	132.4	154.5
	5	155.5	130.6	107.5	67.8	0.7	0.4	0.5	-	14.7	38.9	66.4	104.2
	10	159.7	133.4	117.9	76.5	0.6	0.5	0.2	-	13.4	35.4	53.2	95.4
	15	161.8	140.6	122.5	89.4	0.8	0.2	0.1	-	11.1	31.0	51.6	86.5
	20	163.2	142.3	128.3	89.6	0.9	0.4	0.3	-	10.0	28.4	45.8	80.7
NAS	0	120.1	69.6	37.0	16.7	1.0	0.9	0.6	-	48.8	96.5	131	149.6
	5	156.6	124.3	79.4	54.5	1.4	1.2	0.7	-	12.6	44.6	90.4	120.3
	10	157.2	129.4	89.4	64.2	1.0	1.0	0.6	-	12.3	40.4	81.7	106.3
	15	161.8	140.5	114.3	78.3	0.9	0.6	0.3	-	12.0	30.7	57.1	93.1
	20	159.3	142	125.4	80.6	0.8	0.5	0.2	-	9.5	28.6	46.9	89.9
AS	0	119.8	70.2	37.6	17.2	1.0	0.8	0.5	-	48.1	97.2	130.3	150.4
	5	154.7	123.2	78.6	52.5	0.9	0.5	0.4	-	14.3	47.8	91.5	122.4
	10	158.4	127.7	90.5	62.1	1.1	0.3	0.3	-	13.5	42.5	82.4	109.5
	15	159.2	139.9	107.8	75.7	0.8	0.4	0.2	-	12.2	32.2	63.6	98.6
	20	161.4	140.2	127.2	77.4	0.7	0.5	0.5	-	11.4	30.0	47.8	92.1
NS	0	114.0	70.7	36.4	15.9	0.6	0.7	0.2	-	48.2	98.1	130.2	155.6
	5	167.2	148.4	134.1	101.6	1.0	0.8	0.5	-	7.9	22.7	35.7	71.3

*Doses are mentioned as % urea-N basis; ** Values are the average of three replicates.

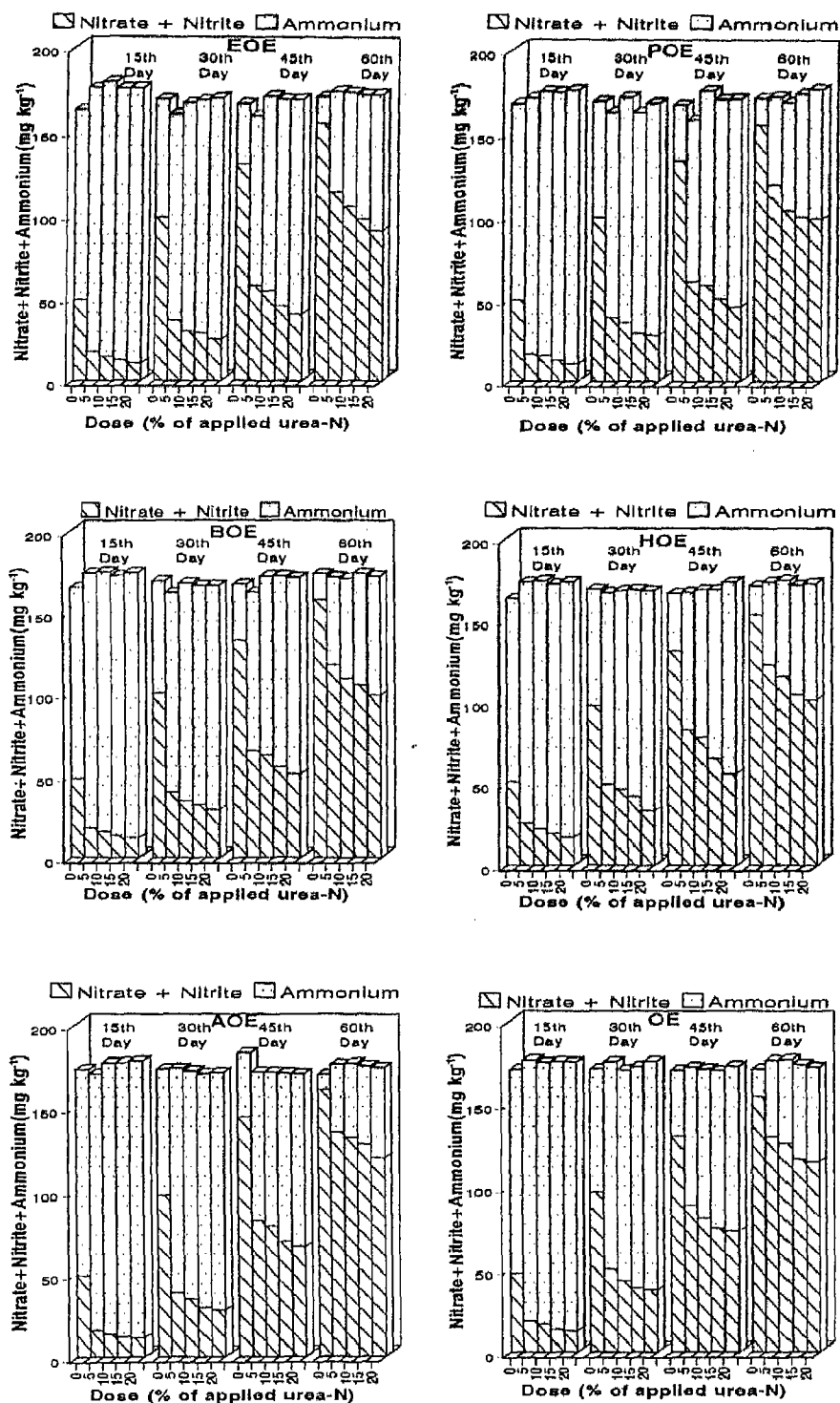


Fig.4.15 Effect of furfural derivatives on ammonium, nitrite and nitrate contents of the incubated soil(the concentrations of the ions are expressed in terms of nitrogen)

accumulation was observed with NA5KN10 and KA5CKN10 treatments. Sahrawat (1973) reported higher values of $\text{NH}_4^+\text{-N}$ as compared to this study. He reported accumulation of $173 \text{ mg kg}^{-1} \text{ NH}_4^+\text{-N}$ on the 15th day at 10% dose whereas in this study, none of the treatments including nitrapyrin crossed the $170 \text{ mg kg}^{-1} \text{ NH}_4^+\text{-N}$ mark. In both the cases 200 mg kg^{-1} urea-N was applied and the initial nitrogen content of the soil was almost same. Differences in physicochemical and microbiological properties of the soil are probably responsible for this difference.

In treatment KA5CKN10, $40.4 \text{ mg kg}^{-1} \text{ NH}_4^+\text{-N}$ was present on the 60th day as compared to $10.2 \text{ mg kg}^{-1} \text{ NH}_4^+\text{-N}$ in the control. Mixture of EOE and CAS with karanjin showed significant increase in the accumulation of $\text{NH}_4^+\text{-N}$ on the 60th day. It can thus be concluded that several of the compounds and/or their mixtures tested in the present study have the potential to significantly conserve $\text{NH}_4^+\text{-N}$, even upto the 60th day of incubation and at low concentration (5%).

4.3.2 Accumulation on nitrite : Among all the treatments tested, not a single one was found to cause significant accumulation of nitrite nitrogen. The synthetic test chemicals OE and AOE showed some $\text{NO}_2^-\text{-N}$ but these were not significantly high. In case AOE, the amount of nitrite accumulated on the 15th day at 5 and 20% doses were 1.0 and $1.6 \text{ mg kg}^{-1} \text{ NO}_2^-\text{-N}$, respectively. In case of all the compounds, on all the days and doses, less than $2 \text{ mg kg}^{-1} \text{ NO}_2^-\text{-N}$ was detected.

In treatments based on karanjin, higher doses (7.5 and 10%) accumulated more than $1 \text{ mg kg}^{-1} \text{ NO}_2^-\text{-N}$ on the 15th day. With increase in dose of karanjin, higher amount of $\text{NO}_2^-\text{-N}$ was detected. The concentration of nitrite detected in soil with karanjin coated urea treatments was found to be directly proportional to the karanjin dose. However, the quantity accumulated was insignificant. Mixtures employing higher doses of karanjin similarly resulted in

Table 4.9 : Effect of coating aids/binders on the ammonium, nitrite and nitrate contents of the incubated soil

Treatment	Dose (%Urea-N)	NH ₄ ⁺ -N mg kg ⁻¹ *			NO ₂ ⁻ -N mg kg ⁻¹ *			NO ₃ ⁻ -N mg kg ⁻¹ *		
		15 th Day	30 th Day	45 th Day	15 th Day	30 th Day	45 th Day	15 th Day	30 th Day	45 th Day
KA	0.0	124.3	83.4	46.3	0.2	0.1	-	38.7	81.2	120.2
	1.0	125.4	88.3	47.2	0.5	0.4	0.1	35.7	81.6	121.7
	2.5	130.2	89.5	48.3	0.6	0.2	0.1	34.0	80.1	119.6
	5.0	136.2	92.1	50.2	0.7	0.3	0.1	29.7	78.4	117.2
NA	0.0	125.7	82.4	48.2	1.0	0.8	0.3	37.6	82.1	119.7
	1.0	127.3	87.6	46.6	0.4	0.1	0.1	34.1	80.5	123.6
	2.5	131.6	90.4	50.6	0.5	0.2	Trace	31.7	80.6	115.4
	5.0	141.5	98.3	57.4	0.2	0.3	Trace	25.6	72.5	111.5
CKA	0.0	124.3	83.4	46.3	0.2	0.1	-	38.7	81.2	120.2
	1.0	129.6	89.1	47.9	0.3	0.2	0.1	34.1	80.4	118.7
	2.5	131.2	91.3	47.8	0.3	0.1	-	34.3	78.1	115.3
	5.0	137.8	95.2	49.4	0.1	0.2	0.1	28.4	75.8	112.8
C	2.0	128.9	87.4	46.8	0.4	0.2	0.1	33.9	80.4	119.1
KO	0.0	12507	82.4	48.2	1.0	0.8	0.3	37.6	82.1	119.7
	1.0	125.2	82.7	47.9	1.0	0.8	0.3	35.2	81.5	118.3
	2.5	125.4	83.8	49.2	0.7	0.6	0.2	34.5	80.4	118.2
	5.0	128.7	84.8	50.1	0.9	0.7	0.2	32.5	79.1	117.4
NS	0.0	126.6	83.7	49.3	1.1	0.7	0.5	36.5	83.6	120.4
	5.0	159.1	146.4	128.2	0.7	0.7	0.6	7.1	23.4	38.6

* Values are the average of three replicates

Table 4.10 : Effect of karanjin coated urea products (along with coating aids) on the ammonium, nitrite and nitrate contents of the incubated soil

Treatment	Dose KN (% Urea-N)	NH ₄ ⁺ -N mg kg ⁻¹ *				NO ₂ ⁻ -N mg kg ⁻¹ *				NO ₃ ⁻ -N mg kg ⁻¹ *			
		15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day
Control		123.1	82.8	45.7	11.8	0.2	0.1	-	-	39.6	82.6	121.1	151.1
	1.0	127.6	89.3	46.1	11.5	0.2	0.3	0.1	-	34.2	72.3	114.6	149.7
	2.5	128.8	90.8	48.8	12.8	0.3	0.3	0.1	-	32.1	69.8	111.2	147.6
	5.0	136.5	97.3	57.6	16.3	0.5	0.3	-	-	26.6	64.3	104.7	144.1
	7.5	149.0	110.6	70.5	20.2	0.8	0.4	0.1	-	18.8	52.1	95.1	140.3
	10.0	152.4	119.6	82.1	30.1	0.8	0.4	0.2	-	14.3	44.7	84.1	133.2
Control NA5KNx		129.1	84.7	50.7	14.1	0.7	0.8	0.4	-	40.2	84.4	126.8	157.6
	1.0	145.8	100.6	60.4	21.2	0.8	0.7	0.5	-	24.3	72.9	113.3	149.1
	2.5	146.4	105.7	62.2	23.4	0.9	0.8	0.4	-	23.4	67.3	111.1	147.2
	5.0	152.5	116.6	75.4	28.5	0.9	1.0	0.7	-	17.9	58.2	94.6	143.1
	7.5	161.7	130.0	90.3	37.4	1.3	1.0	0.8	-	10.8	40.9	79.2	129.8
	10.0	163.2	138.3	102.6	42.7	1.5	1.1	0.4	-	9.1	36.8	68.1	123.3
Control KA5KNx		123.4	82.8	45.7	11.8	0.8	0.3	-	-	41.1	84.1	123.7	153.2
	1.0	132.5	90.5	47.8	12.9	0.5	0.2	0.1	-	29.1	71.6	118.3	150.2
	2.5	138.2	95.1	52.2	13.6	0.8	0.4	0.1	-	26.3	68.2	113.2	148.3
	5.0	153.8	109.1	68.1	21.5	0.8	0.4	0.1	-	17.4	57.1	97.3	140.6
	7.5	157.3	125.2	81.2	33.2	0.9	0.3	-	-	11.1	44.3	84.7	129.8
	10.0	160.8	132.1	94.3	39.2	1.1	0.8	-	-	9.3	36.9	71.1	121.1
Control CKNx		121.6	80.3	48.4	13.4	0.7	0.4	-	-	37.6	85.1	119.0	148.3
	1.0	129.0	88.2	48.2	13.9	0.4	0.2	0.1	-	31.8	74.9	114.4	147.1
	2.5	137.1	95.6	53.1	15.1	0.3	0.2	0.1	-	27.3	70.1	110.2	145.2
	5.0	150.7	107.2	66.3	19.3	0.4	0.3	0.1	-	19.6	58.9	97.1	142.7
	7.5	156.3	120.1	79.1	27.1	0.3	0.2	-	-	16.2	48.2	88.6	136.4
	10.0	159.1	126.8	92.3	35.6	0.9	0.2	0.1	-	12.6	42.0	74.3	127.1

Continued.....

Treatment	Dose KN (%Urea N)	NH ₄ ⁺ -N mg kg ⁻¹ *				NO ₂ ⁻ -N mg kg ⁻¹ *				NO ₃ ⁻ -N mg kg ⁻¹ *			
		15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day
Control		120.3	80.1	43.2	10.2	0.8	0.3	-	-	41.1	84.1	123.7	153.2
KA5CKNx	1.0	131.7	90.9	56.8	12.8	0.8	0.5	0.1	-	29.0	71.5	118.5	150.4
	2.5	139.9	96.7	53.2	14.7	0.9	0.4	0.1	0.1	24.7	67.1	115.2	147.6
	5.0	154.2	110.5	68.0	20.9	1.2	0.5	0.4	-	16.5	56.5	97.0	142.2
	7.5	158.1	125.6	81.9	34.7	1.1	0.4	0.7	-	10.7	42.2	83.1	127.6
	10.0	159.2	133.1	93.2	40.4	1.0	0.9	0.6	-	9.1	36.9	70.9	120.2
Control		119.7	79.9	44.2	11.6	0.9	0.5	0.2	-	40.4	83.2	124.5	154.7
KO2.5KNx	1.0	128.9	88.4	47.3	12.4	0.5	0.4	0.2	0.1	33.5	113.4	113.4	147.9
	2.5	130.2	94.6	47.2	13.6	0.8	0.7	0.4	-	31.6	113.4	113.4	149.8
	5.0	135.7	99.1	59.4	15.7	0.8	1.0	0.7	0.3	28.4	103.1	103.1	146.1
	7.5	148.2	110.1	68.2	25.8	1.0	0.5	0.2	-	17.4	94.3	94.3	138.6
	10.0	153.1	117.3	84.1	30.8	1.2	0.9	0.6	0.1	14.5	83.7	83.7	135.1
Control		119.7	79.9	44.2	11.6	0.9	0.5	0.2	-	40.4	83.2	124.5	154.7
NS5		160.4	148.4	128.8	98.1	1.4	0.9	0.8	0.4	6.1	19.6	40.4	71.6

* Values are the average of three replicates

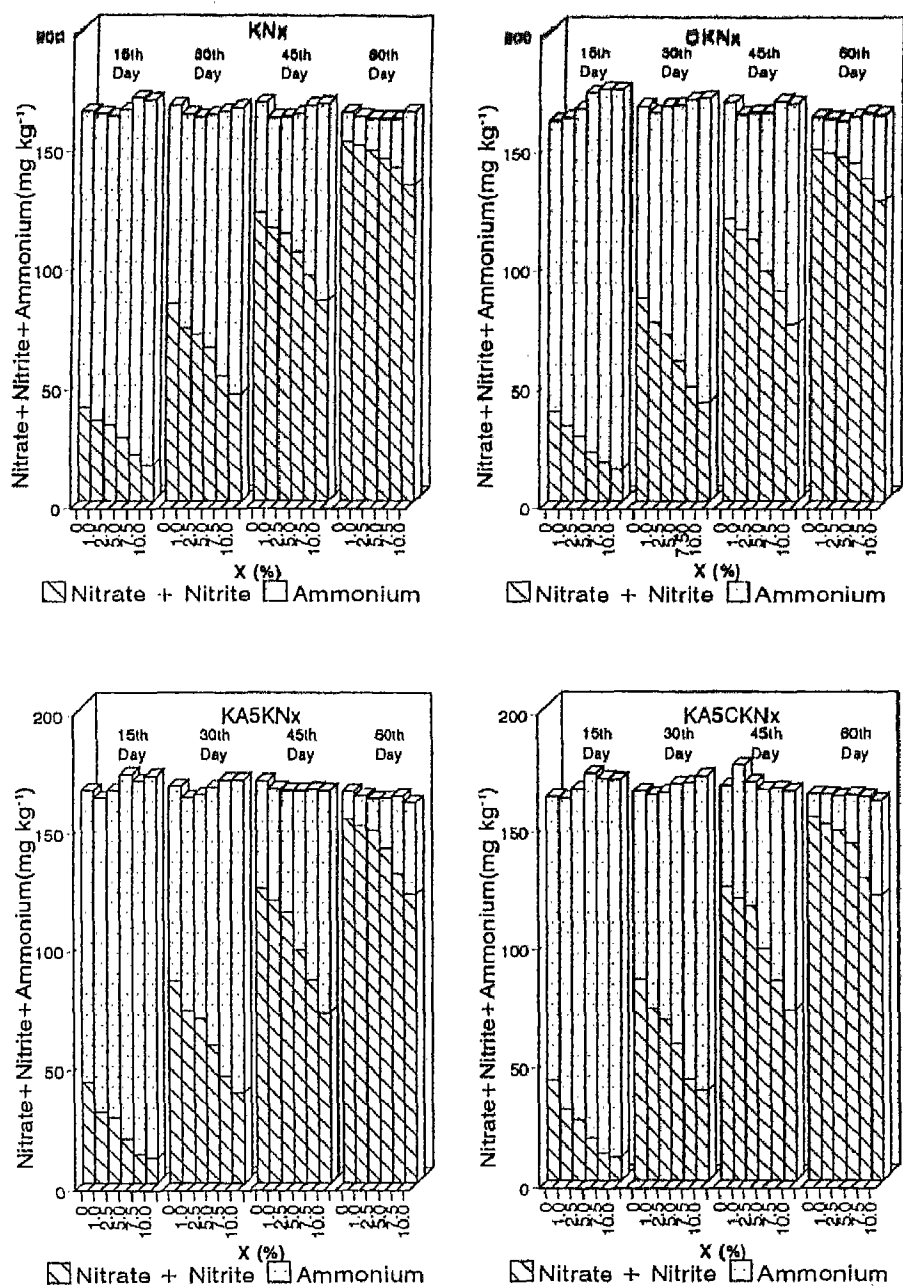


Fig.4.16 Effect of some karanjin coated urea products (along with coating aids) on the ammonium, nitrite and nitrate contents of the incubated soil (the concentration of the ions are expressed in terms of nitrogen)

accumulation of relatively higher amount of NO_2^- -N. On the 60th day in most treatments (with some exceptions) NO_2^- -N could not be detected and on the 45th day, rarely more than $0.5 \text{ mg kg}^{-1} \text{ NO}_2^-$ -N was detected.

It is well known that under aerobic conditions the faster rate of NO_2^- -N oxidation leads to low level of nitrites, quite often undetectable. The first step of NH_4^+ to NO_3^- conversion is slower and hence rate limiting. The chemicals screened in the present study, apparently have no significant adverse effect on *Nitrobacter* species, which is responsible for oxidising nitrite to nitrate in soil. The amount of nitrite detected in this study with karanjin was even lower than reported in the earlier data at the same dose (Sahrawat, 1973 and Kuzvinzwa, 1974).

It is thus prudent to conclude that none of the synthetic derivatives led to the accumulation of high levels of NO_2^- -N even at 20% dose. Karanjin showed a tendency to inhibit the *Nitrobacter* species but at the tested concentration, it did not lead to the accumulation of toxic quantities of nitrite.

4.3.3 Accumulation of nitrate : The NO_3^- -N produced at various intervals showed a similar trend, as revealed by the NI% data. Among the test chemicals EOE, POE, BOE, CAS and DCAS showed less amount of nitrate at low doses (5%). The data for the 15th day of these treatments were 16.5, 16.1, 17.2, 13.1 and $14.7 \text{ mg kg}^{-1} \text{ NO}_3^-$ -N, respectively. The value in control for these treatments varied from 46-49 $\text{mg kg}^{-1} \text{ NO}_3^-$ -N. The data indicate that these derivatives significantly inhibited the conversion of ammonium to nitrate. These figures, though higher than nitrapyrin ($7.9 \text{ mg kg}^{-1} \text{ NO}_3^-$ -N), showed significant effect on *Nitrosomonas* as compared to control.

4.4 Physicochemical properties of the coated urea products

The Fertilizer (Control) Order (1985) prescribes the following specifications for coated urea fertilizer (Schedule 1, Part A, FCO, 1985).

Table 4.11 : Effect of coating vs. solution application of EOE and CAS on the ammonium, nitrite and nitrate contents of the incubated soil

Treatment (%Urea-N)	Application method	NH ₄ ⁺ -N mg kg ⁻¹ *				NO ₂ ⁻ -N mg kg ⁻¹ *				NO ₃ ⁻ -N mg kg ⁻¹ *			
		15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day
Control		126.8	75.5	39.7	8.6	1.0	0.9	0.1	-	48.9	99.5	136.7	162.4
EOE 5	Coating	160.5	126.0	112.9	57.3	1.1	0.7	0.4	0.1	14.9	35.1	57.0	117.7
	Solution	158.4	132.1	113.4	56.4	1.2	0.7	0.4	-	16.1	37.3	56.8	115.5
EOE 10	Coating	164.4	139.6	118.5	68.5	0.9	0.9	0.3	-	12.1	29.3	53.0	105.5
	Solution	157.5	141.0	117.2	66.5	1.0	0.6	0.3	-	12.3	30.3	53.2	107.4
CAS 2.5	Coating	148.7	114.0	80.3	43.7	0.8	0.9	0.4	-	20.5	56.4	92.0	127.1
	Solution	147.8	112.4	80.7	43.9	1.1	0.7	0.4	-	21.4	59.3	91.4	126.4
CAS 5	Coating	160.1	140.5	120.6	65.0	0.9	0.9	0.6	-	12.0	33.2	52.9	107.1
	Solution	159.9	142.0	119.2	66.4	0.9	0.6	0.3	-	12.6	31.4	53.7	105.8
CAS 7.5	Coating	159.5	142.4	122.5	72.7	0.8	0.7	0.3	-	11.0	31.6	50.1	102.3
	Solution	162.7	143.6	121.8	70.6	0.7	0.4	0.4	-	10.9	30.5	50.2	103.3
Control		120.2	78.6	43.2	12.4	0.9	0.6	0.3	-	41.4	81.8	122.4	155.1
NS 5		161.9	148.5	129.2	96.2	1.5	1.0	0.7	0.5	6.4	20.2	40.1	72.6

* Values are average of three replicates.

Table 4.12 : Effect of mixtures of karanjin and furfural derivatives on the ammonium, nitrite and nitrate content of the incubated soil

Treatment (% of Urea-N)	NH ₄ ⁺ -N mg kg ⁻¹ *				NO ₂ ⁻ -N mg kg ⁻¹ *				NO ₃ ⁻ -N mg kg ⁻¹ *			
	15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day
Control	121.9	72.8	37.6	9.7	0.7	0.4	0.1	-	47.1	95.8	132.7	159.1
EOE5KN2.5	160.1	136.2	113.2	54.3	1.2	0.8	0.5	-	14.1	34.0	56.0	118.2
EOE5KN5	163.0	140.7	118.2	57.4	1.4	1.0	0.5	-	12.5	29.6	52.2	115.4
EOE5KN7.5	165.5	141.4	123.9	64.2	1.4	1.1	0.6	0.1	11.5	29.6	46.3	104.0
CAS5KN2.5	160.1	139.6	118.3	69.2	0.6	0.5	0.3	-	11.4	32.8	52.6	103.7
CAS2.5KN5	156.5	130.1	103.7	40.6	1.0	0.8	0.6	-	16.2	40.5	68.4	131
Control	120.2	78.6	43.2	12.4	0.9	0.6	0.3	-	41.4	81.8	122.4	155.1
NS5	161.9	148.5	129.2	96.2	1.5	1.0	0.7	0.5	6.4	20.2	40.1	72.6

* Values are the average of three replicates

Table 4.13 : Effect of mixtures of alcohol extract of karanj seed kernels and furfural derivatives on the ammonium, nitrite and nitrate content of the incubated soil

Treatment (% Urea-N)	NH ₄ ⁺ -N mg kg ⁻¹ *				NO ₂ ⁻ -N mg kg ⁻¹ *				NO ₃ ⁻ -N mg kg ⁻¹ *			
	15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day	15 th Day	30 th Day	45 th Day	60 th Day
Control	124.1	43.9	40.1	17.1	1.1	0.6	0.8	0.1	46.1	96.4	129.2	154.8
KA5CAS2.5	154.2	124.7	86	55.6	1	0.8	0.6	0	16.5	47.3	87.5	117.8
KA5CAS5	160.9	140.3	119.5	67.1	0.8	0.9	0.3	0.1	11.4	36.2	51.8	105.00
KA5EOE2.5	152.6	121.6	82.7	55.4	0.9	1	0.5	0	17.3	48.8	89.3	117.9
KA5EOE5	157.4	133.6	113.1	55.2	0.7	1	0.6	0	13.6	37.8	58.2	119.4
NS5	162.0	141.3	130.4	90.4	0.6	0.6	0.6	0.1	8.7	28.3	40.8	80.8

* Values are the average of three replicates

Urea (coated) 45%N (While free flowing) :

1. Moisture content by weight maximum 0.5%
2. Total nitrogen content per cent by weight, content with coating, maximum 45%
3. Biuret % by weight 1.5
4. Particle size - 90% of the material shall pass through 2.8 mm IS sieve and not less than 80% by weight shall be retained on 1 mm sieve.

The physicochemical properties of the coated products are presented in Table 4.14 .

4.4.1 Flowability : All the treatments mentioned in Table 4.14 were freeflowing. Some of the products did show relatively inferior quality as compared to uncoated urea. Those coated with higher doses of the viscous liquids showed little bit stickiness, but those coated with solids were free from this problem. The mixtures coated with both alcohol extracts and oxime ethers showed relatively less freeflowing character. Urea coated with karanj oil showed stickiness. Under most situations, satisfactory flowability could be achieved by properly adjusting the dose of the coating aid. Overall, at the mentioned doses flowingness does not seem to be a limitation for the coated products. In extreme situations it can be improved by mixing the coated products with inert materials like soapstone.

4.4.2 Total N content : Many treatments showing good nitrification inhibition property in this study were found to contain less than 45% N (per cent weight basis). As the amount of material coated is large, per cent nitrogen content expectedly dips below 45 per cent in certain treatments. The lowest amount of total nitrogen detected was 43.22% in KA5CKN10. Though in certain treatments nitrogen percentage fell below the specified 45% level but because of their substantial nitrification inhibition property, these products will

Table 4.14 Physicochemical properties of coated urea products

Coated products	Total N % (w/w)	Molsture % (w/w)	Freeflowingness	Particle size
KN1.0	46.30	0.20	***	96/90
KN2.5	46.02	0.19	***	96/90
KN5.0	45.51	0.19	***	95/89
KN7.5	45.00	0.21	***	95/89
KN10.0	45.50	0.20	***	95/89
CKN1.0	45.97	0.20	**	96/90
CKN2.5	45.65	0.22	**	96/90
CKN5.0	45.09	0.21	***	96/90
CKN7.5	44.61	0.23	***	95/89
CKN10.0	44.10	0.22	***	95/89
KA5KN1.0	45.30	0.25	**	96/90
KA5KN2.5	45.01	0.21	**	96/90
KA5KN5.0	44.56	0.20	***	95/89
KA5KN7.5	44.06	0.24	***	95/89
KA5KN10.0	43.55	0.20	***	95/89
NA5KN1.0	45.36	0.23	**	96/90
NA5KN2.5	45.07	0.21	**	96/90
NA5KN5.0	44.54	0.22	***	96/90
NA5KN7.5	44.01	0.17	***	95/89
NA5KN10.0	43.55	0.21	***	95/89
KA5CKN1.0	45.00	0.21	*	96/90
KA5CKN2.5	44.67	0.20	**	96/90
KA5CKN5.0	44.20	0.22	**	95/89
KA5CKN7.5	43.70	0.24	***	95/89
KA5CKN10.0	43.22	0.25	***	95/89
KO2.5KN1.0	45.85	0.21	**	96/90
KO2.5KN2.5	45.51	0.22	**	96/90
KO2.5KN5.0	45.01	0.21	**	96/90
KO2.5KN7.5	44.52	0.25	***	95/89
KO2.5KN10.0	44.03	0.22	***	95/89
CEOE5	45.30	0.14	**	96/90
CEOE10	44.60	0.15	**	96/90
CCAS2.5	45.74	0.21	***	95/89
CCAS5.0	45.23	0.19	***	95/89
CCAS7.5	44.42	0.18	***	95/89
EOE5CKN2.5	44.82	0.19	**	96/90
EOE5CKN5.0	44.34	0.18	***	96/90
EOE55CKN7.5	43.90	0.17	***	95/89
CAS5CKN2.5	44.80	0.15	***	95/89
CAS2.5CKN5	44.69	0.17	***	95/89
KA5EOE2.5	45.78	0.22	**	96/90
KA5EOE5	44.22	0.21	**	96/90

*** = Excellent; ** = Good; * = Poor

provide more nitrogen to plants as compared to the ordinary urea prills complying with the per cent nitrogen requirement. So lower nitrogen content with most of these products need to be ignored and cannot be considered as a disadvantage. Even in the treatment in which lowest percentage of nitrogen was detected, the difference with the prescribed limit was less than 2% whereas as compared to control the same product inhibited nitrification by more than 40% on the 45th day.

4.4.3 Size distribution : Particle size analysis of the test coated products confirmed to the FCO specifications.

4.4.4 Moisture content : None of the coated products contained more than 0.25% (w/w basis) moisture, which was less than the prescribed requirement (Table 4.15). Alcohol extract coated products showed somewhat higher amount of moisture content. The hygroscopic nature of the alcohol extract may be the reason for it. The products coated with furfural derivatives were found to contain less (>0.2%) amount of moisture. Thus, all the test samples satisfactorily met with the requirement.

4.5 Effect of test chemicals on germination percentage

Germination test with the synthetic furfural derivatives showed that the most active derivatives like EOE, POE, CAS and NAS did not adversely effect the germination of chickpea seeds, even at the highest dose (40 ppm, soil basis, equivalent to 20 % of applied urea-N). All the test compounds and control showed around 80% germination. The vigour of the seedlings also did not show any significant difference than over control.

Table 4.15 : Effect of EOE, POE, CAS and NAS on the germination of chickpea seeds

Compound*	Dose (ppm, soil basis)	Germination percentage**
Control	-	82
EOE	10	79
EOE	40	82
POE	10	81
POE	40	82
CAS	10	81
CAS	40	80
NAS	10	83
NAS	40	81

* EOE (N-O-ethyl furfural oxime), CAS (furfurylidene-4-chloroaniline), NAS (furfurylidene-3-nitroaniline) POE (N-O-isopropyl furfural oxime).

** Treatment means are average of five replicates.

SUMMARY AND CONCLUSIONS

The research study entitled "Development of slow nitrogen release urea formulations employing *Pongamia glabra* Vent. isolates and some furfural derivatives" was carried out with the objective of developing nitrification inhibitors based on indigenous sources to increase the nitrogen use efficiency (NUE) and to minimize environmental pollution from nitrogenous fertilizers. Twelve furfural derivatives belonging to three classes namely, furfural oxime ethers, furfural chalcones and substituted furfurilidene anilines (furfural Schiff bases) were synthesized and tested for nitrification inhibitory activity. An effort has also been made to develop technology for coating Karanjin, a furanoflavonoid present in karanj seed kernels, on the surface of urea prills in order to develop cheap, ready to use, slow release urea formulations. The physicochemical properties of the developed products were studied and compared with the specifications for such products prescribed in the Fertilizer Control Order (FCO).

Salient results of the study are presented below :

1. Furfural oxime ethers and furfural Schiff bases showed potential activity as nitrification inhibitors but the furfural chalcones, in general showed poor activity. Excellent activity of oxime ethers and Schiff bases has been attributed to the aldimino moiety ($-\text{CH}=\text{N}-$) attached to the furan ring.

2. Among the furfural oxime ethers, EOE (N-O-ethyl furfural oxime) was the most active followed by POE (*iso*-propyl furfural oxime) and BOE (N-O-butyl furfural oxime). Difference in activity between the EOE and POE was statistically nonsignificant at most of the doses and periods but in certain cases EOE showed significantly better activity. Similarly, the differences in activity between POE and BOE in many cases were not significant particularly upto

30th day. HOE (N-O-*n*-hexyl furfural oxime) performed poorly whereas AOE (N-O-allyl-furfural oxime) performed well initially but its activity did not last for long. EOE, POE and BOE remained active upto the 45th day, after which the activity fell rapidly.

3. Activity of oxime ethers increased with an increase in dose but this increase in activity was not proportionate with the doses. On the 15th day, at higher dose (20% of the applied urea-N), EOE (79.67% NI), POE (79.16% NI) and BOE (76.99% NI) approached nitrapyrin (83.14% NI at 5% dose) in activity. At lower dose (5%), the activity of these compounds though considerable was lower than nitrapyrin.

4. The structure activity relationship (SAR) showed that NI activity of the furfural oxime ethers decreased with the increase in chain length of the alkyl group. Introduction of double bond in the side chain showed high activity initially but it did not persist for long. On the basis of decreasing activity, the oxime ethers can be arranged as follows :



5. Furfural oxime, an intermediate used in the synthesis of furfural oxime ethers, itself possessed good activity, but the activity was not long lasting. The persistence of activity of furfural oxime ethers were significantly more than the intermediate oxime.

6. Chalcones in general showed poor activity. Though one of them, APC [3-(furan-1-yl)-1-phenyl-prop-2-en-1-one] showed considerable activity on the 15th day but the effect was short lived. Introduction of hydroxy group in phenyl ring further reduced the activity.

7. Among the Schiff bases, the chlorinated products like CAS (furfurylidene-4-chloroaniline) and DCAS (furfurylidene-3,4-dichloroaniline) showed considerable activity upto 45 days. At 20% dose, CAS was at par with

nitrapyrin upto the 30th day and was comparable to nitrapyrin upto the 60th day. Further, dose higher than 15% failed to improve the activity significantly and hence this dose can be considered as maximum. NI activity decreased with increase in chlorine atoms or the position of substitution in phenyl ring influenced the activity significantly. Nonchlorinated Schiff bases like NAS (furfurylidene-3-nitroaniline) and AS (furfurylideneaniline) showed high activity initially. At lower doses their activity fell rapidly with time and at higher doses, their activity persisted upto the 45th day. In order of decreasing activity the Schiff bases can be arranged as follows :



8. When the NI activity of three classes of nitrification inhibitors were compared among themselves, the activity of EOE and POE were found to be comparable to CAS and DCAS, though the latter two compounds showed marginally better activity. Among the various tested compounds EOE, POE, CAS and DCAS hold promise.

9. The alcohol extracts of karanj (KA) and neem seed kernels (NA) could be employed as useful binding aids to coat nitrification inhibitors like karanjin. Five per cent dose of the alcohol extracts can be considered as optimum for this purpose. KA and NA at 5% dose imparted the desired degree of stickiness for the purpose of coating and holding the inhibitory compounds besides imparting certain degree of nitrification inhibition property (34.36% and 23.45% NI at 5% dose for NA and KA, respectively on the 15th day), of their own.

10. Coating of karanjin (KN) on urea with coal tar (C) as a binder significantly improved the NI activity as compared to the treatment where karanjin was coated without any binder. Urea coated with karanjin employing the alcohol extracts of karanj/neem as coating aids, inhibited nitrification to a greater extent as compared to the coal tar based

products. Karanjin coated along with KA and CKA (coal tar + KA) did not show significant difference in activity.

11. Nitrification inhibition property of karanjin coated urea increased rapidly with an increase in the dose of karanjin. In treatment KA5CKN10 [alcohol extracts of karanj seed kernels (5%) + coal tar (2%) + karanjin (10%)], 76.91% inhibition was observed on the 15th day. This treatment was comparable to nitrapyrin which after same time interval showed 82.59% NI. The nitrification inhibition activity of karanjin decreased considerably with time but its application at higher doses led to the increase in the persistence of activity. At 10% karanjin, employing alcohol extracts as binders more than 40% inhibition was observed on the 45th day. Thus, 10% dose of karanjin, can be considered effective for nitrification inhibition.

12. At lower dose of karanjin, nitrification inhibition activity of NA coated products were marginally superior than the KA coated products. With an increase in the dose of karanjin, the activity of KA coated products matched with NA coated products. NA has been found to impart somewhat more persistent activity than KA.

13. The karanjin coated products, in terms of decreasing activity, can be arranged as follows :

$$\text{KNx} \geq \text{KO2.5KNx} > \text{CKNx} > \text{KA5KNx} \geq \text{KA5CKNx} \geq \text{NA5KNx}$$

The karanjin coated products with alcohol extracts as binders hold promise and need to be evaluated further for improvement in NI activity.

14. Application of EOE and CAS coated urea failed to show improvement when compared with their application in solution form. In the laboratory studies with small amount of soil, the test chemicals could be applied with high efficiency in solution form, which is not possible in the field. Hence, the coated products can be expected to perform better

in the field. Coated products performed at par with the solution application in the laboratory.

15. Urea coated with mixture of synthetic furfural derivatives and karanjin generally did not show any synergistic or antagonistic activity. However, one treatment CAS2.5KN5 (coal tar 2% + furfurylidene-4-chloroaniline 2.5% + karanjin 5%), on 30th and 45th day showed substantial increase in activity over the constituents. At lower dose of karanjin (2.5%), the mixture usually showed the activity pattern of the synthetic partner whereas at its higher doses (more than 5%) the additional effect due to karanjin was observed. At higher doses of karanjin, activity increased but not substantially.

16. Mixtures of alcohol extract of karanj and furfural derivatives failed to improve the NI activity over the constituents.

17. Mixture of alcohol extract of karanj seed kernels and the synthetic derivatives showed almost similar activity as showed by the mixture of synthetic derivatives with karanjin. At the same dose of synthetic derivatives, there was not much difference in intensity of activity between karanjin and alcohol extract of karanj, but karanjin increased the persistence of activity. The added advantage associated with KA is its utility as a binder for coating the synthetic derivatives.

18. None of the treatments accumulated nitrite to a significant level.

19. The physical properties of the coated fertilizers matched the Fertilizer Control Order (FAI,1995) specifications except for the nitrogen content. Though some of the treatments contained as low as 43% nitrogen (as compared to the minimum requirement of 45%-N for coated products) but their nitrification inhibition values indicated that such products will ultimately supply substantially more nitrogen to plant as compared to the common urea prills. Other physical properties like moisture content, freeflowingness and particle size of the coated products passed the FCO specifications.

20. Germination test with chickpea seed suggested that the successful test chemicals did not hinder the germination even at their highest doses of application.

It can thus be concluded that there exists a great potential for utilizing indigenous raw materials like karanj extractives and synthetic derivatives from furfural for improving the nitrogen use efficiency of urea and minimising environmental pollution from nitrogenous fertilizers. Performance of karanjin can be improved substantially by coating karanjin on urea and using mixtures of neem and/or karanj extractives as binder. Such products are needed to be field tested to evaluate their performances in practical situation. Treatments like KA5CKN10, KA5KN10 and NA5KN10 will be suitable for such evaluation. Simple synthetic derivatives of furfural showed significant nitrification inhibitory activity. Structure activity relationship studies indicated that more furfural derivatives are needed to be synthesized and tested. Further study on these chemicals, particularly with the furfural Schiff bases and furfural oxime ethers, on field scale are needed to evaluate their performance in practice. Any success achieved in this field will save not only the costly resources but also will help to minimise environmental pollution.

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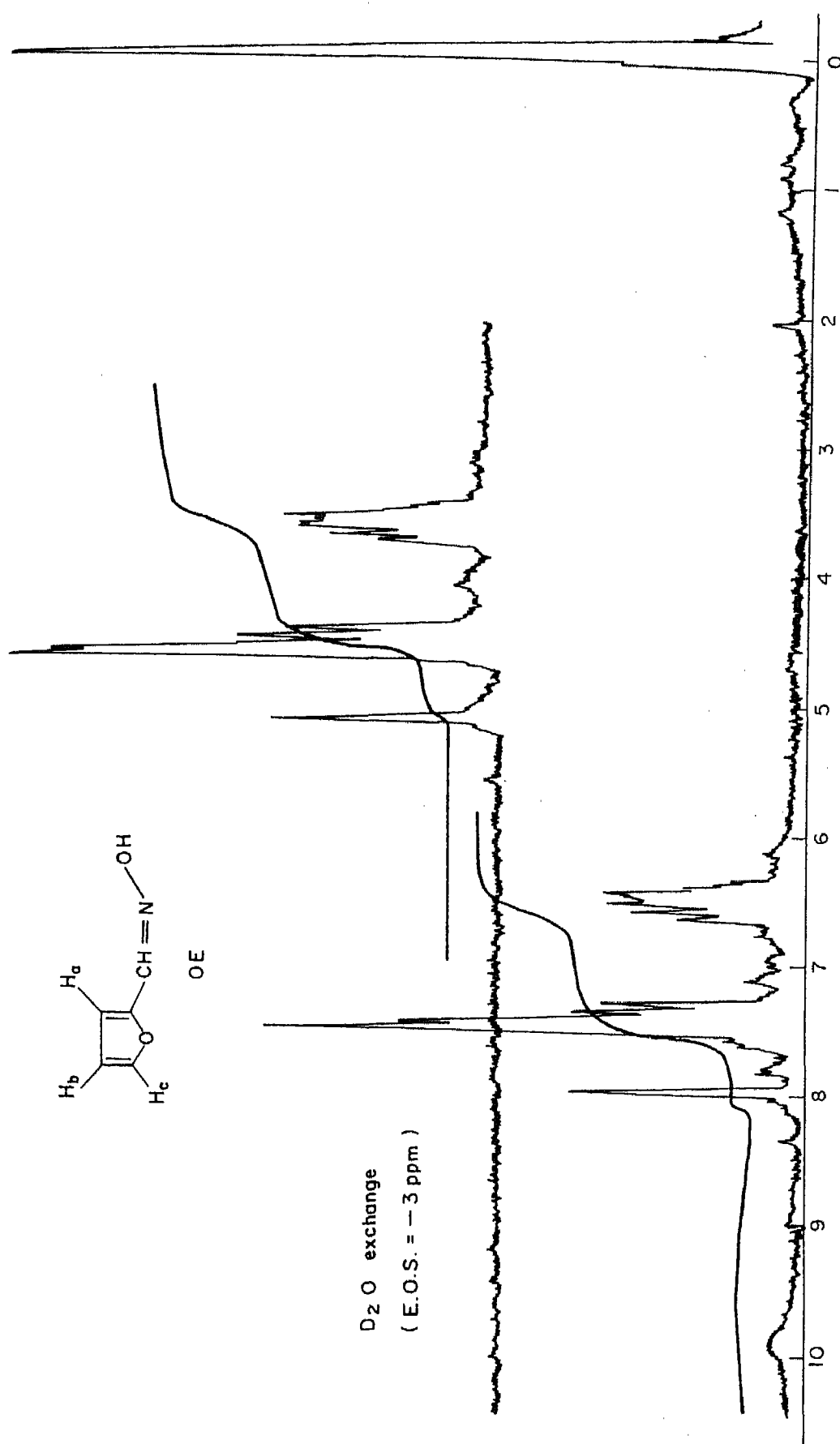
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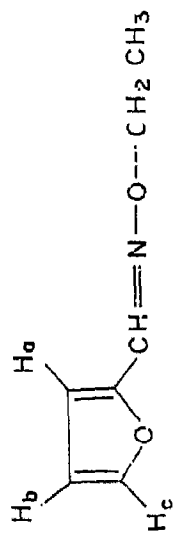
APPENDIX

**[^1H NMR (PMR) SPECTRUM
of synthesized furfural derivatives]**

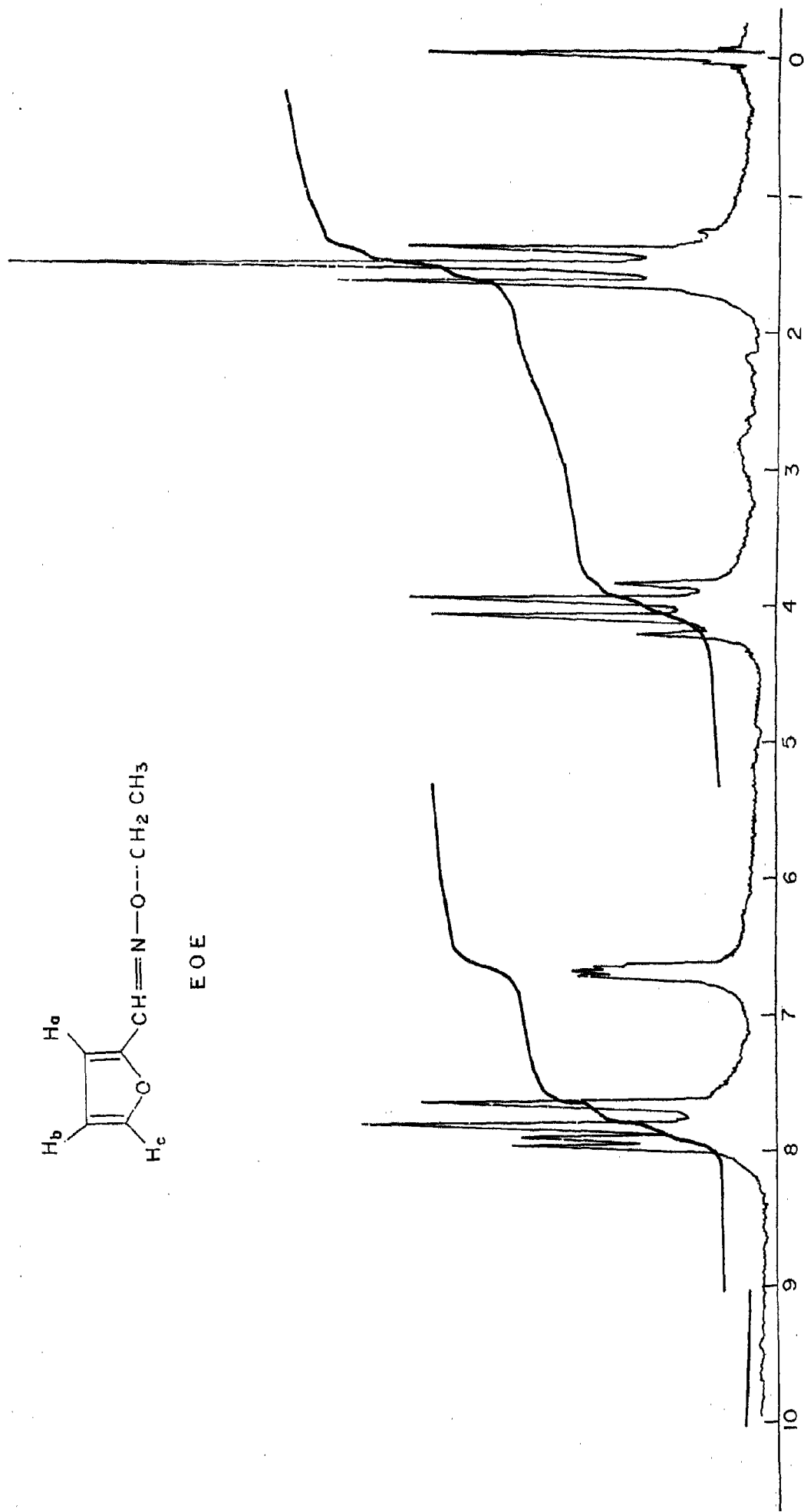


D₂O exchange
(E.O.S. = - 3 ppm)

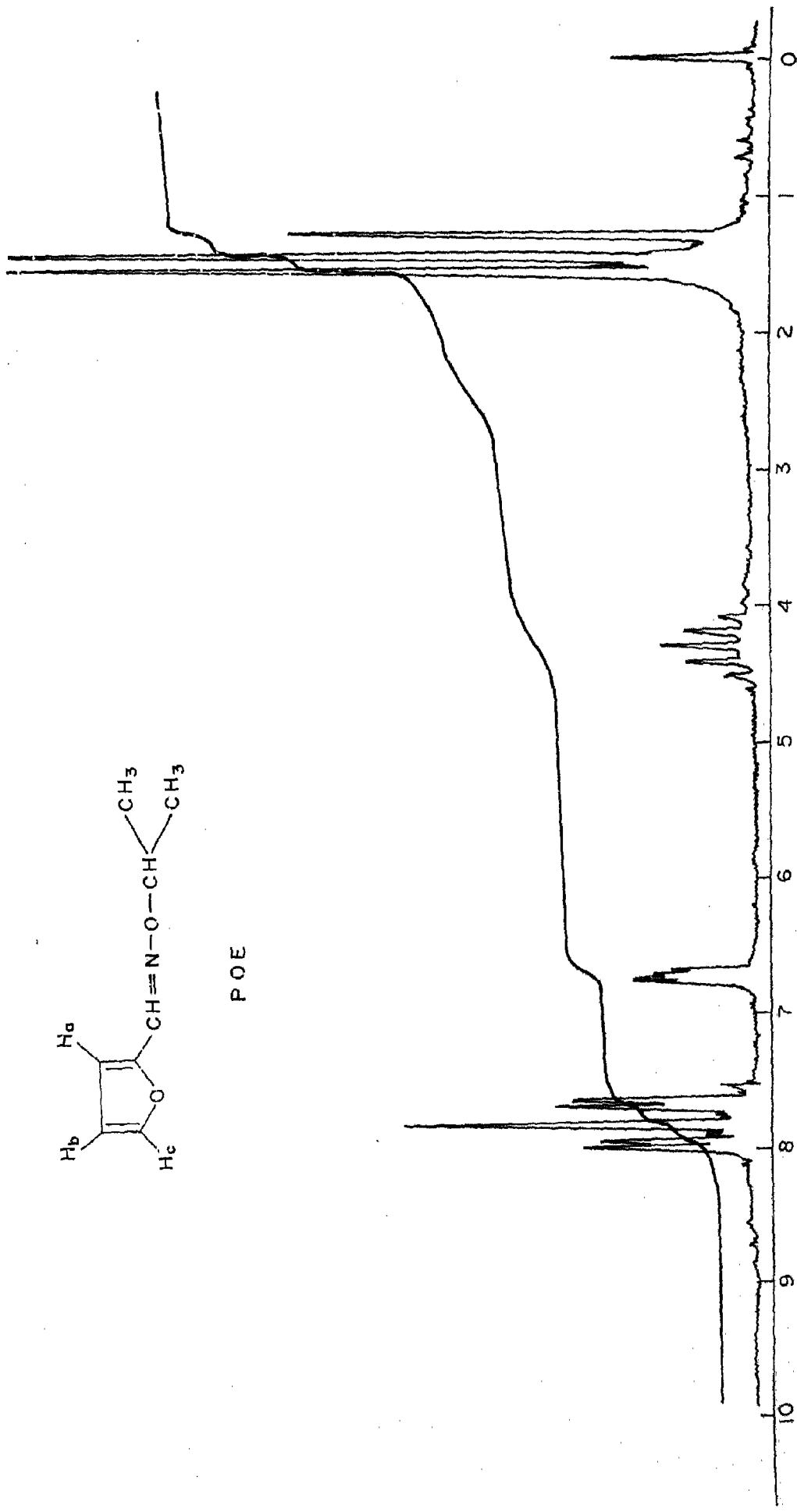
Spectrum 1 : PMR spectrum of furfural oxime



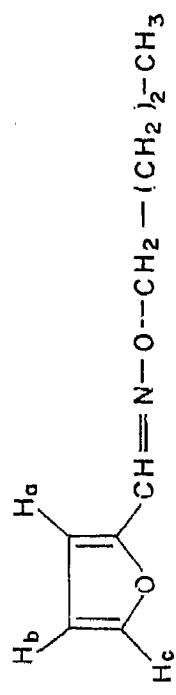
EOE



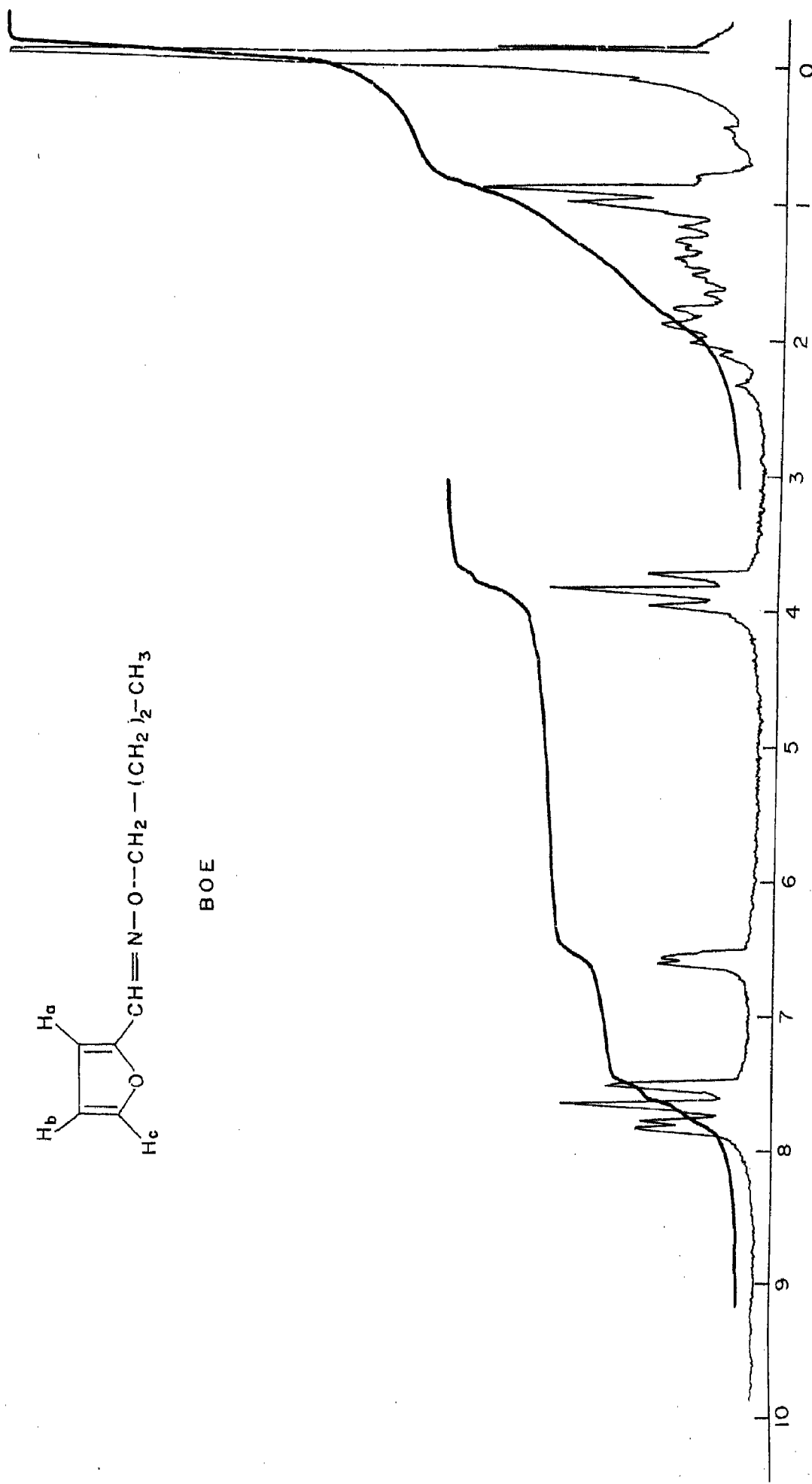
Spectrum 2 : PMR spectrum of N-O-ethyl furfural oxime (EOE)



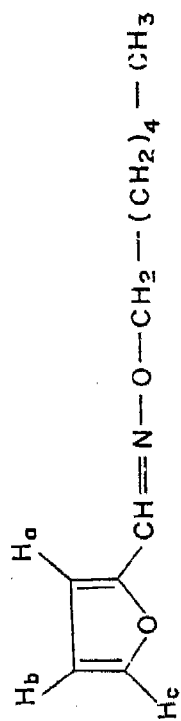
Spectrum 3 : PMR spectrum of N-O-isopropyl furfural oxime (POE)



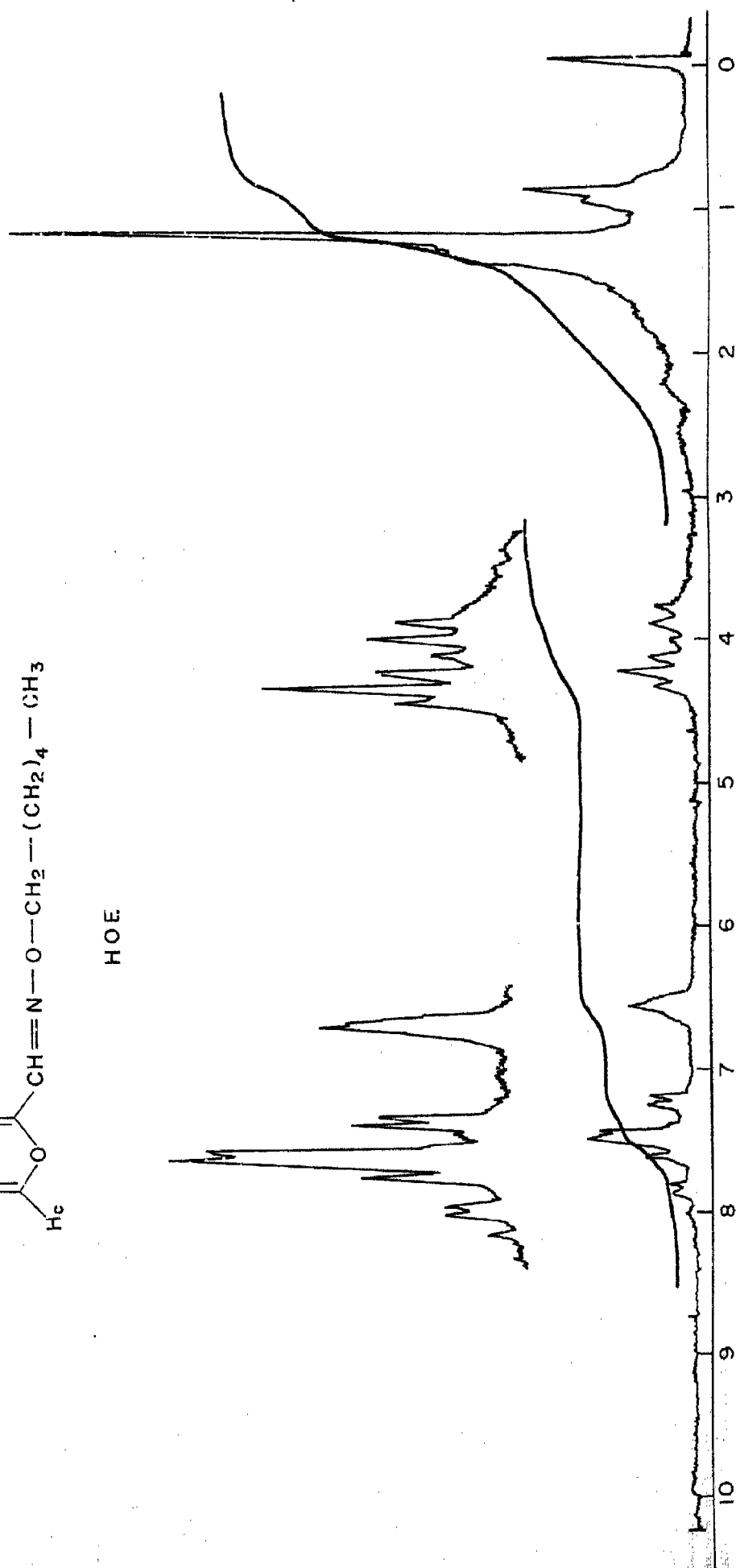
BOE



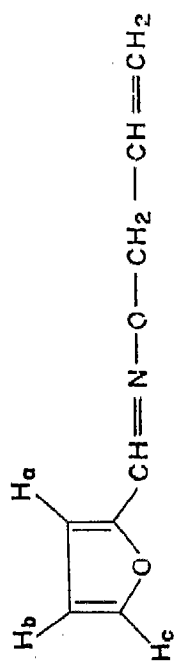
Spectrum 4 : PMR spectrum of N-O-butyl furfural oxime (BOE)



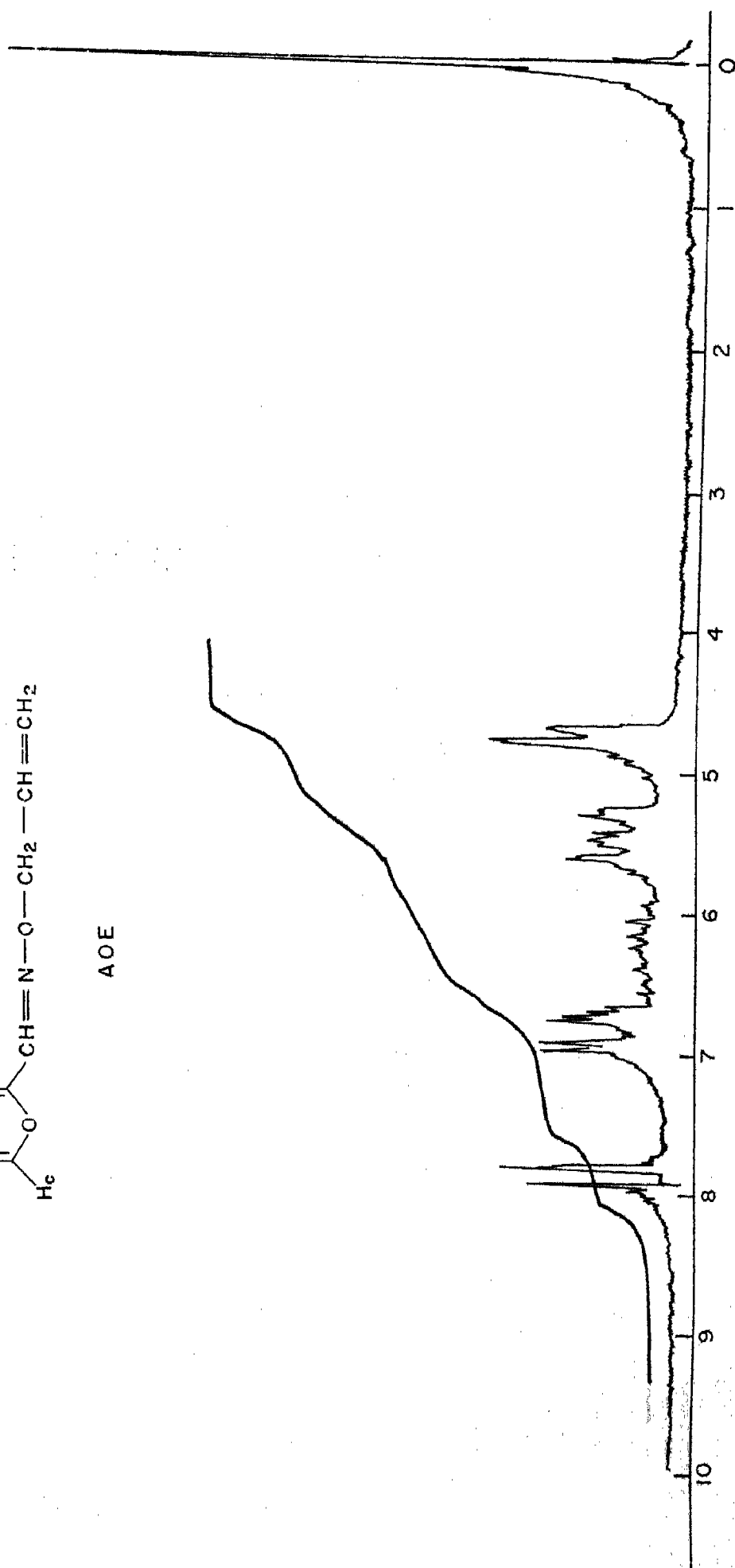
HOE



Spectrum 5 : PMR spectrum of N-O-hexyl furfural oxime (HOE)



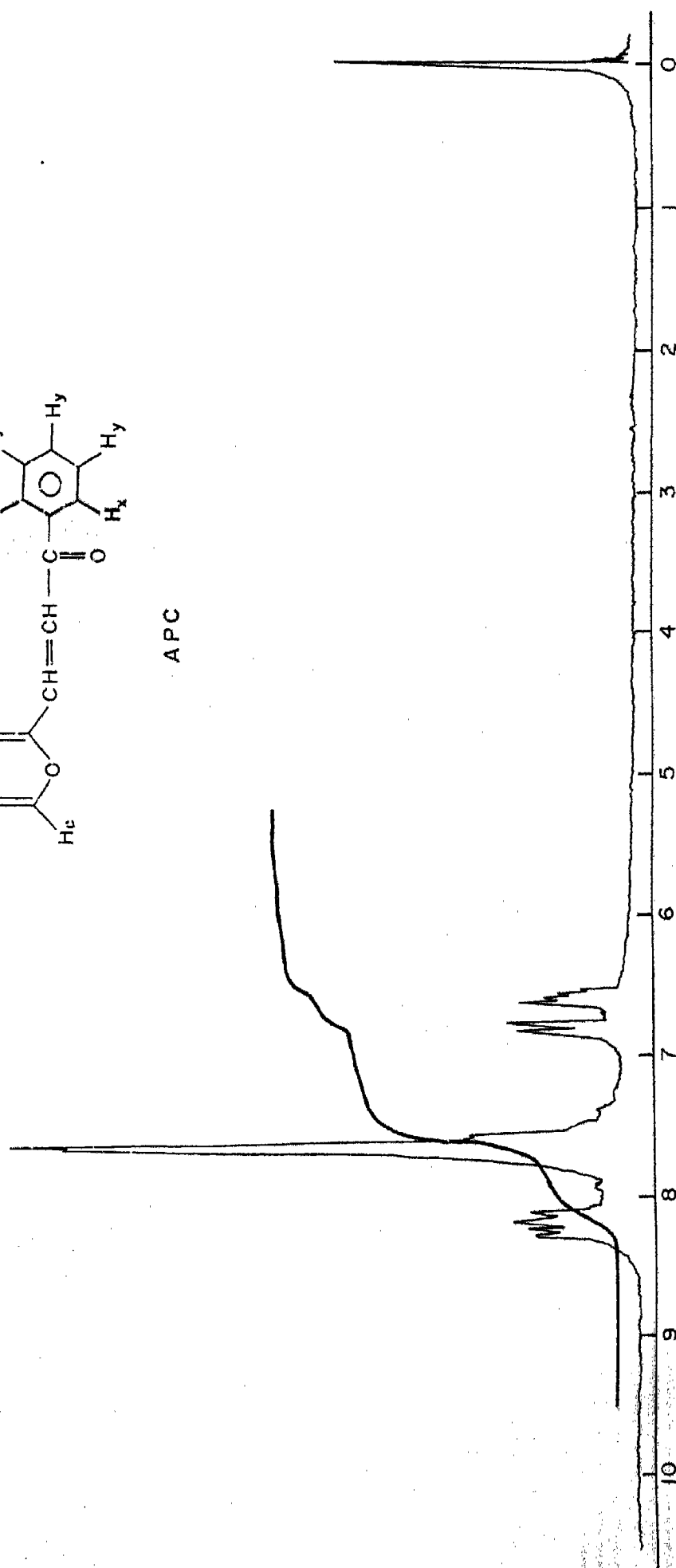
AOE



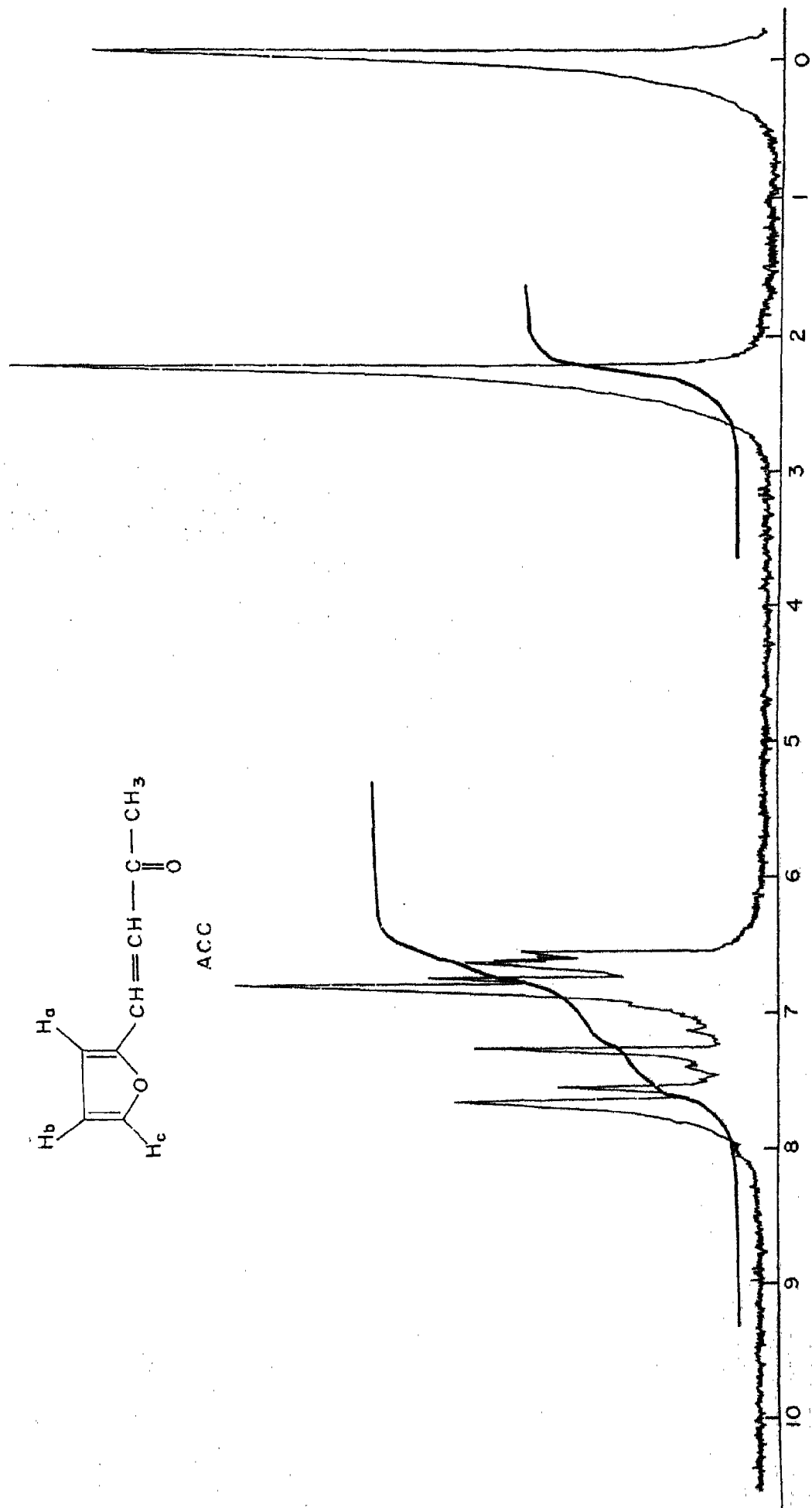
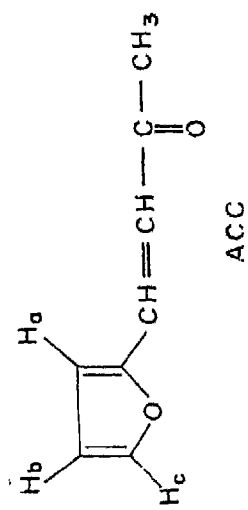
Spectrum 6 : PMR spectrum of N-O-Allylfurfural oxime (AOE)



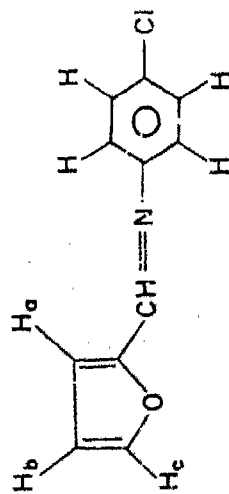
APC



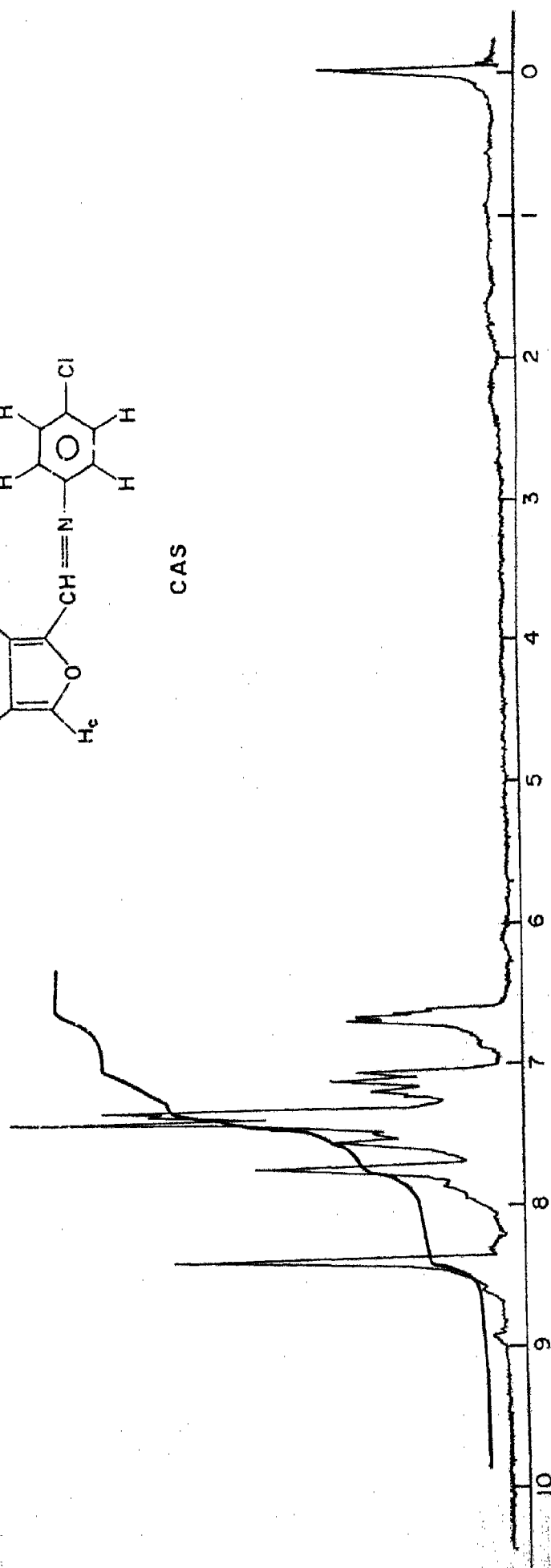
Spectrum 7: PMR spectrum of 3-(furan-2-yl)-1-phenylprop-2-en-1-one (APC)



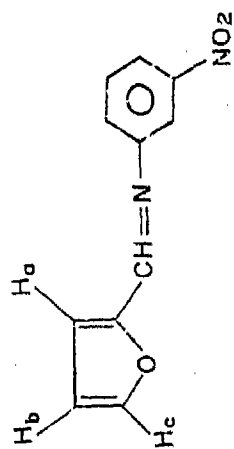
Spectrum 8 : PMR spectrum of 4 - (furan - 1 - yl) - but - 3 - en - 2 - one (ACC)



CAS



Spectrum 9 : PMR spectrum of furfurylidene -4 - chloroaniline (CAS)



NAS

T-6745

