STUDIES ON NEEM CAKE AS SOURCE OF NITROGEN ITS EFFECT ON SOIL MICROBES AND AS AN INHIBITOR OF PESTICIDES DEGRADATION

BY

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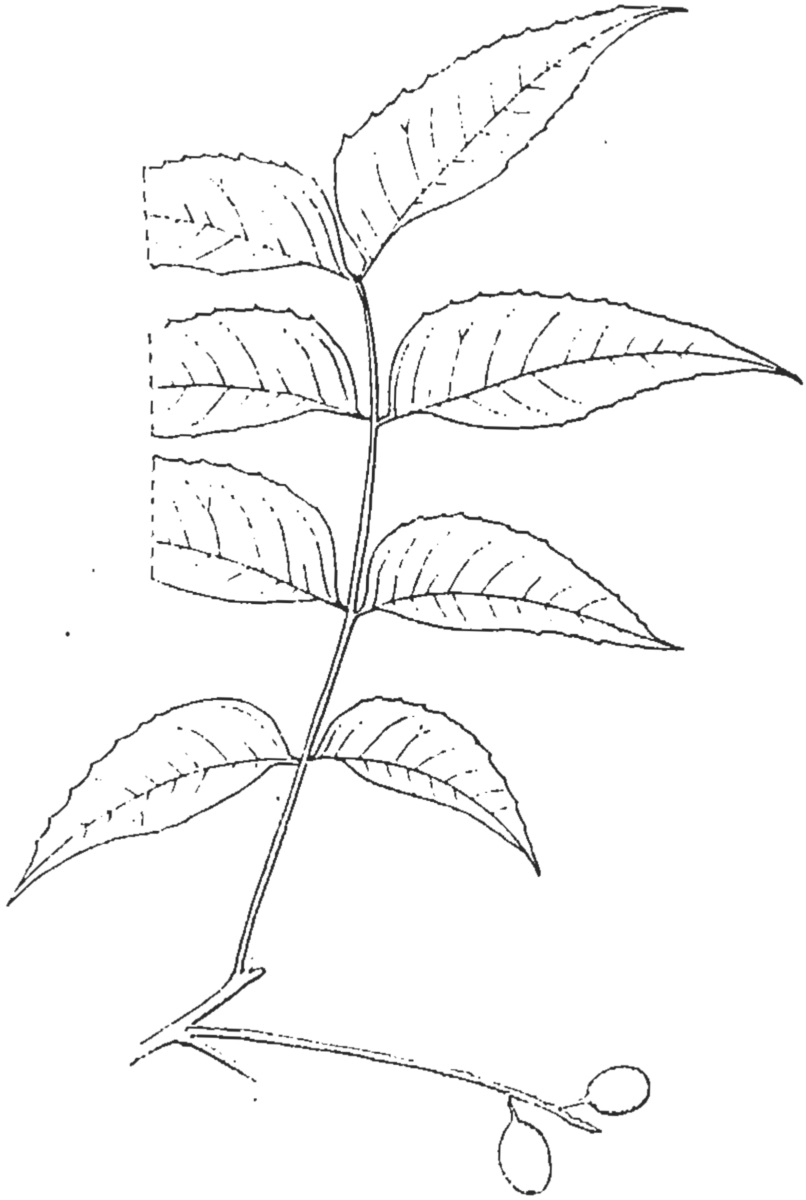
(Prof. Dr. M. A. Siddiqui)
Director Research

Examiner:

AZADIRACHTA INDICA A. Juss.

Synonym	*Melia azadirachta* L.
Family	Meliaceae
Kannada name	Bevu
English name	Neem



C O N T E N T S

CHAPTER-II:

CHAPTER-III:

10. NEEM CAKE AS AN INHIBITOR OF PESTICIDE DEGRADATION:

SUMMARY

Poor management of eco-system in Pakistan is the main sourc of environmental degradation; the major being soil and water Massive use of agro-chemicals involves the risk of interferin with the quality of eco-system, dangerous from fertility an pollution point of view.

To cope with the situation, and to overcome environmenta hazards within our own resources, the research on neem cake (product left-after oil extraction from neem seeds) was initiate to find its property as a fertilizer. General observatio revealed that neem cake is a good nitrogen source; whe experiments were conducted in-vitro. Paddy soil supplemented wit neem cake (n-hexane extracted) at the rate of 1% and 5%(w/w produced two and four times higher ammonia nitrogen than un- supplemented soil, respectively after 10 days of application. However nitrogen release was delayed if the soil was supplemente with the ground neem cake. The expeller extracted neem cake gav a slight and slow formation of ammonia nitrogen after 10 day with 5% & 10% amendment, neem seed powder after 15 days & the water extracted neem cake after 30 days, whereas no response o ammonia nitrogen production was observed with neem oil, amendment.

The studies on the effect of both type of neem cakes (hexane extracted & expeller extracted) on symbiotic and nc symbiotic nitrogen fixing bacteria have revealed that it had adverse effect on its growth with the exception that n-hexa extracted neem cake stimulated the growth of certain species Rhizobium-in vitro.

Investigations regarding the effect on the inhibition degradation of insecticides (Carbofuran 3G, Thiodan 35EC a Basudin 10G) revealed that neem cake amendment alongwi insecticide fortification increased the persistence of all tl three insecticides from their normal period of degradation. was observed that micro-organism are responsible for tl degradation of these insecticides. The natural balance of sor micro-organisms is disturbed by the addition of neem cake whic helps in avoiding degradation of these insecticides, ultimatel the persistent percentage increases.

Keeping in view of the various aspects studies in vitrc neem cake if used in field will not have any adverse effect i the environment. The utilization of this product must be mad aware to the farmers as neem tree is a native of Indo-Pak Sub Continent. Use of neem products in agriculture will be a ste towards self-help and minimize environmental pollution due to th indiscriminate use of chemical fertilizers. It is better t switch over to organic matter as fertilizer which is the basis o green revolution.

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LIST OF TABLES

LIST OF FIGURES

PHOTOGRAPHS

GENERAL INTRODUCTION

Neem, Azadirachta indica (A. Juss.) is a tree belonging to member of the Mahogany family Meliaceae. Neem tree is attractive, broad-leaved evergreen that can grow up to 30m height and 2.5m in girth. The roots penetrate the soil deeply and if injured they produce suckers. This suckering tends to be especially prolific in dry localities. Its flowers are small, white and are borne in axillary clusters. Neem honey is also popular and reportedly contains no trace of azadirachtin (main chemical identified for insecticidal property). Its fruit is ellipsoidal, smooth, yellow or greenish yellow in colour comprising of sweet pulp enclosing a seed. The seed is composed of a shell and kernel (sometimes 2 or 3 kernels) each about half the seed weight. On average neem kernels contain between 2 & 4 mg of Azadirachtin per gram of kernel. The neem tree normally begins bearing fruit after 3-5 years and can produce up to 50 kg of fruits, annually. It may live for more than two centuries.

Neem is native to the whole indian sub-continent throughout all the South and Southeast Asia, including Pakistan, Sri Lanka, Thailand, Malaysia, and Indonesia. The tree has also been established in Fiji, Mauritius, the Caribbeans and many Countries of Central and South America. The tree is easily propagated both sexually and vegetatively. Normally grown by seed, the seeds are not viable for long. It is generally considered that after 2-6 months in storage it will no longer germinate. However, some recent observation of seeds stored in France indicated that seeds without endocarp had an acceptable germinative capacity (42%) after more than 5 years.*

Neem often grows rapidly, almost every where except in freezing or extended cold and can not stand waterlogging. It gives good growth on dry infertile sites. Some common names of neem in various parts of the world are given in table-1.

*Information from Rocderer and Bellefontaine cited in "Neem a tree for solving global problems" 1992, National Academic Press, Washington D.C., page 26.

TABLE-1

COMMON NAMES OF NEEM IN VARIOUS PARTS OF THE WORLD

COUNTRY/LANGUAGE	COMMON NAMES
English	Neem, indian lilac
French	Azadira d'inde; azadira margousier, azidarac
Portugese	Margosa, (Goa)
Spanish	Margosa, nim
German	Niembaum
Hindi	Neem, nimb
Burmese	Tamar, tamarkha
Urdu	Nim, neem
Punjabi	Neem
Tamil	Vembu, Veppan
Sanskrit	Nimba, nimbou,arishtha (reliever of sickness)
Sindi	Nimmi
Sri Lanka	Kohomba
Farsi	Azad darakht-i-hindi (free tree of india),nib
Malay	Veppa
Singapore	Kohumba,nimba
Indonesia	Mindi
Nigeria	Dongoyaro
Kiswahili	Nwarubaini (muarobaini)

Source:

Neem: A Tree for solving global problems. National Academy Press, Washington D.C. 1992, p.28.

Azadirachtin is structurally similar to insect hormones called ecdysones which control the process of metamorphosis. Azadirachtin seems to be an "ecdysone blocker".

Neem has demostrated considerable potential as a fertilizer. For this purpose neem cake and neem leaves are especially promising.

The residue left after the oil extraction varies widely in composition of protein, carbohydrate, crude fibre, fat and ash as given in (Table-2) along-with comparison analysis of neem cake sample used.

TABLE-2

COMPOSITION OF TWO TYPES OF NEEM CAKE

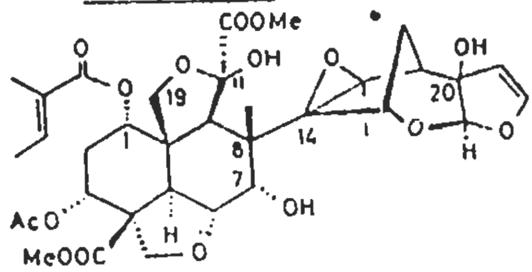
S.No.	Various Tests	Broad Ranges*	n-hexane extracted Neem Cake	Expeller extracted Neem Cake
1.	Protein	13-35%	42.27%	12.8%
2.	Carbohydrate	26-50%	40.16%	34.28%
3.	Crude Fibre	8-26%	9.09%	31.54%
4.	Fat	2-13%	2.1%	13.5%
5.	Ash	5-18%	6.38%	7.88%

* Taken from the Book entitled "Neem - A tree of solving Global Problems" Chapter 8 "Industrial products", report of an Adhoc Panel of the Board on Science and Technology for International Development, National Research Council, National Academy Press washington, D.C. 1992. P.74.

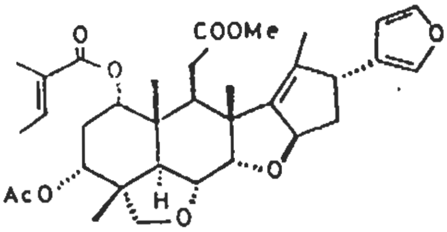
This so called neem cake has considerable local potential. It contains more Nitrogen, Phosphrous. Potassium, Calcium and Magnesium than farmyard manure. Plowed in to soil it protects plant roots from nematodes and white ants probably due to its content of the residual liminiods. So far 9 neem liminoids have been demonstrated. Azadirachtin, salannin, meliantriol, nimbin and nimbidin are the best known and seems to be most singificant. The structures are presented in Figure-1. The residual neem cake obtained aften removal of oil by n-hexane still contains the main active liminiod ingredient.

Agriculture is the mainstay of Pakistan's economy in terms of providing food and foreign exchange earnings for which it faces a tremendous challenge in modernizing agriculture and making its allied more productive, to maintain this economy better crop production is required. Balanced fertilization generally helps good yield and efficient agriculture input. With the increase in population and demand for quick and more production of crop, chemical fertilizers are regarded as the most significant single input in modern agriculture.

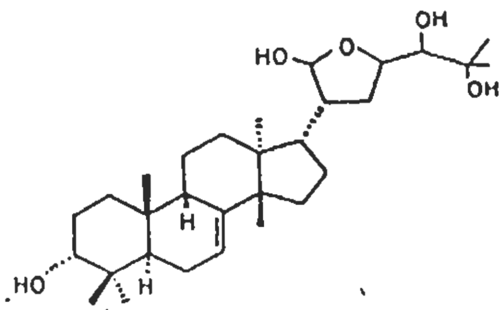
CHEMICAL STRUCTURES OF NEEM'S MAIN INGREDIENTS



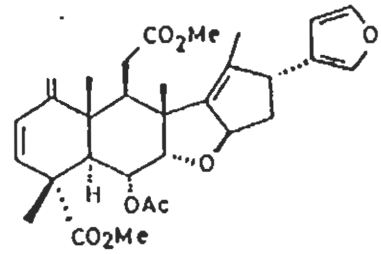
Azadirachtin



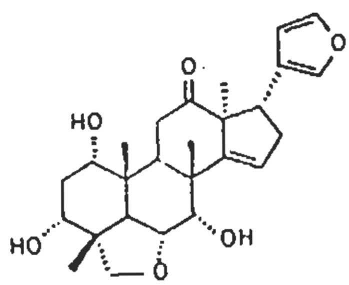
Salannin



Meliantriol



Nimbin



Nimbidin

Fig: 1

In Pakistan, fertilizers contribution for 1984 had been estimated to be 44, 29, 66, and 28% to wheat, rice, maize, and sugarcane production, respectively (Saleem, 1985). When the amount of nutrient fertilizer used per unit cropped area are calculated, the present nutrient consumption in Pakistan is about 61 kg per ha, (Saleem, et. al. 1989). With the increase use in % of fertilizer Nitrogen it is evident that fertilizer has become one of the agriculture's important tools, and on closer examination it is clear that there are a number of problems to be over come in the increased use of fertilizers.

To over come the increased demand of fertilizers it has become important to exploit new techniques and match the best method/source of fertilizer during soil crop management. In this respect efforts are being made to the integrated use of organic and mineral fertilizers (Hussain, et al. 1988)because the application of organic manure alone or in combination with fertilizers helps both in proper nutrition of crop plant (Talashilkar & Vimal, 1986) and maintenance of soil fertility (Salim, et al. 1988). Research programs with neem tree and its products are underway throughout the world, the commercial availability of margosan-o the first neem oil extract has been registered as an insecticides in U.S.A.

Neem tree possess a variety of noval properties which are of potential use in pest management programme (Fig-2). Besides oil its another product produced from the seed of the tree functions as a fertilizer, the residue obtained after extraction of the oil from the seed kernel known as press cake or Neem cake. According to a survey report of sindh area the residual cake left after the extraction of oil from different samples of Karachi, Hyderabad, Larkana, and Shikarpur is 65, 54 and 58%, respectively (Kazmi, et.al. 1991).

Literature survey reveals that the neem cake exhibits the properties of insecticides, nitrification retardation and inhibiter of pesticide degradation (Parmar, 1986). It exhibits systemic insecticidal and apparent fertilizer properties when added to the water in rice paddy culture. These fertilizer effects are due to the biological action, it has been reported that the fertilizer action in flooded rice culture due to arresting the development of grazing in vertebrates as well as nitrifying bacteria (Grant, et. al. 1983). In paddy rice the

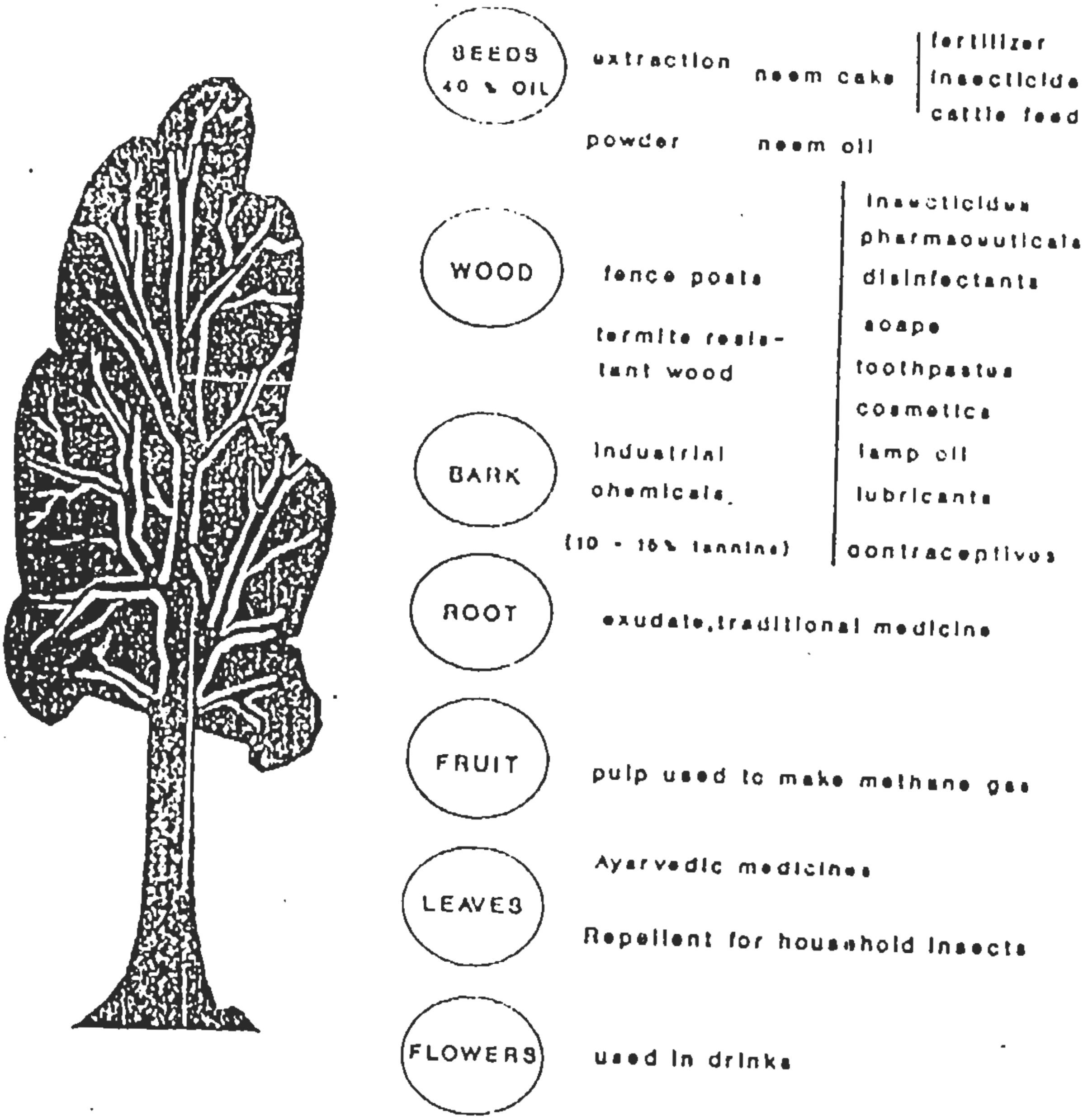


Figure-2. Neem Tree and Its Products

Source: Update: Neem - A new Era in pest control products. The IPM practitioner 9(10) p.1-8, 1987, Bio-Integral Resources Centre, Berkeley CA.

ostracod *H. luzonensis* limits the establishment and growth of nitrogen fixing blue-green algae inhibition of ostracod increases nitrogen availability and consequently, significantly increases rice yield (Ketkar, 1974).

OBJECTIVES OF THE STUDY:

In view of the background this work on the use of neem cake in Pakistan was initiated with the following objectives.

i) Evaluation of Nitrogen released from Neem cake amended soil.

ii) To study the Interaction of neem cakes with beneficial soil microbes (Rhizobium sp.) & nitrifying bacteria in-vitro.

iii) To investigate Neem cake as an inhibitor of pesticide degradation in paddy soil, the insecticides tested for degradation are viz Carbofuran 3G, Basudin 10G, and Thiodan 35 EC.

EVALUATIONOF NITROGEN RELEASE

FROM

NEEM CAKE AMENDED SOIL

ABSTRACT

Soil supplemented with neem cake (n-hexane extracted from neem seed) at the rate of 1% and 5%w/w produced two and four times higher ammmonia nitrogen than unsupplemented soil, respectively after ten days of application. However, nitrogen release was delayed if the soil was supplemented with the ground neem cake. Neem oil did not help in the production of additional nitrogen.

INTRODUCTION:

The ever increasing demand for N fertilizers coupled with the appallingly low recovery of the applied nitrogen by crops is of great concern in modern agriculture. In soil, N is generally lost through volatilization leaching, run-off and de-nitrification mechanisms. The conditions of shallow and deep placement of Ammonium sulhate in paddy soil is shown in Figure-3. Application of slow nitrogen release fertilizers or of the nitrogenous fertilizers with various nitrification retarders is a promising approach to minimise these losses.

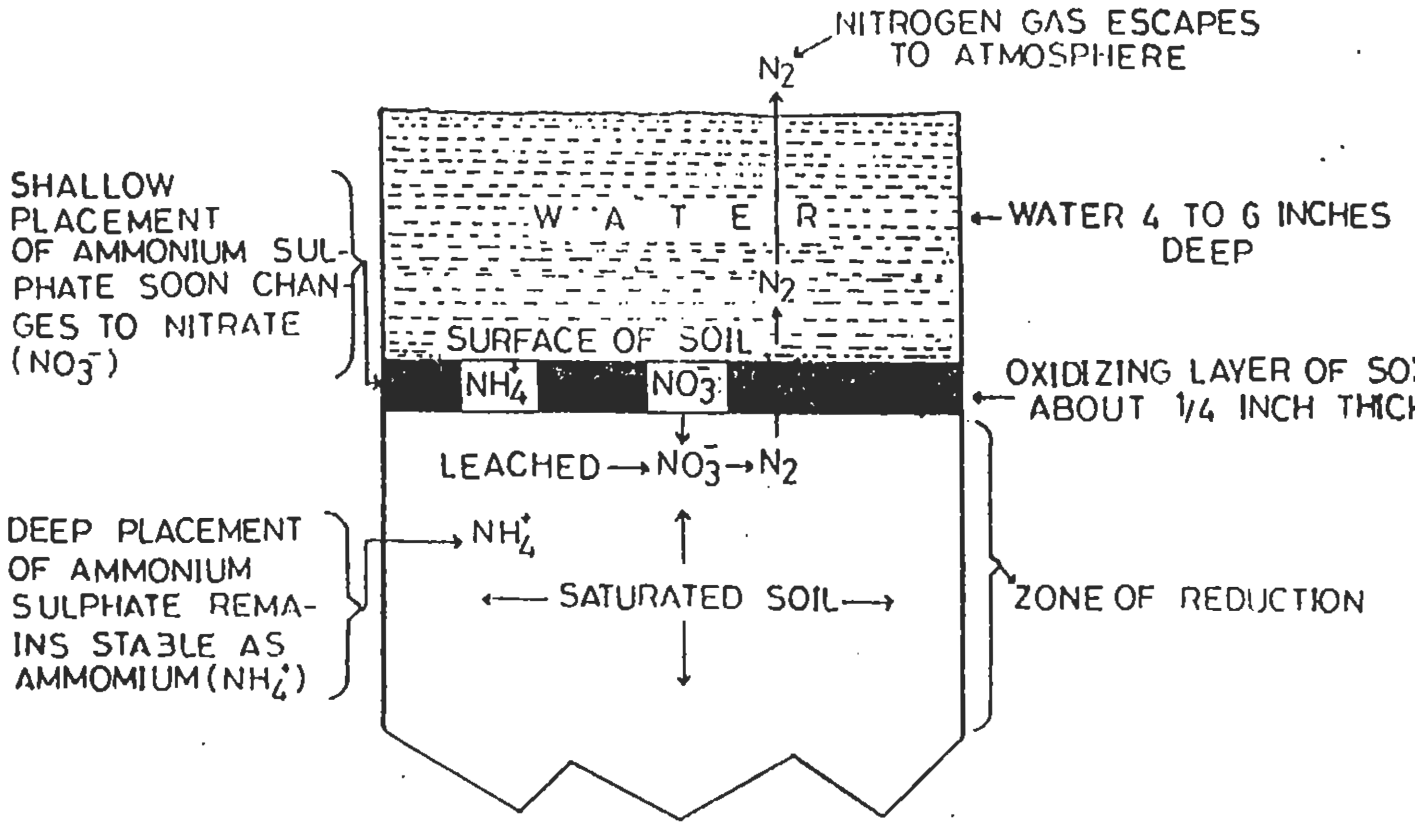


Fig :- 3 Schematic illustration of a paddy condition showing the shallow aerated zone of soil at the soil surface and deeper anaerobic zone.

Source: Patrick, W.H. Jr. & K.R. Reddy(1977) "Fertilizer Nitrogen Reaction in Flooded Soils" in SEFMIA, proceedings of the International seminar on Soil Environment and Fertility Management in Intensive Agriculture (Tokyo: The Society of the Science of Soil and Manure), pp. 275-281.

Control of nitrification of added ammoniacal fertilizers in soils has been receiving increased attention in recent years or enhancing fertilizer efficiency by minimising leaching/ volatilization of fertilizer N from the crop rhizosphere in the form of No3-N. Regulated nitrification also results in reducing nitrate pollution of surface and sub-surface water. For regulating nitrification , many chemicals have been tried which are either mixed with ammoniacal fertilizers or applied as coatings to the fertilizer granules. Most of these chemicals are imported and their high cost restricts their large scale use in this country. As a substitute, neem cake has been used for mixing with ammoniacal fertilizers.

REVIEW OF LITERATURE

Long before in indian agriculture when the chemical fertilizer was not introduced, the oil seed cake, particularly the peanut (Arachis hypogaea), Castor (Ricunus communis) and Mohwa (Bassia latifolia), were used as a source of plant nutrients for cotton and sugarcane crops. Chandra and Shrikhande (1955) reported that the neem cake contained 7.1% N and mineralized only to 61% while castor cake contained 4.6% Nitrogen and mineralized to 71% the lower nitrification of neem cake was due to the presence of neem bitters. Later it was detected that when neem cake was extracted with hot alcohol its nitrification increased to 55.6%.

The utility of neem products using seed kernal and it's extraction seed crush, deoiled cake, oil etc. as rewarding adjuvants nitrogenous fertilizers has been demonstrated in many field trials, these formost mechanism of nitrogen regulation is due to nitrification retardation. Korah and Singhate (1968) showed that neem cake released ammoniacal nitrogen and the extracted neem cake was a better nitrogenous manure than the unextracted cake. Mineralization studies of neem cake, groundnut cake nitrified maximum 68.2% followed by safflower cake 64.1% neem kernal cake 50.4% and neem seed cake 44.8% (Barde, 1970). Bains, et. al. (1971) was the first to show under field condition that treatment of urea with acetone extract of dried and crushed neem kernal well proven nitrification inhibitor and obtained a 22% increase in rice yield with a saving of 100 kg N/ha. Utilized N effectively for grain yield in IR 8 rice (Sarma, 1972). Urea 60 kg N/ha coated with neem extract at 0.5, 1.0 and 2 kg increased the grain yield of wheat over the control (Khandelwal et. al. 1977). Application of 100 kg urea + 33 kg urea neem cake to rice crop higher yield of grain as compared to 100 kg urea alone (Katti, et. al. 1976). Neem cake blended urea applied to rice variety java gave higher yield than untreated urea, (Oommen, et.al 1977). In the rice crop variety (triceni) split application and complete basal application of neem cake coated urea were superior to application of complete basal urea, (Abraham, et.al 1976).

Mishra, et. al. (1972) studied decomposition of organic matter (Susbania and Bajra) stubble plus neem cake, applied at the rate of 0.5% soil pH 7.8 incubated at 30°C. (Ketkar, 1974) found that application of urea with neem cake increased rice yield by about 400 kg/ha. Ketkar (1976) also reported the advantage of applying neem cake along with urea in sugarcane field. Sharma and Prasad (1980) observed that finely powdered neem cake mixed at 20% of urea increased recovery of urea N by the rice crop from 28% to 47%. Ketkar (1983) carried on agronomic studies on neem cake varying in oil content from 2.4 to 18.7% N alongwith coaltar as an adhesive binding material, gave promising results in terms of yield and efficient use of N fertilizer in rice and sugarcane. Grant, et. al. (1983) assayed crushed neem seeds and neem cake for N analysis and found 2.14 and 2.5% N, respectively by microkjeldahl method. In a comparison of the nitrification rates of neem seed crush - coated and plastic - coated urea found a lower rate in the former (Chhonkar, 1984). Bhatia, et. al. (1985) failed to obtain any significant increase in grain yield of wheat when urea was combined with neem cake as compared with urea alone, however drilling of Nitrogen plus neem cake at a rate of 120 Kg/ha led to a higher uptake of N than did broadcast application. Surve and Daftardar (1985) employed urea blended with neem cake, deoiled neem cake, neem oil, neem oil extractive and Karanja cake and found that all the neem products were superior to Karanja cake in their effect on grain production in rice. Prakasa and Singh (1985) reported that the neem cake - coated urea was effective only at higher levels of N. i.e 300 and 400Kg/ha/year.

In view of the research done by various workers the present study has been planned to evaluate the amount of ammoniacal nitrogen released by different forms of neem cake, neem seed powder, and neem oil, amended in paddy field soil.

MATERIALS

PREPARATION OF TEST MATERIAL:

The dropped dry neem fruits were collected by sweeping under neem trees in the year 1989 from the Karachi University campus during July, brought to laboratory washed, shade dried and ground to a fine powder (30 mesh) on an electric/mechanical grinder. This powder was named as Neem seed powder.

PREPARATION OF NEEM CAKE FROM NEEM SEED POWDER:

i) SOXHLETS EXTRACTION:

The oil from neem seed powder was extracted with n-hexane (40-60°C) in a soxhlets extraction apparatus for 8 hrs. The extract thus collected was concentrated on rotary evaporator at 30°C to get the neem oil. The residue remained in the thimble was collected and named as Neem cake (n-hexane extracted unground) (Photograph-1).

ii) GROUND NEEM CAKE:

This neem cake was further ground to a fine powder and named as ground neem cake.

iii) **WATER EXTRACTION:**

Neem seed powder was soaked in tap water for 12-16 hrs and taking out the water extract by passing through a piece of muslin cloth the residue thus collected on the muslin cloth was named as Neem cake (water extracted). (Photograph-2).

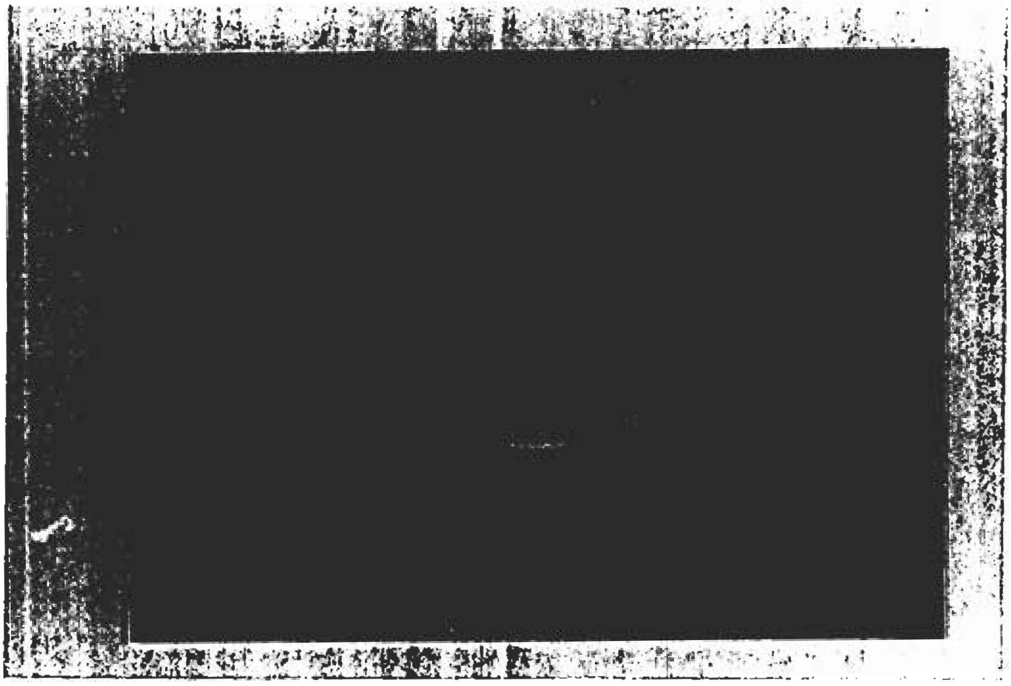
iv) **EXPELLER EXTRACTED:**

The oil from Neem seed was extracted by the commercially available expellers in the market. The residue thus collected from the expeller after the extraction of oil was named as Neem cake (expeller extracted) (Photograph-3)

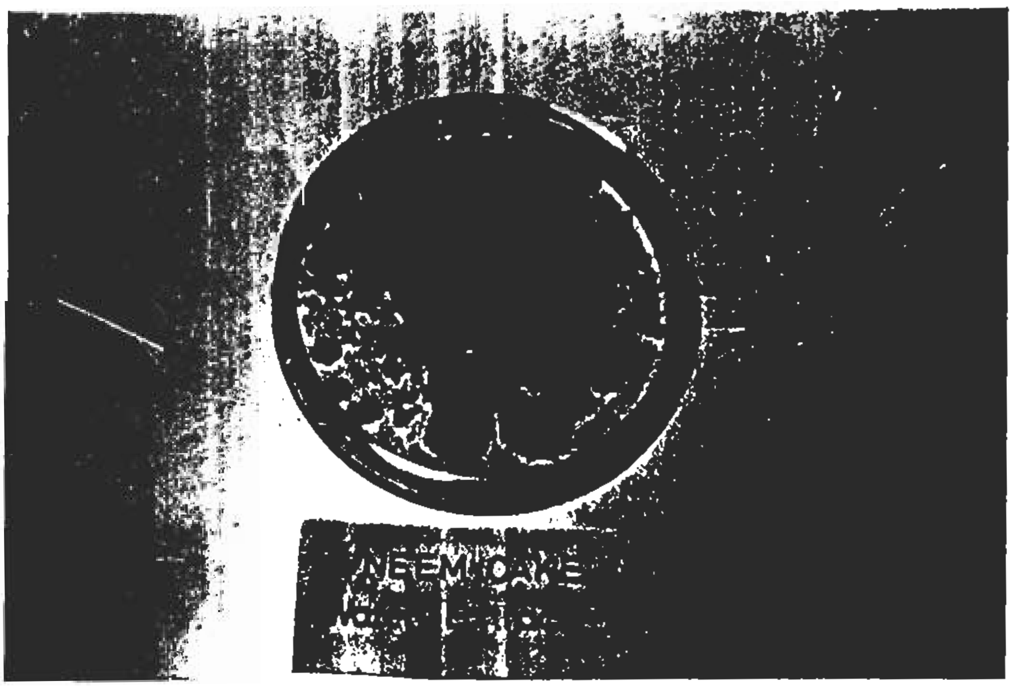
v) **EXPELLER COLLECTED NEEM CAKE EXTRACTED BY SOXHLET EXTRACTION:**

The neem cake mentioned at (iv) was dark brown nearly black in colour which showed the presence of oil was ground and extracted further with n-hexane by soxhlet extraction. The residue remained was also named as Neem cake (Expeller-n-hexane extracted)(Photograph-4).

PHOTOGRAPH-1

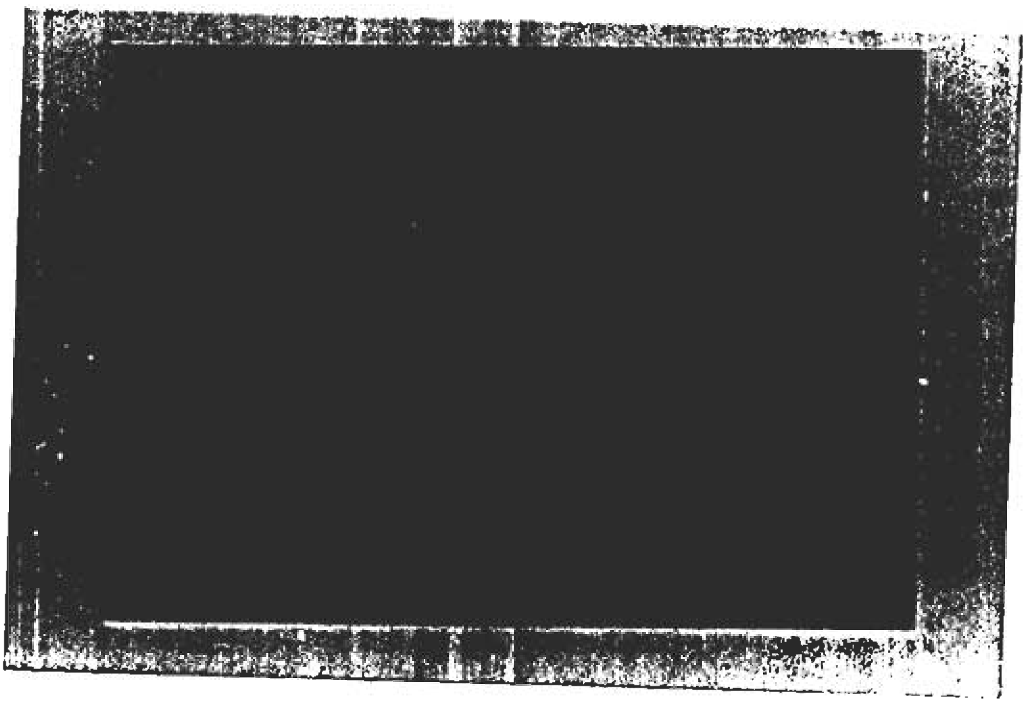


PHOTOGRAPH-2



PHOTOGRAPH-3

PHOTOGRAPH-4



The different types of neem cake used as test material for the evaluation of ammoniacal N release were named symbolically in the experiment as:

NC1 :- Neem cake (n-hexane extracted) Unground.

NC2 :- Neem cake (n-hexane extracted) ground.

NC3 :- Neem cake (water extracted).

NC4 :- Neem cake (Expeller extracted).

NC5 :- Neem cake (Expeller neem cake extracted further by n-hexane).

In addition to the above materials the neem seed powder and Neem oil were also used as test material in this experiment.

NSP:- Neem Seed Powder.

NO:- Neem Oil.

METHODS

1. INCORPORATION OF SOIL WITH NEEM CAKE, NEEM SEED POWDER AND NEEM OIL:

The five different types of neem cake and neem seed powder were incorporated manually at the rate of 1, 5 & 10% in 50 gm w/w in soil collected from paddy field (Analysis report of soil is given in Table 3). Neem oil was applied at the concentration of 0.1, 0.5 and 1%w/w per 50 gram soil in glass test tubes. After the treatment the tubes were plugged with cotton wool and kept at room temperature. Moisture content of the treated and untreated soil samples was maintained by adding distilled water. All the treatments were run in triplicate.

2. DETERMINATION OF NH4-N AND NO3-N+NO2-N IN SOIL WITH NEEM CAKES, NEEM SEED POWDER AND NEEM OIL:

Ammonium nitrogen (NH4-N) liberated in soil due to the incorporation of different types of neem cake, neem seed powder and neem oil was determined at zero hour and after 1,5,10,15,30 and 60 days interval using steam distillation microkjeldahl method (Black, 1965). Similarly the nitrate and nitrite (No.3 + No.2-N) was also determined by the same method.

TABLE-3

Soil Analysis Report

1.	Texture		Loamy clay
2.	Particle size fraction		
	a.	Sand	33.8%
	b.	Silt	28.4%
	c.	Clay	25.8%
3.	Total N		0.045% (NH_4+NO_3 = Total N)
4.	Total P		7.0 PPM
5.	Total K		70.0 PPM
6.	Organic matter		0.8%
7.	pH		8.5%
8.	Moisture holding capacity		Generally good
9.	EC of saturation extract		2-3 ds/m

Data obtained on the liberation of NH4-N and No3+No2-N at different intervals was subjected to factorial analysis of veriance (FANOVA) by completely randomized design procedure (Gomez and Gomez, 1984). The follow-up of (FANOVA) consisted of Duncans multiple range test for the comparison of pair-wise means, the test was performed at the probability level of 0.05. The analysis was accomplished using the COSTAT package.

3. INCORPORATION OF SOIL WITH NEEM CAKE AND INSECTICIDES (CARBOFURAN, DIAZINON AND THIODAN):

The three insecticides (Carbofuran 3G, Diazinon 10G and Thiodan 35EC) were added to soil (prior treated with neem cake) at the recommended rate separately (750g(a.i)/ha, 17.5Kg/ha, 1Kg/ha) in triplicate. The flask containing the soil, Neem Cake and Insecticide were incubated at room temperature for different time periods. This experiment was done to observe any adverse effect on N release from neem cake in the presence of these insecticides.

A Zero hour reading was also observed before incubation. After 1,5,10,15,30 and 60 days incubation intervals NH_4-N & NO_{3+} NO_2-N released was determined by steam distillation micro kjeldahl method as mentioned in section-2.

4. CHEMICAL ANALYSIS OF NEEM CAKE FOR DETECTION OF NPK:

Neem cake (n-hexane extracted and Expeller extracted was amended in soil(paddy field)@ 1%w/w. The soil analysis for NPK was done at soil testing laboratory, NARC. The report reveals that the difference amongst the two type of neem cakes in respect of NH4-N is remarkable whereas Phosphorous and Potassium were slightly different. As compared to blank both the neem cakes are rich in NPK (Table-4).

TABLE-4

Chemical analysis of paddy field soil amended with 1% Neem Cake

Sl. No	Type	(N) PPM NH_4+NO_3-N	(P) PPM Phosphorous	(K) PPM Potassium
A	Soil (Blank)	9.2	10.2	68
B	Soil amended with Neem Cake (the cake collected after n-hexane extraction)	15.3 (6.1)	20.0 (9.8)	294 (226)
C	Soil amended with Neem Cake (the cake collected after expeller extraction)	12.8 (3.6)	18.2 (8.0)	314 (246)

Remarks: The figures in paranthesis are the difference amongst the two types of Neem Cake. NH_4-N as compared to blank is remarkable whereas for P and K there is slight difference.

RESULTS AND DISCUSSION:

Ammonium nitrogen and Nitrate+Nitrite-nitrogen production in soil amended with neem cakes, neem seed powder and neem oil, calculated by applying equivalence 1 ml of 0.01 N Hcl=140 ug N (Lab. Manual, 1983) is presented in table-5 and 6.

It was observed that neem cake prepared by n-hexane extraction (Unground and ground) produced more ammonium nitrogen than that by water and expeller extracted neem cake. Unground neem cake produced more ammonium nitrogen than the ground cake as the release 8N in the soil was delayed. Conversely ground cake although produced comparatively less amount of nitrogen yet its release was faster (table-5).

Maximum nitrogen production in unground cake @ 1, 5 & 10% concentration was after 30, 15 and 30 days of treatment, respectively. In the ground cake maximum nitrogen production was after 10,30 and 15 days at the respective concentration. A similar trend was observed in the production of Nitrate + Nitrite Nitrogen by ground neem cake (n-hexane extracted) and unground (water extracted) (table-5).

TABLE-5

AMMONIUM NITROGEN, NITRATE NITRATE NITROGEN PRODUCED AT DIFFERENT INTERVAL BY THE SOIL SUPPLEMENTED WITH DIFFERENT FORMS OF NEEM CAKE

Forms of neem cake	Rate of application (% w/w)	NH_4-N (ug) at indicated days					NH_3-NO_2-N (ug) at indicated days				
		0	10	15	30	60	0	10	15	30	60
NC1	1	42	42	56	126	42	28	28	28	70	28
	5	70	280	210	140	70	42	42	84	98	56
	10	112	420	462	450	140	42	56	154	168	70
NC2	1	42	84	84	70	42	28	42	42	42	28
	5	42	168	218	140	70	28	84	98	98	70
	10	56	238	280	280	140	28	140	182	196	84
NC3	1	42	42	42	42	42	28	28	28	28	28
	5	42	42	42	70	84	28	28	28	42	84
	10	42	42	42	70	140	28	28	28	42	98
NC4	1	42	42	56	56	70	28	28	28	28	28
	5	42	56	70	70	84	28	28	28	42	84
	10	56	70	70	84	126	28	28	28	42	98
NC5	1	42	42	56	56	42	28	28	28	28	28
	5	42	56	70	112	84	28	28	28	42	42
	10	56	70	112	140	140	28	28	28	42	42
CONTROL	0	42	42	42	42	42	28	28	28	28	28

KEY:

NC1 = Neem Cake (n-hexane extracted unground)
NC2 = Neem Cake (n-hexane extracted ground)
NC3 = Neem Cake (Water extracted unground)
NC4 = Neem Cake (Expeller extracted)
NC5 = Neem Cake (Expeller, extracted further with n-hexane)

TABLE-6

AMMONIUM NITROGEN PRODUCED AT DIFFERENT TIME INTERVAL BY THE SOIL SUPPLEMENTED WITH NEEM SEED POWDER AND NEEM OIL

Material	Rate of Application (% w/w)	Ammonium Nitrogen (ug) at Indicated days					
		0	05	10	15	30	60
NSP	1	42	42	42	42	42	42
	5	42	42	42	57	70	84
	10	42	42	56	196	210	180
NO	0.1	42	42	42	42	42	42
	0.5	42	42	42	42	42	42
	1.0	42	42	42	42	42	42
CONTROL	0	42	42	42	42	42	42

KEY:

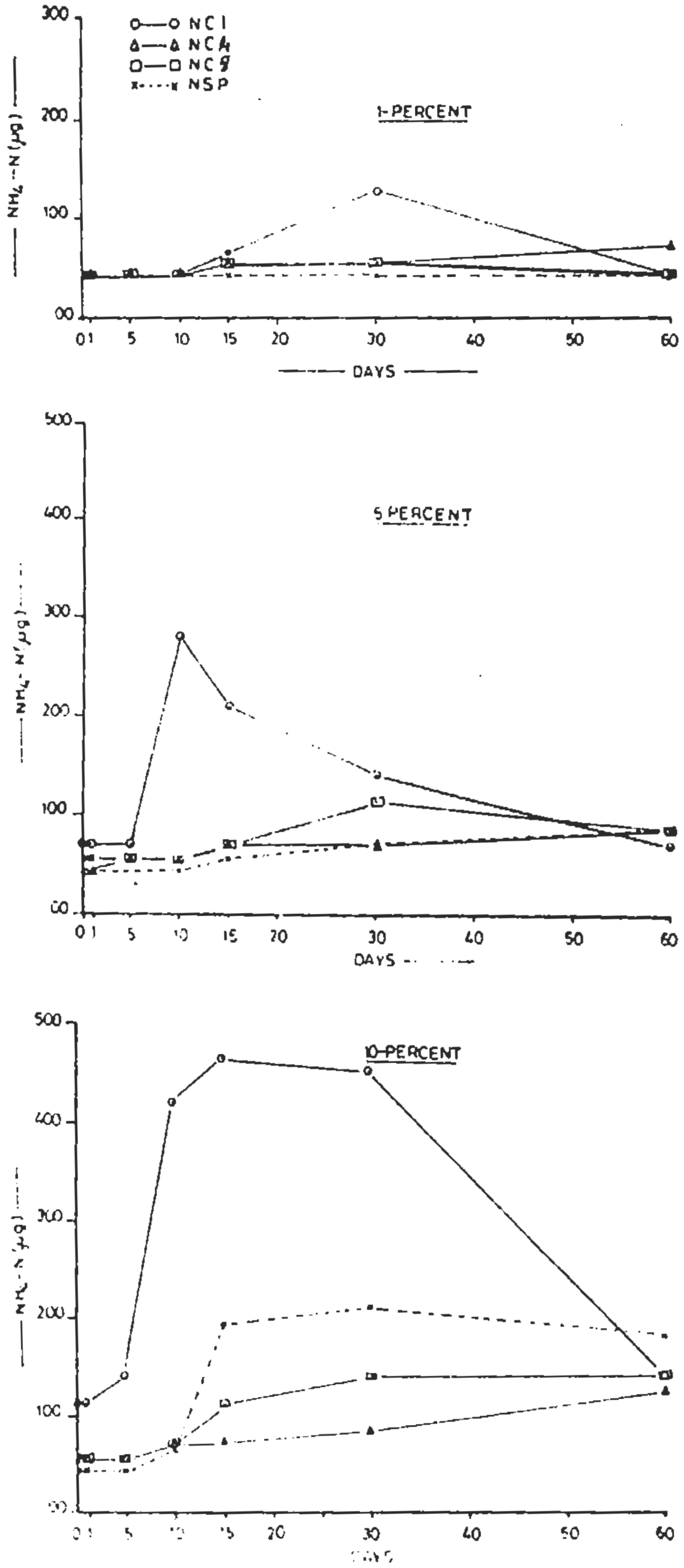
NSP = Neem seed powder.
NO = Neem Oil.

Treatment were found to be significantly different tested as 0.05 probability level (see appendix-A).

Soil supplemented with neem seed powder did show some increase in Ammonia nitrogen production but at higher concentration (Table-6), the production of nitrogen was delayed upto 60 days. Neem seed oil did not help in nitrogen production (Table-6). The neem seed kernal powder as such or extracted with water having more oil did not result in the faster production of nitrogen. Similarly the Expeller extracted neem cake did not showed much increased in ammonia nitrogen production. The expeller neem cake extracted further with n-hexane showed increase after 15 and 30 days. A comparison of liberation of NH_4-N from soil amended with 1,5 & 10% of NC1, NC4 and NC5 along-with neem seed powder (NSP)is presented graphically (Figure-4).

This indicated that neem oil if totally or partially present in the neem cake preparation causes inhibition and delayed production of nitrogen in the soil. The unground form delayed nitrogen production as compared with the ground form in which oil was further removed by extraction which correlates with the findings of (Sinha and Gullati 1968) that neem cake is the better nitrogenous manure than the unextracted cake. Neem cake released highest amount of ammoniacal nitrogen the rate of ammonification increased upto 30 days (Korah and Singhate 1968).

Fig. 4 : Liberation of NH_4-N from soil amended with different types/percentages of Neem Cakes



Key: NCI = Neem cake n-hexane extracted.
NC4 = Neem cake expeller extracted.
NC9 = Neem cake expeller extracted further with n-hexane.

CONCLUSIONS:

Following conclusions can be drawn from preceeding studies:

1. The above observations lead us to a conclusion for the maximum and total removal of oil before using the neem cake as supplement for nitrogen production.

2. This means that maximum active components are taken out from neem seed powder, the residue becomes more useful as a source of nitrogen.

3. Present studies demonstrated that neem cake (n-hexane extracted) is a promising source of nitrogen in the treated soil.

4. The effectiveness of neem cake can be improved through increasing its application rate or ensuring its use soon after oil extraction.

INTERACTION OF NEEM CAKE WITH BENEFICIAL SOIL MICROBES

EFFECT OF NEEM CAKE/FERTILIZERS/INSECTICIDES ON SYMBIOTIC, NON SYMBIOTIC NITROGEN FIXING MICROBES, NITRIFYING BACTERIA & TOTA SOIL MICROBIAL BIOMASS:

Abstract

Neem cake amendment in soil at 1-3%w/w had no adverse effec on the growth of symbiotic (four Rhizobium species viz., R japonicun, R. leguninosarum, R. phaseoli and R. fredii) and non symbiotic free living nitrogen fixers bacteria viz., Pseudomona diazotrophicus, Klebsiella planticola and Enterobacter cloacae Neem cake - (expeller extracted), neither inhibited no stimulated the growth of Rhizobium species except for Rhizobiu fredii, whose growth was slightly retarded. The fertilizers viz. urea, NPK and DAP & insecticides viz., Carbofuron, Diazinon an Endosulfan tested at the recommended dose and ten times highe the recommended dose also had no adverse effect on these bacteria.

INTRODUCTION

Flooded rice fields have a wide range of macro and microenvironment. Because the environments differ in redox potentials, physical properties, light status and nutrient sources, they support all kinds of N2-fixing organisms. N2 fixin agents in soil and water are natural "fertilizer factories", promoting their growth and N2 fixing activity which is an important strategy for sustaining rice production. The quantity of N2 fixed in rice field by these agents has been estimated with various levels of accuracy (table-7) and according to data the biological fixation in rice field for nitrogen balance on earth is 135 ha x 10(Area) 30 Kg/ha N2 fixed or 4 metric tons ha x 10 based on (Delwiche, 1970, Hardy and Holsten, 1972). The phototrophic or autotrophic organisms grow at the surface of the soil and in the flood water. Heterotrophic N2- fixing bacteria grow in the soil in association with crop residues or living rice roots.Some live in symbiosis with legume plants that can grow in flood condition, due to this reason rice plants obtain 60-70% of their N from the soil. Therefore crop intensification

TABLE - 7

RANGE OF ESTIMATES OF N, FIXED BY VARIOUS AGENTS IN WETLAND RIC FIELDS AND THEORETICAL MAXIMUM POTENTIAL AND ASSUMPTIONS (AFTER ROGER AND LADHA, 1990)

COMPONENT	REPORTED RANGE OF ESTIMATES (Kg N/ha PER CROP)	THEORETICAL MAXIMUM POTENTIAL (Kg N/ha PER CROP) AND ASSUMPTIONS
BNF associated with Rice Rhizosphere	1 - 7	40 All Rhizospheric bacteria are N, fixer. C flow through Rhizosphere is 1 t/ha per crop. 40 mg N is fixed/g C.
BNF associated with straw	2-4/Kg N/t straw	35 5 t of straw is applied 7 mg N is fixed/g of straw.
Total heterotrophic BNF	1 - 31	60 All C input (2 t/crop) is used by N fixers.
Blue-green algae	0 - 80	70 Photosynthetic aquatic biomass is composed exclusively of N - fixing BGA (C/N=7). Primary production is 0.5 t C/ha per crop.
Azolla	20 - 150 (experimental plots)	225 One Azolla standing crop. is 140 kg N/ha.
	1 - 50 (field trials)	Two Azolla crops are grown per rice crops Ndfa is 80%
Legume/green manures	20 - 260	260 (55 d) Sesbania rostrate is used as green manure. 290 kg N/ha accumulated in 50-60 d. Ndfa is 90%

Ndfa = N derived from the atmosphere

may affect rice fertility if proper N imports do not replenish N taken up from the soil. The replenishment can be attained by:

a) increased biological N source

b) decreased N loss by proper N application

c) increased chemical fertilizers

d) increased N by other sources.

The increased use of nitrogenous fertilizers to enhance crop production has become a major and economically essential part of modern agricultural practices; some studies have shown that the chemical fertilizer interact with soil microbial communities in various ways and consequently influence the normal functioning of the ecosystem (Lundergardh, 1927, Katznelson and Montegut 1960, Guillemat and Montegut 1960 and Leuken, et. al. 1962 and Jong, et. al. 1974).

LITERATURE REVIEW

1. EFFECTS OF VARIOUS FERTILIZERS:

The effect of a combination of agrochemical studies on nitrogen fixation was either decreased or unaffected at high level of urea 80 ug/g and increased at low level of urea 20 ug/g (Jena et.al. 1990). In a laboratory investigation soil samples of fallow land treated with 490 mg/ litre NH_4NO_3 and 58 mg/litre K_2HPO_4 over a period of 340 days showed that the bacterial growth peaked at 20 days in a fertilizer variant. A more active heterotrophic link was observed where mineral nutrients were available, the autotrophic link was more active where supply was limited (Grossman and Russ 1990). The effect of 6.2 t/h vetch (Vicia sativa) and 7.1 t/h rape (Brassica rapus) biomass incorporated as green manure enhanced de-nitrification in a calcareous soil with maize crop (Lehn-Reiser, et. al. 1990). It was studied that prolonged application of fertilizers (135 kg N+90Kg P+90Kg K/ha) and higher rates changed the microbiological population where as manure applied at 40t/ha together with low rates of mineral fertilizer and organic fertilizers had a positive effect on the balance of soil microbiological processes and humus (MerenJuck et.al. 1989).

Continuous application of high rates of nitrogen fertilizer 240-480 kg/ha resulted in a marked deterioration in the biological properties of the soil and decreased the total number of microflora. This unfavourable effect was lessened when fertilization was accompanied by the ploughing in of post harvest residues (Voinova-Raikova 1981).

2. **EFFECTS OF OTHER PLANT RESIDUES, STRAW ETC ALONGWITH NEEM CAKE:**

The experiments with some plant residues (Maize straw or roots, barley straw or roots, potato leaves and sugarbeet leaves) and a commercial humic acid product were added to a silt loam soil at a rate of 0.2%w/w showed that the evolution of NH4 was most pronounced under water logged condition with rice straw and no change with maize roots and humic acid treatment (Cleemput et.al. 1990). Wheat straw and neem cake applied to an alluvial soil at the rate of 1.0, 0.5 and 0.25%w/w showed that the total bacterial and non-symbiotic nitrogen fixing bacterial population increased with wheat straw and reduced by neem cake. But the ammonifying bacteria increased with both the treatments. (Da and Mukherjee 1990).

Addition of straw to paddy soil decreased de-nitrificatic to 2-3% from 20% . (Iimura and Ito 1990). Data on the effect of fresh tree bark, (0, 25, 50, 100, 200 t/ha) were shown to improv soil physical properties at low level (25t/ha) (Rusanova, et. al 1989).

The rate of decomposition of wheat straw in soils treate with urea solution increase the nitrogen level in soil is one o the factors limiting the decomposition process (Charaya et. al 1989). In a 3 years examination findings showed that the wood as amendment increased microbial activity resulting in increase mineralization and N availability. (Weber et. al. 1985).

3.EFFECTS OF DIFFERENT PESTICIDES ALONE AND IN COMBINATION WIT FERTILIZERS:

Generally the organochlorine & organophosphate insecticide did not have adverse effect on nitrification. (Martin and Anderson 1959, Ross 1974). In a submerged alluvial soil, application of carbofuran (4ug/g & butachlor (2ug/g), stimulatec nitrogen fixation in the presence of low and high levels of urea

(Jena, et. al. 1990). At normal rates but higher rates sho adverse effect, as noted that application of Carbofuran at 2 & ppm did not cause much variation in the N conversion but at 1 ppm Nitrobacter Sp. was more sensitive than the Nitrosomonas Sp (Palaniappan and Balasubramanian 1986). Studies showed that carbofuran and diazinon (commercial formulation) alongwith other insecticides did not inhibit Pseudumonas Sp at a rate of 50 ug/ml and Nitrosomonas and Nitrobacter Sp at a rate of 10 ug/ml in pure culture (Ramakrishna and Sethunathan 1983). The application of Furadan at 7.5 ug/ml inhibited Nitrosomonas in pure culture (Kukreja, et. al. 1987). In a nitrification study it was found that commercial endosulfan was more toxic than the technical material towards nitrification activity in a clay loam soil was very slow only 5% of the added NH4 was converted into NO3 with in 70 days (Stratton 1990). Endosulfan was toxic to all the S-oxidizing bacterial isolates (Bezborush et.al. 1990).

The availability of NH4-N, No3-N and P and K was more with Carbofuran applied at 30 or 60 days after transplanting rice (Jadhav and Kadrekar 1984). In carbofuran treated soil an initial bacteriostatic influence followed by subsequent stimulatory effect on *Azotobacter* population and continuous increase i *Pseudomonas* population (Nain et. al. 1984). Study with various pesticides reveals that carbofuran @ 10 kg/ha inhibited the growth of *Rhizobium leguminosarum* (Raddy et. al. 1986) Carbofuran 15 and 30 ppm stimulated rhizobial growth compare with control (Joshi and Kulkarmi 1987).

The insecticides Diazinon and Endosulfan @ 7.5 g/litre d not have harmful effect on nitrogen fixation in *P. vulgaris*. (Michael et. al. 1990). Treatment of red clover in lab or fiel experiments with Basudin (2.5 kg/ha) stimulated N fixation 2-fold (Gukov 1983). Carbofuran along with urea increased th uptake of N by rice (Sathasivam et. al. 1982). Higher yield wa obtained by endosulfan 0.07% spray in combination with fertilize at 25 kg N and 50 kg P205/ha (Sundaraju and Ramgarajan 1987).

Working on different urea insecticide combination involvin Carbofuran, Diazinon and Thiodan along with other insecticide for rice Biddappa et. al. (1980) observed that combine application of urea with insecticides had no deleterious effec on urea transformation in the soil. Sadasivan (1980) observe that the combined application of urea and Carbofuran in pape packets by root zone placement was very effective in enhancin the rice grain yield.

4. EFFECTS OF NEEM CAKE ALONE AND WITH FERTILIZER COMBINATION:

Working on urea mixed neem cake Sarma (1972) found that th grain yield was more when urea was mixed with neem cake at 10 kg N/ha than urea alone. Khandelwal et. al. (1977) reported tha urea 60 kg N/ha coated with neem extract at 0.5,1.0 & 2.0 k increased the grain and straw yield of wheat over the control. Application of 100 kg N as urea + 33 kg neem cake / ha gav higher grain yield than the application of urea alone (Katti et. al. 1976). Residues of neem cake increased the plant yield at all level of application tested (Sharma et. al.1981).

Attri et. al. (1981) suggested the possibility of using neem cake coated urea for increasing the N use efficiency of rice. Saxena (1984) reported the effect of neem cake application with urea 2:10 gave significantly higher yield than control plots in both dry and wet cropping season and due to its fertilizing effect gave increase in rice yield upto 40%. The suggestion of possibility of using neem cake coated urea alongwith other materials were tried by Muni-Ram et. al. (1989)

in a field experiment with four N carrier (Neem cake coat urea, urea super granules, lac coated urea and prilled urea applied at 2 rates of N (60 & 120 kg/ha). The best results we obtained with neem cake coated urea, prilled urea being th least effective. Application of 120 kg N/ha gave better resul than 60 kg N/ha. In an another field experiment conducted in clay loam soil, blending of urea with neem cake and foli feeding saved 50 kg N/ha (Jayable and Chockalingam 1990).

Laboratory experiments showed that neem cake reduced th rate of nitrification in soil for 3 weeks at 0.4 % and 4 weeks at 1.0%w/w the population of nitrite forming bacteria was reduced accordingly whereas nitrate forming bacteria wer unaffected (Mishra et. al. 1975). The NO3 formation was reduce to half the control value when ethanolic extract of neem see cake was added to soil (Sahrawat and Parmer 1975). It was als reported that neem cake and alcoholic extracts of neem seed slows down the nitrification rates (Mishra and Choonker, 1978 an Reddy and Prasad, 1975). Later on Mishra (1976) found that ure mixed with neem cake powder or coated with hot alcohol extract of neem cake significantly inhibited the growth of Nitrosomonas

Keeping in view, the properties of neem cake mentioned in the literature review, present study was designed to investigate the effect of Neem cakes, Insecticides (Carbofuran 3G, Basudin 10G and Thiodan 35EC) and chemical fertilizers (NPK, DAP, and Urea) on the growth of Nitrogen fixing bacteria (Symbiotic and Non-symbiotic free living N2 fixers of rice Rhizosphere), Nitrifying bacteria (Nitrobacter & Nitrosomonas) and total soil microbial biomass, in-vitro and in-situ.

MATERIALS

A. CULTURES:

Lyophilized strains of Rhizobium species viz; *R. japonicum*, R. fredii, R. leguminosarum, R. phaseoli were received from USDA Beltsville Rhizobium culture collection. Agricultural research services ARS-60, USA and free living N_2 fixing bacteria viz Pseudomonas diazotrophicus *IRBG 183 & 184*, Klebsiella planticola *IRBG 185 and* Enterobacter cloacae *IRBG 312* (Associated with Rice Rhizosphere) from Bacterial Germ plasm Resources, Soil Microbiology Division IRRI Los Banos, Laguna, Philippines.

MAINTENANCE AND TRANSFER OF LYOPHILIZED BACTERIAL STRAINS:

The lyophilized cultures of four Rhizobium species and three strains from Rice Rhizosphere free living N_2 fixers were transferred to Nutrient Broth and Nutrient Agar slants. The Rhizobium strains were first transferred to Yeast Mannitol Broth. The cultures were regularly transferred on fresh media after 15 days interval and kept in the refrigerator ($8^{\circ}C$) for the purpose of inoculum used for the experiments from time to time.

B. INSECTICIDES:

The following formulated insecticides were used:

a) Carbofuran (formulation as Furadan granules with 3 % a.i.). M/s. Agricide (PVT.) Ltd.

b) Diazinon (formulation as Basudin granules with 10% a.i.). M/s. Agrochemical (PVT.) Ltd.

c) Endosulfan (Formulation as Thiodan emulsifiable concentrate with 35% a.i.). M/s. National Pesticide Corporation.

C. FERTILIZERS:

Following three fertilizers were purchased from local market:

a) NPK (nitrogen, phosphorous and potassium) (13:13:21).

b) DAP (Diammonium Phosphate) (18-46 D).

c) Urea (46% N.).

D. MEDIA:

The following media were prepared using compositions mentioned against each. The media were heated and autoclaved before use.

NAME OF MEDIUM	COMPOSITION IN G/LITRE OF WATER	
a) Nutrient Agar	Lab-lemco powder	1.0 g
(Oxoid CM3)	yeast extract	2.0 g
	Peptone	5.0 g
	Sodium chloride	5.0 g
	Agar	15.0 g
	pH	7.4
b) Nutrient Broth	Lab- lemco powder	1.0 g
(Oxoid CMI)	Yeast extract	2.0 g
	Peptone	5.0 g
	Sodium chloride	5.0 g
	pH	7.4
c) Yeast Mannitol Broth	Mannitol	10.0 g
(Oxoid CMI)	Yeast extract	1.0 g
	$K_2 H Po_4$	0.5 g
	$Mg So_4.7H_2O$	0.2 g
	Nacl	0.1 g
d) Ammonium-calcium carbonate	$(NH4)_2 So_4$	0.5 g
medium for Nitrosomonas	K_2HPo_4	1.0 g
	$Fe So_4. 7H_2O$	0.03 g
	Nacl	0.3 g
	$Mg So_4.7H_2O$	0.3 g
	$CaCo_3$	7.5 g

e) Nitrite-calcium carbonate medium for Nitrobacter	KNO_2	0.006 g
	K_2HPO_4	1.0 g
	Nacl	0.3 g
	$Mg\ SO_4.7H_2O$	0.1 g
	$FeSO_4.7H_2O$	0.03 g
	$Ca\ Co_3$	1.0 g
	$Cacl_2$	0.3 g

E. **INSTRUMENTS:** The following instruments were used:

Colorimeter Spectronic 20 A.Schimadzu Bausch and Lomb.

Colony Counter.

Autoclave.

Balance (Electric)

Hot plate.

Microscope.

F **GLASSWARES:** The following glasswares wer used:

Conical flasks 1 litre, 250 ml, 100 ml.

Test tubes. (15 cms length, 2 cms diameter).

Petri dishes

Volumetric flasks, 10 ml, 25 ml.

Spirit lamp.

Pipette 5 ml, 10 ml.

Microsyringe: 100 microlitre

Glass tube size : 20 cms length, 2.9 cms diameter.

CONVERSION OF QUOTED RATE OF COMMERCIALLY FORMULATED PESTICIDES INTO PARTS PER MILLION

Conversion from Kg or litre ha^{-1} have been based upon 10^{8} grams of soil ha^{-1} to a depth of 1 cms assuming an average bulk density for soil of 1.0. Thus the recommended dose of 1 Kg/ha is equivalent to 10 ppm (Anderson, 1978).

For preparing different doses of pesticides/Neem cake and fertilizer, the following concentration were prepared.

I. PESTICIDES:

S.No.	FORMULATED PESTICIDES	CONCENTRATION EQUIVALENT TO (RECOMMENDED-DOSE)
a)	Furadan 3G	750 g a.i /ha (RD) 25mg /100 ml media/Soil
b)	Basudin 10G	9 kg/Acre (RD) 20 mg / 100 ml media/Soil
c)	Thiodan 35 EC	1 kg/ha (RD) 1 mg/100 ml media / Soil

II. NEEM CAKE:

S.No	TYPE OF NEEM CAKES	CONCENTRATION USED IN SOIL/MEDIUM
a)	n-hexane Extracted	0.1, 0.5 & 1.0, 2.0 & 3.0%w/w in soil and w/v in medium.
b)	Expeller Extracted	-do-

III. FERTILIZERS AND THEIR CONCENTRATION USED:

S.No.	FERTILIZERS	CONCENTRATION EQUIVALENT TO (RECOMMENDED DOSE
a)	NPK (13:13:21)	120 Kg/Acre(RD)= 3mg g^{-1}
b)	DAP (18:46-D)	37.5 Kg /Acre (RD) 9ug g^{-1}
c)	Urea (46 % N)	120 Kg / ha (RD)120ug g^{-1}

METHODS

A. EFFECT OF NEEM CAKE/FERTILIZERS ON NITROGEN FIXING SYMBIOTIC BACTERIAL STUDY

NEEM CAKE/FERTILIZERS FORTIFICATION-IN VITRO

The two types of neem cake i.e the n-hexane extracted an expeller extracted were added at the rate of 0.1, 0.5 and 1.0%w and the fertilizers at recommended dose mentioned at sl.No.III the medium 30ml Yest Extract Mannitol Broth(YEMB) and 30 nutrient Broth, separately. The YEMB tubes were inoculated with all the four Rhizobium strains (R. japonicum, R. leguminosaru R. phaseoli and R. fredii) and the NB with Ps. diazotrophicus Encloacae and Kl. planticola. The inoculum used was 24 hrs. ol culture and 6 drops/tube was used for inoculation. One set control medium without any inoculation was also kept. All th experiments was run in triplicate. The tubes were incubated (fo 24 hrs. at room temperature 30^{o}-32^{o}C). At different time interva starting from Zero hrs & after (1,2,3,4, and 5 days) treatment, ml aliquot sample from each treatment was drawn aseptically ar microbial growth monitored on colorimeter by measuring th optical density at 560nm.

Data on optical density was subjected to factorial analysi of variance (FANOVA) by completely randomized design procedur (Gomez and Gomez, 1984). The followup of (FANOVA) consisted c Duncan's multiple range test for the comparison of pair-wis means, the test was performed at the probability level of 0.05 The analysis was accomplished using the AMSTAT package.

II. EFFECT OF NEEM CAKES/FERTILIZERS ON NON-SYMBIOTIC NITROGEN FIXING BACTERIA.

Neem cake/fertilizer fortification - in situ:

Twenty four hour old cultures (2 ml) of the four differen bacteria viz Pseudomonas diazotrophicus (183 and 184) "Klebsiell planticola and Enterobacter cloacae were added in sterilized 10 soil of paddy field fortified with 1, 2 and 3%w/w neem cake (n hexane extracted) and (Expeller extracted) and the fertilizer (Urea, NPK & DAP) at their recommended doses separately in 100 m flask. The flasks were incubated at room temperature (30^{o}C) 2 and 48 hrs. with treated and untreated control in triplicate The population of these bacteria were studied by Plate dilutio technique (Wasksman 1957). Colonies were counted by colon counter and compared with the control after 24 and 48 hrs incubation.

C. EFFECT OF INSECTICIDES ON SYMBIOTIC & NON-SYMBIOTIC NITROGEN FIXING BACTERIA:

Insecticide fortification:

Pesticides (Carbofuran 3G, Basudin 10G and Thiodan 35 EC) at the dosage 750g a.i./ha., 9kg/acre and 1kg/ha. were added in autoclaved medium (Nutrient Agar) before solidifying. The nutrient agar slants were inoculated with the cultures of all the four Rhizobium strains separately in triplicate and also with Pseudomonas diazotrophicus, Klebsiella planticola and Enterobacter cloacae separately in triplicate the cultures for inoculum purpose was maintained in Nutrient Broth. One loopful was streaked on Nutrient Agar slants fortified with pesticides. Incubated at room temperature 28°-30°C. Growth was observed visually after 24 hrs and compared with control; the blank Nutrient Agar slants (i.e. without pesticide).

D. EFFECT OF NEEM CAKE/FERTILIZERS AND INSECTICIDES ON TOTAL MICROBIAL COUNT ON NUTRIENT AGAR:

The mixed culture inoculum of soil suspension (2 ml) was added to 10g sterilized soil incorporated with Neem cake (n-hexane extracted) and Neem cake (Expeller extracted) @ 1, 2 & 3%w/w, in 100ml sterilized flasks separately, Urea, NPK & DAP (Recommended doses) and insecticides (Carbofuran, Basudin & Thiodan)at RD separately in triplicate. The insecticides treatment in combination with neem cake (n-hexane extracted) an Neem cake (Expeller extracted) were also studied.

The flasks were incubated at room temperature for 24 hours and 48 hours. After 24 & 48 hrs the population of the colonies o bacteria on agar plates were observed (Wasksman, 1957) an compared with the control.

E. EFFECT OF NEEM CAKE/FERTILIZERS/INSECTICIDES ON NITRIFYING BACTERIA:

Both types of neem cakes were incorporated in soil @ 0.1 0.5 and 1%w/w in a 500 ml flasks separtely. The flasks wer incubated at room temperature and soil tested by Most Probabl Number method for Nitrosomonas and Nitrobacter after 15 days an one month treatment with neem cake. Similarly the fertilizer (Urea, NPK and DAP) and insecticides (Carbofuran, Basudin an Thiodan) were incorporated in soil at recommended doses. All th experiments were run in triplicate. Observations were taken afte 15 days and one month of treatment by the method recommended fo Nitrobacter presence and Nitrosomonas (Black, 1965).

RESULTS AND DISCUSSION

A & B) EFFECT OF NEEM CAKE/FERTILIZER ON SYMBIOTIC & NON SYMBIOTIC BACTERIA:

The influence of neem cake-I (n-hexane extracted) an neem cake-II (Expeller extracted) was observed by measuring th absorbance on the spectrophotometer taken at different tim intervals and doses, to compare their effect. The tables 8A, 8B 8C, 8D give the results of the (ANOVA) analysis for optica density. It is evident from the results that n-Hexane extracte neem cake showed stimulation in all the doses applied ($p<0.05$). The increase in measurement of optical density was gradual wit the increase in doses i.e. stimulation was proportional to th concentration of neem cake added to the media being the highes at 1% dose. It was noted that the initial lag phase period wa reduced as the dose increased compared to that of contro ($p<0.05$).

TABLE - 8A

OPTICAL DENSITY OF RHIZOBIUM PHASEOLI

TIME IN DAY	TYPE OF NEEM CAKE	CONTROL	DOSE IN PERCENTAGE		
			0.1	0.5	1.0
1	n-Hexane extracted	0.057 P	0.080 OP	0.087 OP	0.117 NO
2	"	0.117 NO	0.217 M	0.200 L	0.587 H
3	"	0.213 M	0.407 JK	0.607 H	0.833 G
4	"	0.390 K	0.947 F	0.980 EF	1.100 C
5	"	0.980 EF	1.167 B	1.267 A	1.267 A
1	Expeller extracted	0.057 P	0.057 P	0.080 OP	0.097 OP
2	"	0.117 NO	0.167 MN	0.290 L	0.462 I
3	"	0.213 M	0.273 L	0.447 IJ	0.597 H
4	"	0.390 K	0.373 K	0.497 I	0.797 G
5	"	0.980 EF	0.950 EF	1.013 D	1.000 DE

LSD= 0.05138 at alpha = 0.05
Coefficient of Variation = 6.28%

* Values in each column and row followed by the same letters are not significantly different at alpha=0.05

** Each value is a mean of three replicates.

TABLE - 8B

OPTICAL DENSITY OF RHIZOBIUM LEGUMINOSARUM

TIME IN DAY	TYPE OF NEEM CAKE	CONTROL	DOSE IN PERCENTAGE		
			0.1	0.5	1.0
1	n-Hexane extracted	0.090 R	0.097 R	0.130 R	0.380 NO
2	"	0.343 O	0.497 LM	0.693	1.033 G
3	"	0.520 L	0.877 J	1.200 DE	1.267 CD
4	"	0.997 GH	1.200 DE	1.300 BC	1.367 AB
5	"	1.067 FG	1.300 BC	1.367 AB	1.433 A
1	Expeller extracted	0.090 R	0.097 R	0.160 QR	0.207 PQ
2	"	0.343 O	0.247 P	0.340 O	0.473 LM
3	"	0.520 L	0.430 MN	0.490 LM	0.647 K
4	"	0.997 GH	0.897 IJ	0.953 HI	1.167 E
5	"	1.067 FG	1.000 GH	1.033 G	1.133 EF

LSD= 0.07267 at alpha = 0.05
Coefficient of Variation = 6.32%

* Values in each column and row followed by the same letters ar not significantly different at alpha=0.05.

** Each value is a mean of three replicates.

TABLE - 8C

OPTICAL DENSITY OF RHIZOBIUM FREDII

TIME IN DAY	TYPE OF NEEM CAKE	CONTROL	DOSE IN PERCENTAGE		
			0.1	0.5	1.0
1	n-Hexane extracted	0.107 R	0.097 R	0.130 R	0.380 NO
2	"	0.117 R	0.497 LM	0.693	1.033 G
3	"	0.500 M	0.877 J	1.200 DE	1.267 CD
4	"	0.913 GH	1.200 DE	1.300 BC	1.367 AB
5	"	1.067 D	1.300 BC	1.367 AB	1.433 A
1→	Expeller extracted	0.107 R	0.137 QR	0.200 OPQ	0.247 O
2→	"	0.117 R	0.223 OP	0.243 O	0.397 N
3→	"	0.500 M	0.350 N	0.393 N	0.593 KL
4→	"	0.913 GH	0.590 L	0.663 JK	0.793 I
5→	"	1.067 D	0.963 EFG	1.000 DEF	1.000 DEF

LSD= 0.07267 at alpha = 0.050
Coefficient of Variation = 6.32%

* Values in each column and row followed by the same letters are not significantly different at alpha=0.05.

** Each value is a mean of three replicates.

TABLE - 8D

OPTICAL DENSITY OF RHIZOBIUM JAPONICUM

TIME IN DAY	TYPE OF NEEM CAKE	CONTROL	DOSE IN PERCENTAGE		
			0.1	0.5	1.0
1	n-Hexane extracted	0.060 P	0.080 OP	0.137 NO	0.253 M
2	"	0.113 OP	0.407 L	0.610 K	0.960 FGH
3	"	0.600 K	0.767 I	0.980 EFG	1.200 B
4	"	0.980 EFG	1.067 CD	1.060 D	1.300 A
5	"	0.997 DEFG	1.067 CD	1.200 B	1.300 A
1	Expeller extracted	0.060 P	0.057 P	0.087 OP	0.190 MN
2	"	0.113 OP	0.353 L	0.400 L	0.423 L
3	"	0.600 K	0.590 K	0.690 J	0.897 H
4	"	0.980 EFG	0.927 GH	0.940 FGH	1.200 B
5	"	0.997 DEFG	1.033 DE	1.033 DE	1.133 BC

LSD= 0.07267 at alpha = 0.05
Coefficient of Variation = 7.06%

* Values in each column and row followed by the same letters are not significantly different at alpha=0.05.

** Each value is a mean of three replicates.

The results of all the four <u>Rhizobium</u> species ar similar in case of n-hexane extracted neem cake. But the expeller extracted neem cake showed different results with different species of <u>Rhizobium</u>. For <u>Rhizobium phaseoli</u> an <u>Rhizobium japonicum</u> there is a slight stimulation with dose 0.5 & 1% as compared to control, slight stimulation was observed wit <u>Rhizobium leguminosarum</u> at the 1% dose, whereas sligh retardation in growth of <u>Rhizobium fredii</u>.

With these observation it is obvious that the expeller neem cake contains oil more than the n-hexane extracted neem cake which is the main factor affecting the growth of the bacteria, but it is not completely inhibiting. One can conclude that neem cake when applied in soil will not effect the population of bacteria belonging to the genus i.e. <u>Rhizobium</u>.

Similarly neem cake n-hexane extracted) increased the colony count of <u>Pseudomonas diazotrophicus</u>, <u>Klebsiella planticola</u> and <u>Enterobacter</u> cloacae on Nutrient Agar plates whereas the NC (Expeller extracted) showed no increase as compared to control. The chemical fertilizers (NPK, Urea, & DAP) which are normally applied in fields for better crop yield were also tested at recommended doze (Table-9) and ten times higher

TABLE-9

EFFECT OF NEEM CAKE/FERTILIZERS/INSECTICIDES ON THE GROWTH OF RHIZOBIUM SP.

(Percentage

	R. phaseoli	*R. legum*	*R. japonicum*	*R. fredii*
Control	100	100	100	100
Neem Cake (n-hexane extracted)	150	155	140	160
NPK	100	100	100	100
DAP	100	100	100	100
Urea	100	100	100	100
Carbofuran	100	100	100	100
Endosulfan	100	85	100	100
Basudin	100	100	100	100

*The growth measured in the control (untreated) has been considered as 100%.

Table-10

EFFECT OF NEEM CAKE/FERTILISER/INSECTICIDES ON THE GROWTH OF NON-SYMBIOTIC BACTERIA

(Percentage)

Neem Cake/ Fertilizer	Rate of application %	Pseudomonas diazotrophicus	Klebsiella planticola	Enterobacter cloacae
Control		100	100	100
NC I	1%	100	100	100
	3%	200	200	200
NC II	1%	100	100	100
	3%	200	200	200
NPK	RD	100	100	100
	10 RD	100	100	100
DAP	RD	100	100	100
	10 RD	100	100	100
Urea	RD	100	100	100
	10 RD	100	100	100
Carbofuran	RD	100	100	100
Endosulfan	RD	100	100	100
Basudin	RD	100	100	100

Key:

NC1 = n-hexane extracted
NCII = Expeller extracted
NPK = Nitrogen Phosphorous & Potassium
DAP = Diammonium phosphate
RD = Recommended dose
10 RD = 10 times high recommended dose
*The growth in the control is considered as 100%.

than the recommended dose. All the fertilizers showed neither stimulation nor inhibition on all the four Rhizobium species and the four different N2 fixers tested of rice Rhizosphere viz: (Pseudomonas diazotrophicus, Klebsiella planticola, Enterobacter cloacae) table-10.

C) EFFECT OF INSECTICIDES ON SYMBIOTIC & NON SYMBIOTIC BACTERIA:

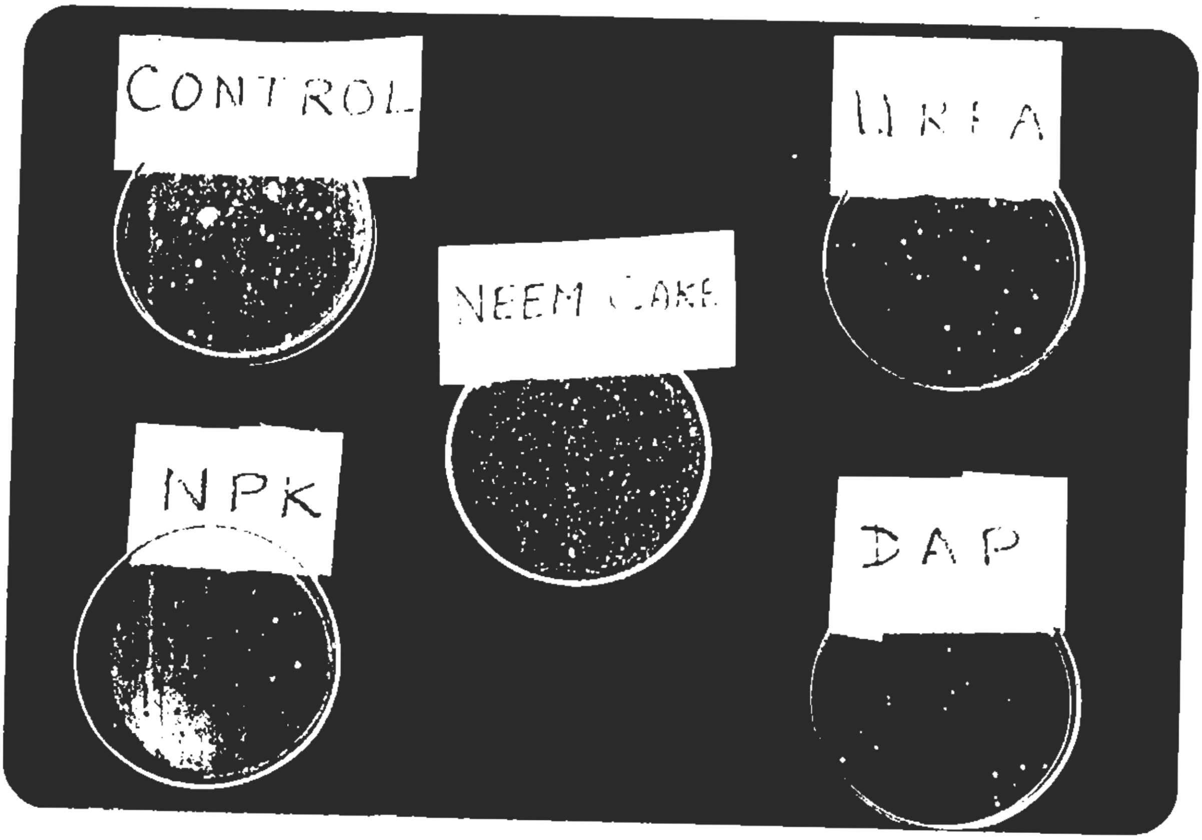
The three insecticides viz; Carbofuran, endosulfan and Diazinon tested at recommended dose showed no adverse effect on the growth of Ps. diazotrophicus, Kl. planticola, Enterobacter cloacae (table-10) and the three Rhizobium species viz R. japonicum, R. phaseoli and R. fredii except there is slight retardation in the growth of R. leguminosarum with endosulfan (table-9).

D) EFFECT ON TOTAL MICROBIAL BIOMASS WITH NEEM CAKE/FERTILIZER/:

Physical observation was taken after 24 hrs treatment by observing the shape of colonies. Generally all the three fertilizers tested have shown the same type of growth as the control. The neem cake treatment @ 1, 2 and 3%, as compared to control is giving different types of tiny colonies which are numerous and off-white yellowish colour, the big size white opaque and filamentous colonies present in the control plates are

PHOTOGRAPH-5

EFFECT OF NEEM CAKE/FERTILIZER ON TOTAL SOIL MICROBIAL BIOMASS



not absent in the treated plates (Photograph-5). These tiny colonies were isolated separately for identification. This isolate was sent to USA and got identified by using fatty acid analysis, a method which considered to be specially good for the aerobic eubacteria. The strain was identified as Pseudomonas specie.

E) EFFECT OF NEEM CAKE/INSECTICIDES/FERTILIZERS ON NITRIFYING BACTERIA:

No adverse effect on the growth of Nitrosomonas and Nitrobacter with the fertilizers and insecticide and NC (Expeller extracted) was observed except NC (n-hexane extracted) inhibited the growth of Nitrosomonas at 1% (table-11) as reported earlier (Mishra, 1975 & 1976).

CONCLUSIONS:

The overal conslusion drawn while comparing both the types of cakes, n-hexane extracted and Expeller extracted both showed stimulation in growth of symbiotic and non-symbiotic bacteria. The n-hexane extracted neem cake being more stimulant. Thus it can be concluded that if neem cake is applied as a fertilizer in soil it will not disturb the balance of Rhizobium species thus maintaining the naturally occuring soil fertility.

TABLE-11

EFFECT OF NEEM CAKE ON NITRIFYING BACTERIA

(Percentage)

Type of Neem Cake	Rate of Application (%)w/v	Nitrosomonas Sp.	Nitrobacter Sp.
NC-I	0.1	100% growth	100% growth
	0.5	100% growth	100% growth
	1.0	100% inhibition 0% growth	100% growth
NC-II	0.1	100% growth	100% growth
	0.5	100% growth	100% growth
	1.0	100% growth	100% growth

KEY:

NC-I = n-hexane extracted.

NC-II = Expeller extracted.

NEEM CAKE AS AN INHIBITOR OF PESTICIDES DEGRADATION

NEEM CAKE AS AN INHIBITOR OF PESTICIDES DEGRADATION

Abstract

Experiments were conducted to observe the influence of two types of Neem cake [n-hexane extracted (NCI) and expeller extracted (NCII)] on the persistence of Diazinon 10G, Carbofuran 3G and Thiodan 35EC. Results revealed that with the amendment of both the types of Neem Cake @ 1, 2 & 3%w/w prolonged the degradation period as compared to the normal period without the Neem Cake amendment. The percentage increase in Neem Cake amendment increases the persistence of insecticides. It was noted that expeller extracted Neem Cake showed little influence on the persistence of these insecticides relative to soxhlet extracted Neem Cake. Treatment of soil with commercial formulation of Basudin, Carbofuran and Thiodan 10-days after Neem Cake application did not cause the increase in persistence. To obtain a good response, treatment of soil with insecticides and application of Neem Cake need to be done at the same time.

Literature Review:

The use of pesticides and fertilizer now a days has shown steady increase but the interaction between these agro-chemica in the agricultural environment are little understood. Nitrog fertilizers are probably the most important input, for increasi the production of agricultural crops, including rice. T application of nitrogen fertilizer showed little influence on t persistence of Carbofuran in Philippine flooded rice soil (Maah clay) with a high native nitrogen content (Siddarampa, et.a 1978). Rajagopal, et. al. (1984) reported that the applicati of ammonium sulphate at the equivalent of 50 and 100 mg N kg flooded soils influenced the persistence of Carbaryl a Carbofuran depending on soil type. It was reported th Carbofuran along with urea increased the up take of N by ric (Sathasivam, et. al. 1982). Inhibition of degradation Carbofuran by 3% neem cake (alcohol extracted) incorporat granules has been reported that % degradation was 53.50% after days compared to 91.90% and 85.6% in commercial granules, (Rav 1979).

The addition of urea did not effect the insecticid activity of Endosulfan (Baskaran and Premkumar 1985). The highe persistence of 3G of Carbofuran was showed by linseed oil surfa coating (Mukerjee, et.al. 1984).

Ammonium sulphate and urea, but not potassium sulphat increased the persistence of carbaryl in a flooded lateral so with a low native nitrogen content (0.04%) but not in an alluvi soil with a higher nitrogen content (0.11%). Likewise, ammoni sulphate increased the alluvial soil. (Rajagopal et.al 1984) Addition of urea, zinc sulphate and sodium bicarbonate t carbaryl along with other insecticides did not increased thei shelf-life, urea did not effect much in the pH value (Kelya Singh and Sheo-Prasad 1982). Treatment of soils with commercia formulation of Diazinon and Parathion to which LAS (Linear alky benzene sulfonate) and ABS (alkyl benzene sulfonate) has bee added to increase the persistence of insecticidal residues (Lichtenstein, 1966).

Inorganic N at a typical fertilization level appeared slightly stimulate the enhanced degradation of Carbofuran (Hend and Richardson, 1988).

Experiments with neem cake amendment along with insectici fortification were planned to observe the role of neem cake wh applied as a N source in rice field on the persistence degradation of the insecticides viz. Carbofuran 3G, Basudin 1 and Thiodan 35EC.

EXPERIMENTAL METHODS:

Three insecticides, namly, Carbofuran 3G, Endosulfan 35EC and Diazinon 10G were used for undertaking residual persistence studies. The material and methods for the two lateral referred insecticides viz; Basudin and Endosulfan differ with Carbofuran Therefore, the details methodology is given as under:

I) CARBOFURAN RESIDUE STUDY:

MATERIALS:-

A. **REAGENTS AND SOLVENT:**

These were: Methanol extra pure (Merck), Ether, n-hexane (Distilled), NaoH, p-Nitrobenzene-Diazonium tetrafluorobarat (Sigma) and Filter Paper(Whatman No.1).

B. **GLASSWARES;**

Conical Flasks (250 ml), Microsyrige (100 ml), Funnel size 2.5 inch dia, Volumetric Flask 5ml and Spraying bottles (100 ml).

C. **INSTRUMENTS:**

Rotary Evaporator and Shaker.

D. **INSECTICIDE:**

a. FORMULATION:

Carbofuran (Furadan 3G) M/s Agricide Ltd.

b. Reference Standard and its metabolites: (Received from EPA Research Triangle Park N.C.)

i) Carbofuran - 98.9%

ii) 3- Keto Carbofuran 98.4%

iii) Hydroxy Carbofuran 99.8%

E. **CHROMATOPLATES:**

Silical Gel 60 A Flouresent at 254 nm. Size. 20 x 20 cm 25 um layer (whatman) Made in USA.

METHODS

1.1 SAMPLING AND PREPARATION OF SOIL:

Soil samples of paddy field for experimental purpose wer received from Muridke, Dist Sheikhupura and Rice researc Institute, Kala Shah Kaku dated 13.10.1990 and 31.10.1990. Thes were stored at 8°C at the original moisture levels until th experiments were initiated. The physical and chemica characteristics of the soil is given in Table-3 (Chapter-I).

The moist soil samples were used for preparing the mixe culture inoculant of microorganisms by shaking one gm soil in 10 ml sterile water.

1.2 SOIL SAMPLE PREPARATION FOR TREATMENT:

a) The soil sample for treatment purpose were air dried, finel powdered and sieved to exclude roots and stones etc.

b) Soil sample were checked for the traces of insecticid (Carbofuran) by the method standardized in section 1.4. of thi chapter.

c) Soil sample was sterilized by autoclaving at 121°C for 30 minutes and checked for microbial count by plate dilution technique.

1.3 SOIL TREATMENT:

About 20g of soil (Sterilized) was placed in Erlenmeyer flasks 250 ml for the following neem cake treatments:-

I) Neem cake (n-hexane extracted) mixed @ 1, 2 and 3%w/w separately.

II) Neem cake (Expeller extracted) mixed @ 1,2 and 3%w/w separately.

Mixed culture inoculum of soil microorganisms (10 ml) mentioned in section 1.1 was added to the treated soil along with the insecticide carbofuran 3G (0.167 g a.i. = 5010 ug) calculated as ug a.i/g of dry soil equivalent to Recommended Dose which is 750 g a.i/ha.

A control soil sample (with and without inoculum) untreated with neem cake was also maintained containing the insecticide. After the addition of inoculum, insecticide and neem cake the soil was flooded with 10ml distilled/sterilized water a moisture was maintained during the incubation period.

The treated and untreated soil in flasks were incubated a room temperature for different time periods for residue analysis

1.4 STANDARDIZATION OF METHOD:

Preliminary studies were carried out to standardize th efficacy of analytical procedure for the detection of pesticid used, the parent compound (Carbofuran) and its two metabolite (3-Keto carbofuran & Hydroxy Carbofuran (available). Th percentage recovery of spiked and unspiked samples were studied the method demonstrated good recovery (2 mg/ml) and is very sensitive economical and quick. It offers the ability to analyse both the metabolites and parent compound by using one procedure(Photograph-6).

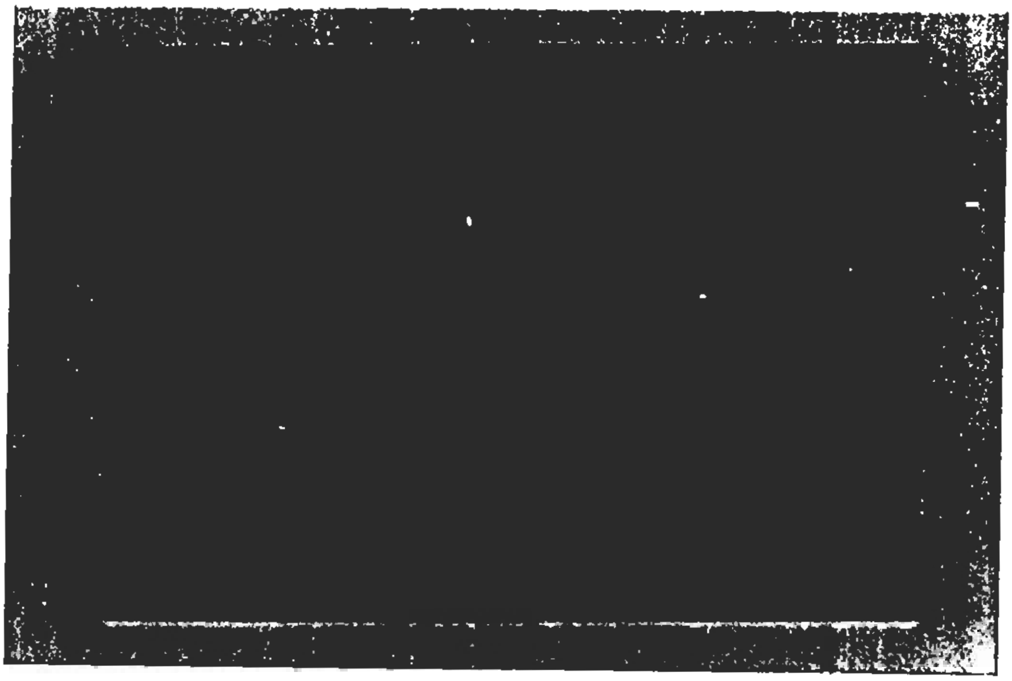
1.5 ANALYTICAL PROCEDURE:

EXTRACTION AND SAMPLES PREPARATION:

The extraction of residues from treated and untreated soil samples was done periodically (0, 1, 5, 10, 15, 30 and 60 days) in three replicates by tumbling the soil with 50 ml methanol for

PHOTOGRAPH-6

FORTIFICATION STUDY OF CARBOFURAN 3G FOR METHOD STANDARDIZATION (TLC PLATE)



one hour on a shaker as described by (Haris, et. al 1984). T
soil was separated from the shaked soil slurry by using whatm
No.1 filter paper in a 250 ml conical flask and pooled upto 1
ml with four times methanol washing. The methanol extracts th
collected was evaporated on a rotatory evaporator at 80°C. T
residues were redissolved in 3 ml methanol for detection by TLC

The extraction was tried with two solvents viz; methanol a
ether. They were both equally good.

1.6 THIN LAYER CHROMATOGRAPHY:

The residues dissolved in methanol were spotted along wi
its standard parent compound and metabolites on a gla
chromatoplate coated with silica gel, 60 A. 250 um thickness. Tl
plates were developed with ether:n-hexane (3:4) as a runnii
solvent for a distance of 15 cms and air dried. The standard
were located by spraying with chromogenic reagents consisting c
2N NaoH in absolute methanol followed by a solution c
p-nitrobenzene diazonium tetrafluroborate (5 mg dissolved in 25m
methanol and 25 ml dietyl ether) in succession (Archer, 1976)
Pink coloured spots developed (Photograph-7) against whit
background showing the presence of carbofuran and it
metabolites. The RF values noted are presented in the (Table-12)
The thin layer chromatography determination is quite sensitiv
for the soil extract with no interference of any other soi
substance.

PHOTOGRAPH-7

PERCENTAGE RECOVERY OF CARBOFURAN 3G FROM TREATED/UNTREATED SOIL

Metabolite

Parent Com

S = Standard Insecticide.

A = Formulated Insecticide without Inoculum-Untreated.

B = Formulated Insecticide with Inoculum-Treated.

C-1,3% = Insecticide with Inoculum and 1,3% Neem cake (n-hexane extracted).

E-1, 3% = Insecticide with Inoculum and 1,3% Neem cake (Expeller extracted).

TABLE-12

RF VALUES OF CARBOFURAN AND ITS METABOLITES

Compound	Rf Values
Carbofuran	0.49
Hydroxy Carbofuran	0.35
3-Keto-7-Phenol	0.57

Micrograms detectable by TLC are 2 for carbofuran and 1 for th

phenols and hydroxy carbofuran.

ENDOSULFAN 35 EC AND DIAZONON 10 G RESIDUE STUDY:

MATERIALS:

A. REAGENT AND SOLVENTS:

These were: Ammonium hydroxide 25%, Charcoal (activate for 4 hrs. at 120°C, Florisil (60-100 mesh) BDH tivated), Acetone-distilled, n-hexane-distilled and H2SO4 (anhydrous).

B) GLASSWARE:

Glass tubes size 20 cms length 2.9 cms dia, Glass columns size (2.5 cms dia 34 cms length jet size 4cms length 0.1 cms dia), Micro syringe 100 ml, Volumetric Flask 10 ml/5ml and Conical Flask 250 ml.

C) INSTRUMENTS :

Gas Liquid Chromatograph series 204. Pye Unicam and Rotary evaporator.

D) INSECTICIDES:-

a) Formulations

1) Diazinon (Basudin 10G)
2) Endosulfan (Thiodan 35EC)

b) Reference standards

1) Diazinon - 99.9%
2) Endosulfan - 99%

METHODS

1) Sampling and preparation of soil same as mentioned at 1.1 i revious section.

2) Soil samples for treatment. Same as mentioned at 1.2 i previous section.

3) SOIL TREATMENTS:

EXPERIMENT NO. I:

About 25 g of soil (sterilized) was placed in glass tubes with cotton wool plugs for the following treatment:

i) Insecticides at RD [7Kg/Acre for Basudin 10 G]
[1Kg/ha for Thiodan 35 EC]
without inoculum - Untreated control.

ii) Insecticides + inoculum - Treated control.

iii) Insecticides+inoculum+Neem cake - n-hexane extracted @ 1, 2 & 3%w/w separately.

iv) Insecticides+inoculum+Neem cake - Expeller @ 1, 2 & 3%w/w separately.

Mixed culture inoculant (10 ml) mentioned at (1.1) was added to this treated soil.

After the addition of inoculum, insecticides and neem cak the soil was flooded with 10 ml distilled/sterilized water an moisture was maintained during the incubation period. The treate soil tubes were incubated at room temperature for different tim period.

STANDARDIZATION OF METHOD:

Preliminary studies were carried out to standardized the efficiency of analytical procedure for the detection of pesticides from treated and untreated soil samples. The extraction of lower limits (0.1 ppm) of formulated pesticides sample (Thiodan 35 EC Basudin 10G) were studied for the accuracy of method adopted (table-13).

ANALYTICAL PROCEDURE:

The extraction of insecticide residues from the treated and untreated soil samples was done at 0 hr. and them periodically after 1,5,10,15,30 and 60 days in triplicate. The soil samples for analysis were air dried, homogenized and extracted by the method adopted by (Mumtaz, et.al. 1983). The treated soil (25g) was mixed with charcoal (1.0g) and Florisil (1.0g) and placed

TABLE-13

FORTIFICATION STUDY OF INSECTICIDES

Insecticides	Methodology used	% recovery
Thiodan 35 EC	Column chromatography analysis by GLC (ECD)	90%
Basudin 10 G	Column chromatography analysis by TLC and GLC (FID)	95%

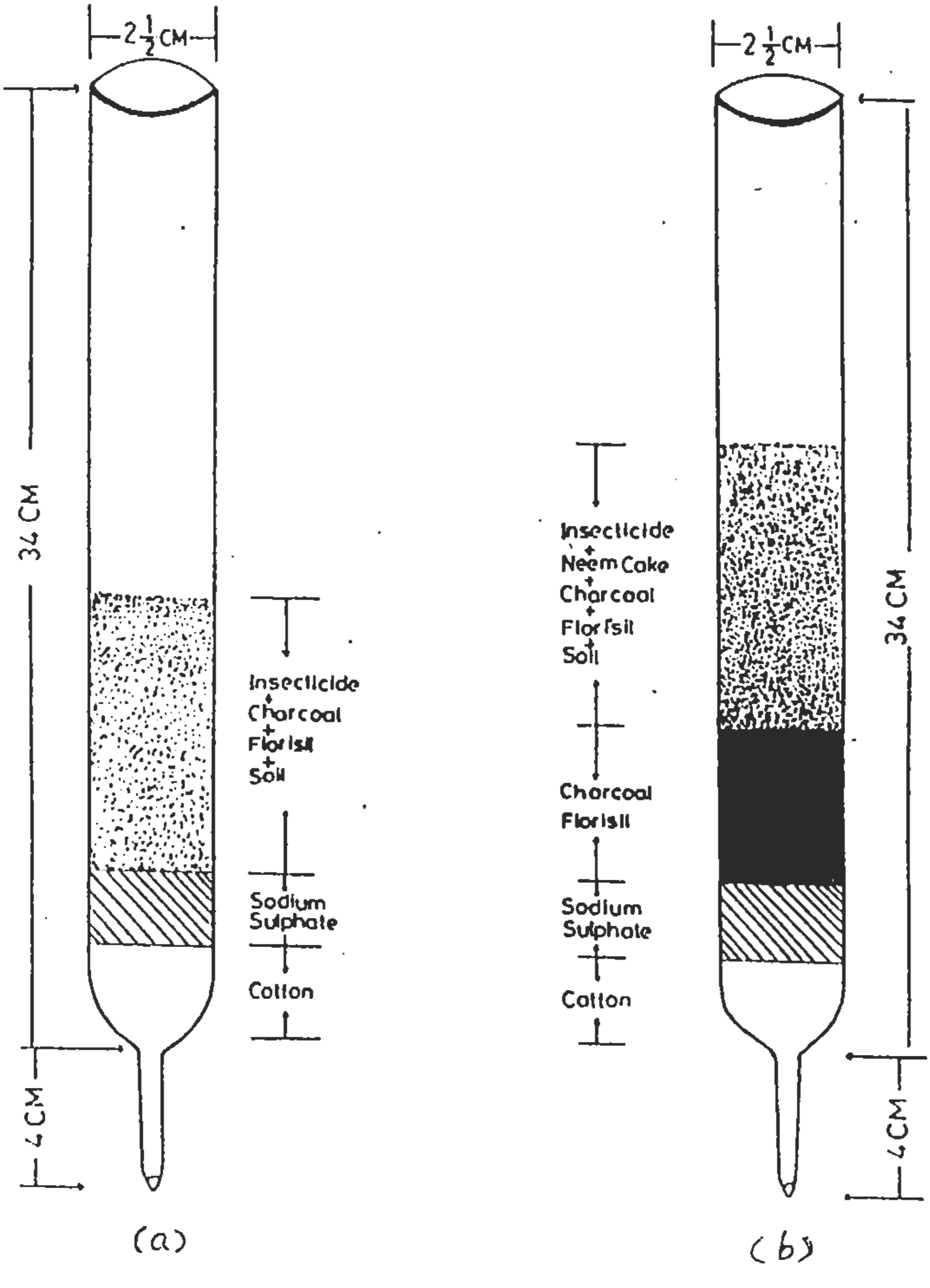
above a layer of sodium sulfate in a column as described figure-5. In the case of samples amended with neem cake, further layer of charcoal(0.5g)+Florisil(10g) was placed betw the soil mixture and the sodium sulfate. The samples w extracted with hexane+acetone (4+1 by volume). The solvent evaporated, the residue taken up in hexane and made up to 5 ml a volumetric flask for GLC analysis.

The lower limit of detection of the method was found to 0.1mg ha^{-1}.

RESIDUE DETERMINATION:

The extract thus obtained were analysed by GLC usi electron capture detector for thiodan 35 EC and flame ionizati detector for Basudin 10G. The parameter of GLC for ECD and F were as follows:

Fig. 5 : Column used for the extraction of Pesticides from soil-amended with & without Neem Cake



Pesticides :-

a) Basudin 10G
b) Endosulfan 35 EC

ECD FOR THIODAN:

Column material: 1.5% SP-2250/1.95% 2401 on 100/120 supe
copport supplied by supelco. Inc (qualit
laboratory chemicals).

Detector temp: 250°C

Injector temp: 175°C

Column temp: 180°C

Attenuation: 512

Chart speed: 120 secs/cms

Model: Pye Unicam Series 204

FID FOR DIAZINON:

Column Material: a)3% OV-101 chrom W-AW-DMCS 100/120

b)7.5% QF-1+5% DC-200

Detector temp: 250°C

Injector temp: 250°C

Column temp: 180°C

Attenuation: 128 & 256

Chart speed: 120 Secs/cms

THIN LAYER CHROMATOGRAPHY:

The extract of Basudin 10 G samples were also analyzed by thin layer chromatography using glass chromatography plates coated with silica gel 60A. 250 mm thickness. The plates after spotting were developed with n-hexane+acetone (distilled) (2:1) for a distance of 15cm and air dried. The spots were located by spraying with (a) 2% solution p-nitrobenzene pyridine) in acetone distilled) and them after heating at 120°C for 10 minutes with (b) 10% solution pentamine. The Rf value calculated for Diazinon was 0.65.

EXPERIMENT NO.II:

This experiment was conducted without sterilization of soil. The wet soil (25 g) was added in the sterilized glass tubes to which the same treatment mentioned at experiment No.I with the exception of adding inoculum separately. The rest of the procedure for extraction and residue analysis were the same with the same interval of days (i.e. 1, 5, 10, 15, 30 and 60 days).

EXPERIMENT NO.III:

To 25 gm sterilized soil neem cake treatment @ 1, 2 & 3%w/w and inoculum were added. After 10 days of Neem cake treatment the insecticide was added at the RD. (Basudin, Carbofuran and Thiodan). After the addition of insecticides extraction was done after 1, 5, 10, 15, 30 days interval for residue analysis.

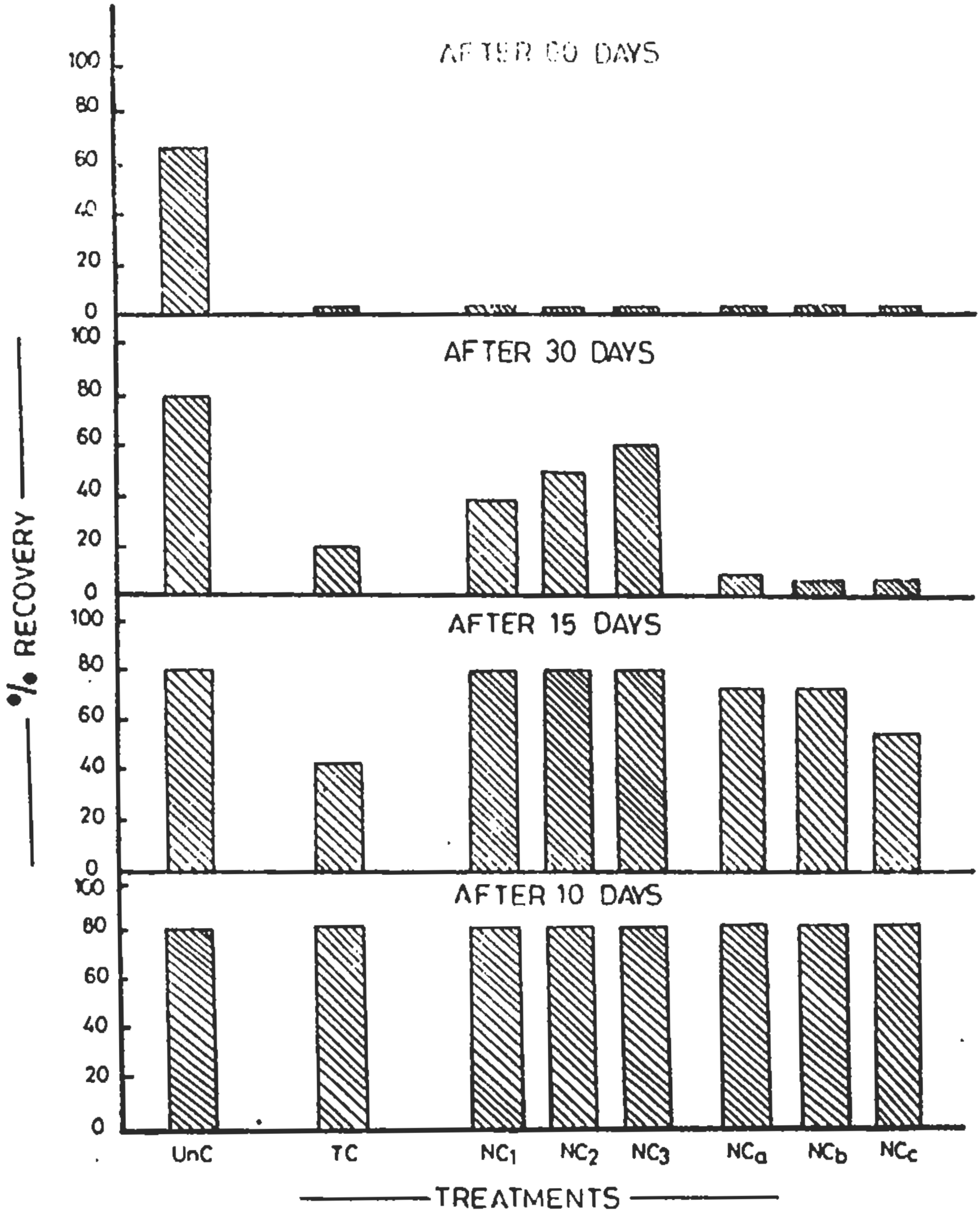
RESULTS AND DISCUSSION

CARBOFURAN:

General observations made during the series c experiments on the persistence of carbofuran 3G in soil amende with and without neem cake are histrographically summarized (Figure-6). The finding reveals that the persistence c carbofuran has increased with the amendment of neem cake upto 3 days as compared to that without amendment of neem cake. Result showed that without amendment the insecticide carbofuran degrade upto (80%) within 30 days and with amendment of neem cake @ 1,2 3% the degradation was reduced to 60,50 & 40% respectively whic reveals that with the increase of neem cake insecticid persistence also increased.

Comparing both the types of neem cake the n-hexane extracte neem cake showed more persistent i.e. upto 30 days wherea expeller neem cake only upto 15 days. Within 15 days the treate control gave 50% persistence of the insecticide but with neem cake (n-hexane) it gave 100% persistence and expeller neem cake 70-80% persistence. The results noted after 60 days were nil i.e. no persistence in treated and untreated soil of the parent

Fig. 6: PERSISTENCE OF CARBOFURAN 3G IN SOIL WITHOUT AND WITH AMENDED NEEMCAKE



KEY :–

UnC = Unioculated soil as Untreated control.

TC = Inoculated soil as Treated control.

NC = Neemcake [Nc_1, Nc_2, Nc_3 = Soil amended with n-hexane extracted neemcake @ 1,2,& 3% resp.].

Nc_a, Nc_b, Nc_c = Soil amended with expeller neemcake @ 1,2,& 3% resp].

compound with a clear spot of the presence of metabolit (Photograph-7). The % of the metabolite could not be calculate due to the non-availability of standard of this metabolite Mostly from literature study hydrolysis is the major route o degradation of all carbamate insecticides in soil and wate environment (Kuhr and Dorough 1976). The hydrolysis product o carbamate insecticides are generally less toxic than the paren molecule, but it is reported that 1-napthol (metabolite) is a toxic or more toxic than carbaryl to certain organisms and thei activities in the soil (Ramakrishna and Sethunathan 1983).

Results of various researches indicate that microorganisms were involved in the break down process (Venkateswarlu and Sethunathan 1977; Siddaramappa, et. al. 1978; Ahmed, et. al. 1979, Gorder, et. al. 1980 and 1982; Felsot, et. al. 1981 and 1982, Greenhalgh and Belanger 1981, Read, 1983a, Rajagopal, et. al. 1984, Wilde and Mize, 1984). With this background of accelerated microbial degradation, the conclusion drawn is that the addition of neem cake disturbs the population dynamics of those microorganisms responsible for degradation ultimately resulting in the persistence of insecticide.

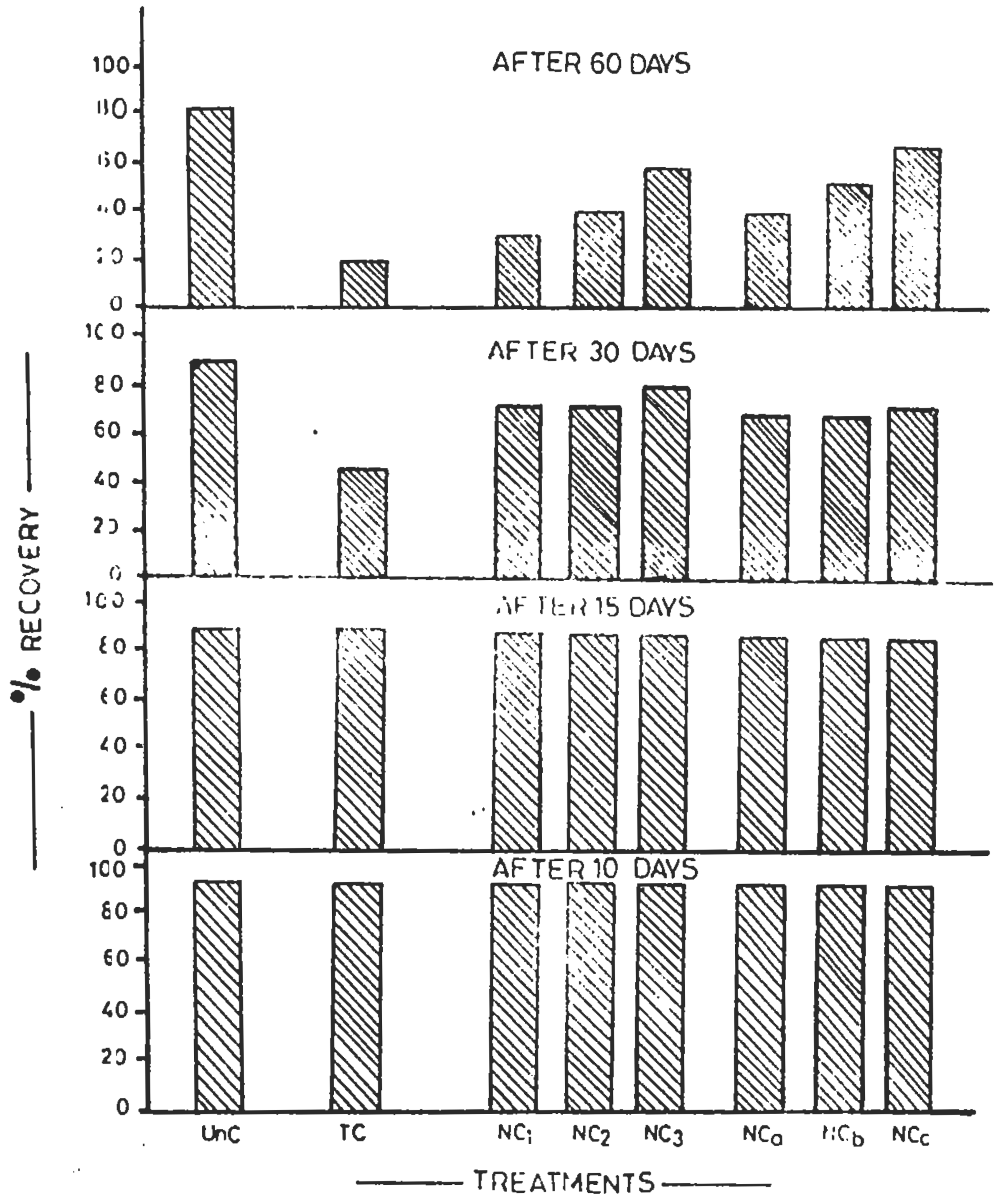
The second experiment conducted in which the insecticide was added after 10 days of neem cake application showed no good effect on the persistence of carbofuran. The degradation percentage was similar to the treated control which reveals that neem cake when applied along with the insecticide gives better response in the persistence of the insecticide.

BASUDIN 10 G:

The persistence of Basudin 10 G in soil amended with and without neem cake has been presented in (Figure-7). The finding reveals that within 15 and 30 days the treated control is showing 50% degradation and neem cake amendment @ 1,2 & 3%; 20, 20 & 10% respectively. The expeller neem cake is similar to n-hexane extracted neem cake until day 30 (Figure-8). The results after 60 days of incubation of treated control is 85% degradation and neem cake amended soil at 1, 2 & 3% 80, 70 & 65% respectively and expeller extracted neem cake a slightly higher i.e. 45, 45, 35. The thin layer chromatography of these samples are presented in (photograph-8) shows no indication of metabolite.

The experiment-II conducted with fortification of insecticide 10 days after neem cake application, showed no indication of prolonging the persistence of insecticide. The treated and

Fig. 7 : PERSISTENCE OF BASUDIN 10G IN SOIL WITHOUT & WITH AMENDED NEEMCAKE



KEY:-

UnC = Unioculated soil as Untreated Control.

TC = Inoculated soil as Treated Control

NC = Neem cake [Nc_1, Nc_2, Nc_3 = Soil amended with n-hexane extracted neem cake @ 1,2,&,3 % resp]

Nc_a, Nc_b, Nc_c = Soil amended with expeller neem cake @ 1,2,&,3 % resp]

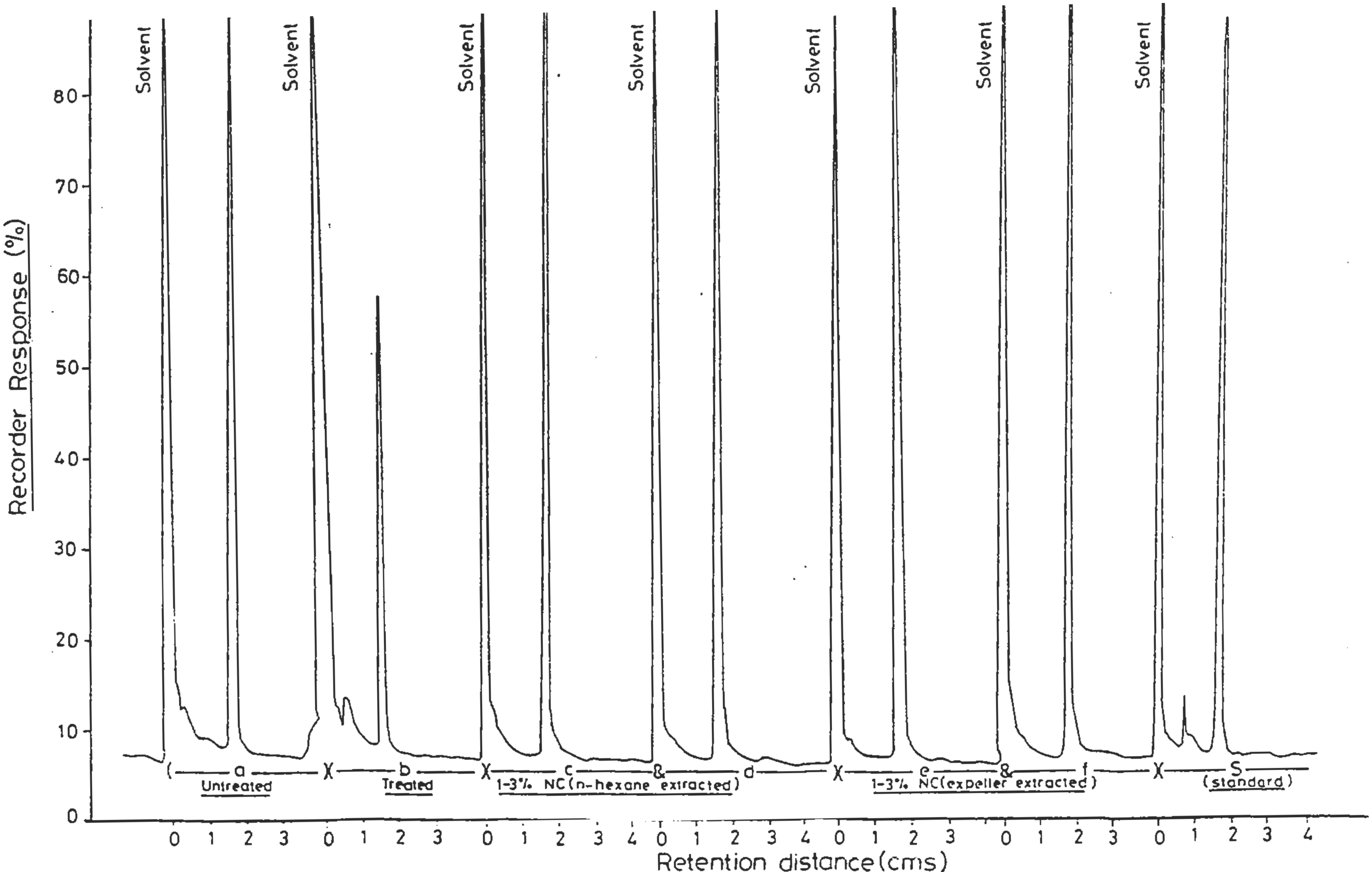
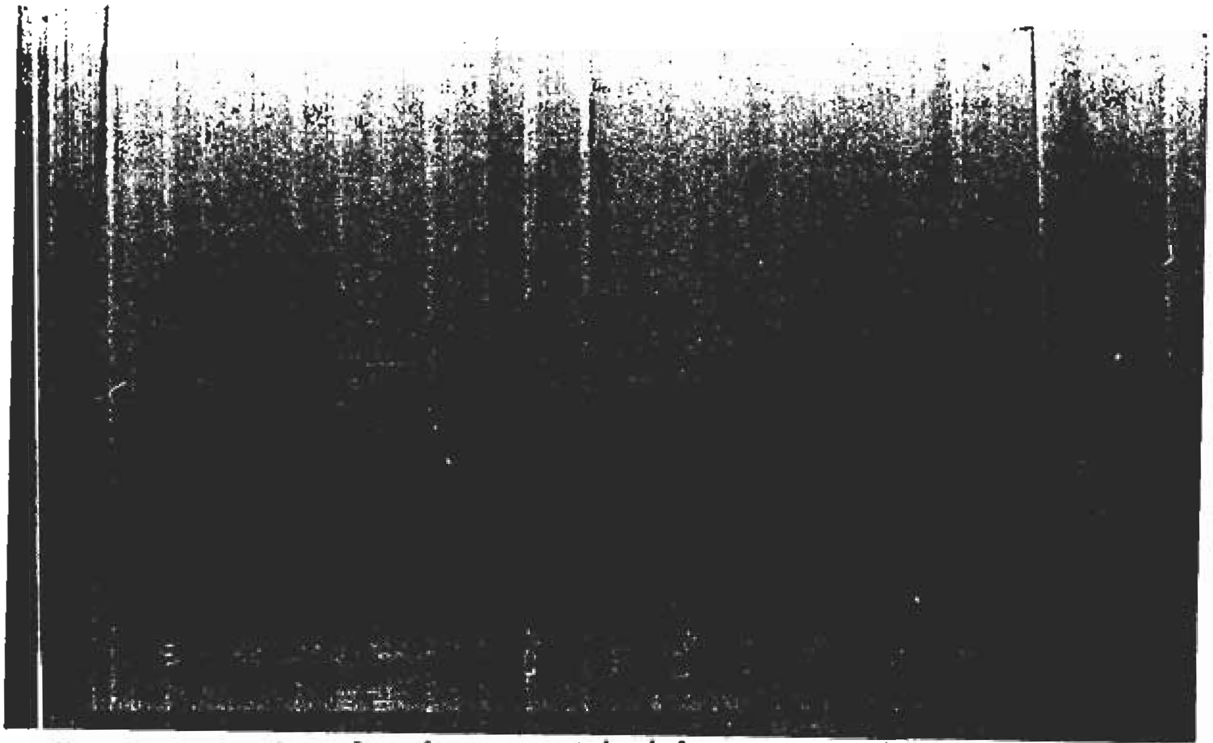


Fig:-8 Gas chromatograms of treated and untreated soil extracts of Basudin 10G after one month treatment with Neem cake(NC).

PHOTOGRAPH-8

PERCENTAGE RECOVERY OF DIAZINON 10 G FROM TREATED/UNTREATED SOIL



S = Standard Insecticide.

SI = Soil+Insecticide (Untreated).

SIC = Soil+Insecticide+Inoculum (Treated).

H = Soil with Insecticide, Inoculum and Neem cake (n-hexane extracted).

E = Soil with Insecticide, Inoculum and Neem cake (Expeller extracted).

Sp = Formulated Insecticide.

untreated sample of soil were giving the same response which concluded that neem cake must be applied along with the insecticide to get better persistence.

ENDOSULFAN 35% EC:

Endosulfan 35% EC contains both the isomers alpha & beta 70 and 30% respectively. It was noted that upto 10-days (Figure-9) no degradation was observed in all the treatments whereas after 15-days (Figure-10) there was slight degradation in alpha & beta isomers in the soil samples treated with the two types of Neem Cake. The treated control (B) showed no degradation upto 15-days but after 30-days (Figure-11) the treated control (B) showed 80% degradation of alpha-isomer and 70% of beta-isomer as reported earlier (Shahida, A. et. al. 1985), whereas the soil samples amendment with Neem Cake-1 caused a good persistence of alpha-isomer leaving nearly 90% in all the treatments i.e. 1, 2 & 3% respectively. After 60-days (Figure-12) the Neem Cake-1 treated soil is showing nearly 50% persistence of alpha-isomer whereas beta-isomer is not detectable indicating that there is no effect on persistence due to addition of Neem Cake I & II.

Fig.9: Persistence of Thiodan 35EC (Alpha & Beta isomers) after 10 days treatment.

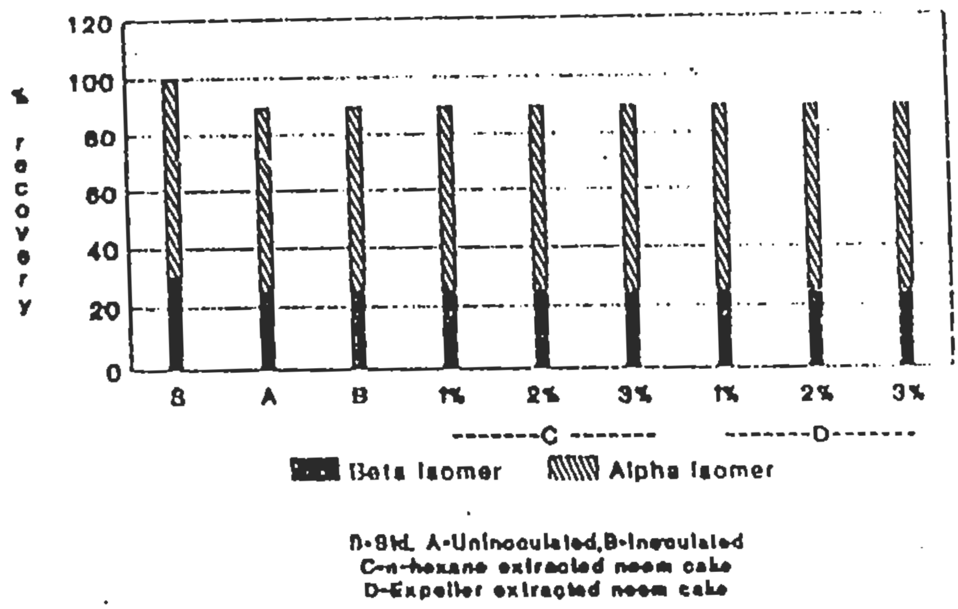


Fig.10: Persistence of Thiodan 35EC (Alpha & Beta isomers) after 15 days treatment.

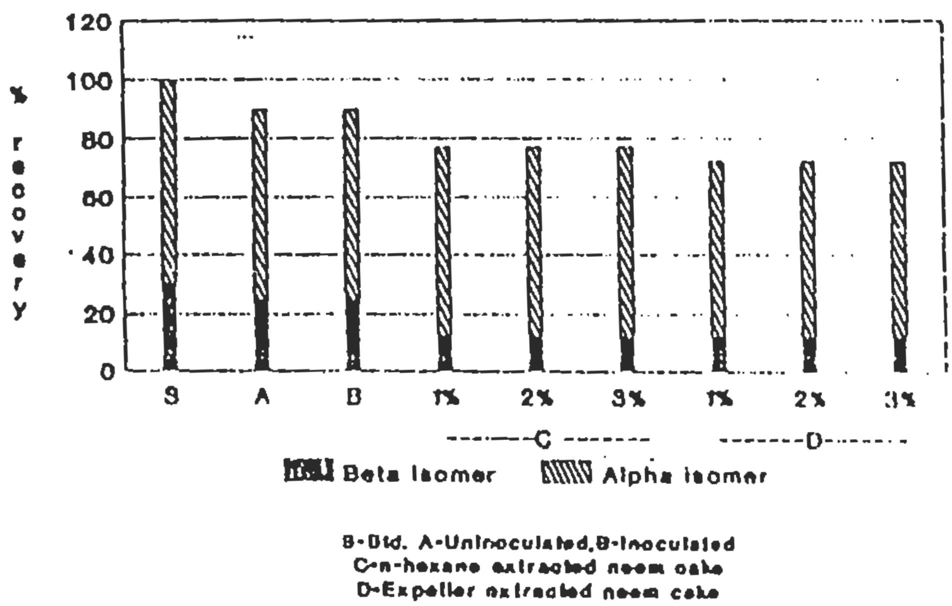


Fig. 11: Persistence of Thiodan 35EC (Alpha & Beta isomers) after 30 days treatment.

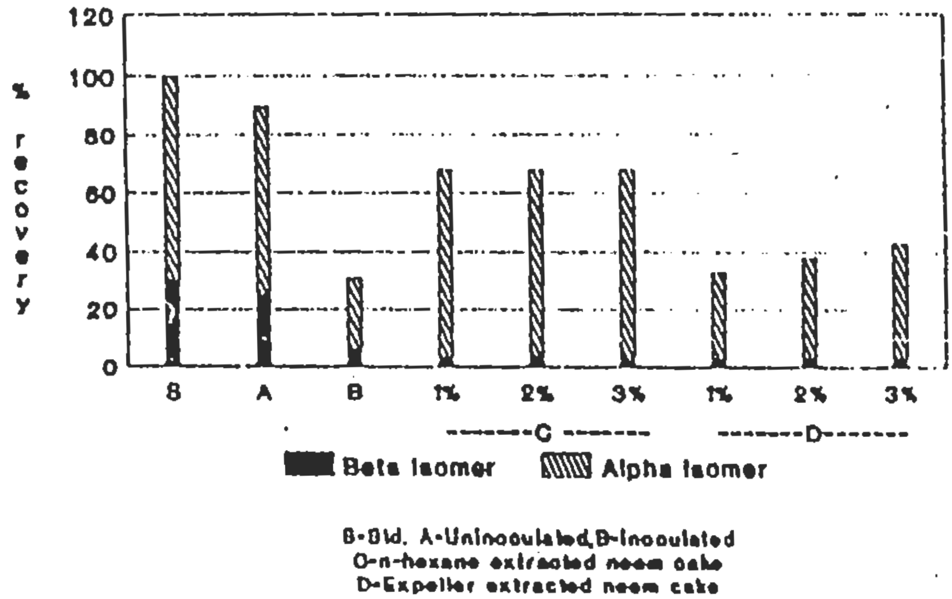
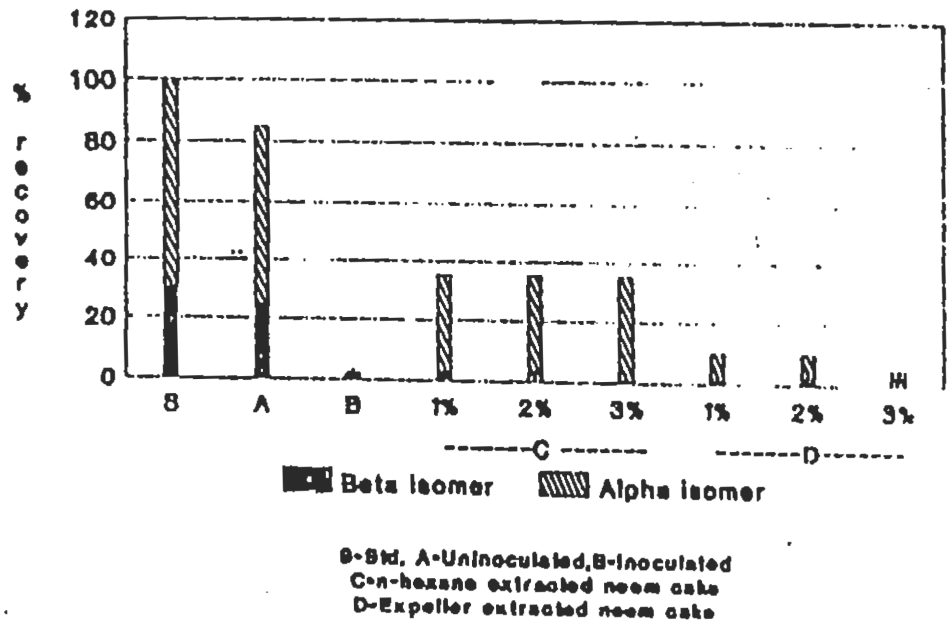


Fig. 12: Persistence of Thiodan 35EC (Alpha & Beta isomers) after 60 days treatment.



Comparing the two types of Neem Cake n-hexane extracted Neem Cake is showing good response on persistence of alpha-isomer only. The gas chromatograms of one month study are presented in (Figure-13). The experiment-II revealed the same type of results as the other insecticide.

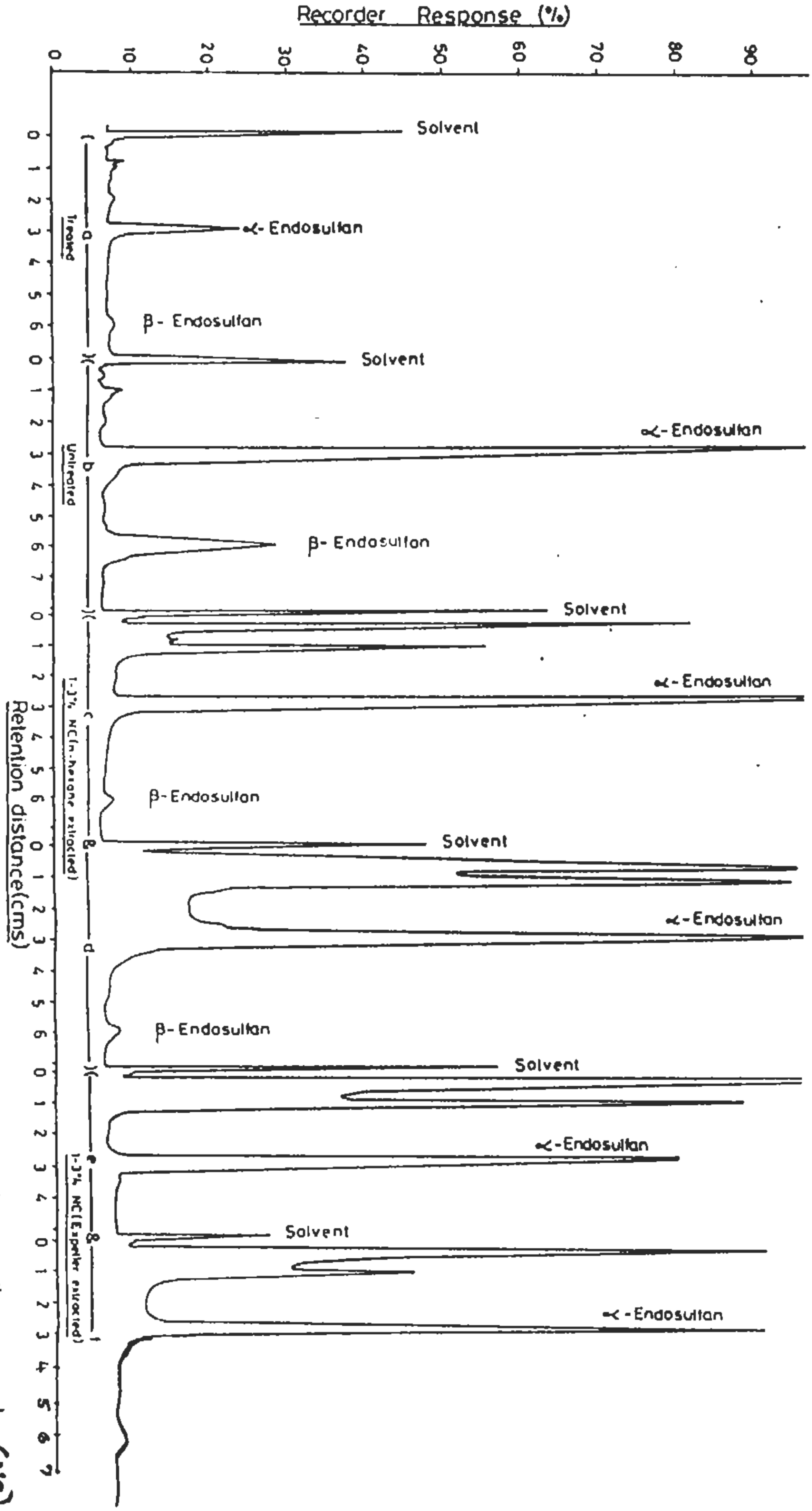


Fig.-13 Gas chromatograms of treated and untreated soil extracts of Thiodan 35 EC after one month treatment with neem cake (NC)

CONCLUSIONS:

The conclusions drawn from these studies are that with the addition of neem cake-I and II no adverse effect on degradation of pesticides (Carbofuran, Endosulfan, Basudin) was noticed. Howerve, it prolongs the period of degradation thus the pesticides persist for a longer period. We can avoid second application of pesticides in the field. With the addition of neem cake it is probably that the natural balance of some soil microorganism is disturbed there by increasing or decreasing the population of some microorganisms. This aspects needs further detailed investigation in order to understand the potential side effects on the fertility and consequently the production capacity of the soil. The experiment conducted with fortification of insecticide after 10 days of neem cake application showed no indication of persistence. These findings reveal that to get better response in persistence neem cake and insecticide must be applied at the same time.

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APPENDIX-A

AMMONIUM - NITROGEN:

SIGNIFICANT DIFFERENCE IN TREATMENTS/TYPE, DOSE AND DAYS TESTED AT 0.05 PROBABILITY LEVEL.

Treatments/Type		Dose		Days	
Ranked means	*n.s. range*	*Ranked means*	*n.s. range*	*Ranked means*	*n.s. range*
151.48	a	148.53	a	105.71	a
108.30	b	101.65	b	105.36	a
66.50	c	59.01	c	101.91	a
60.21	cd	42	d	76.41	b
52.5	d			49.58	c
F=184.17	(p<0.001)	F=301.21	(p<0.001)	F=64.22	(p<0.001)

Treatments were found to be significantly different tested at 0.05 probability level.

NITRATE + NITRITE - NITROGEN:

SIGNIFICANT DIFFERENCE IN TREATMENTS/TYPE, DOSE AND DAYS TESTED AT 0.05 PROBABILITY LEVEL.

Treatments/Type		Dose		Days	
Ranked means	*n.s. range*	*Ranked means*	*n.s. range*	*Ranked means*	*n.s. range*
66.51	a	68.89	a	55.98	a
56.73	b	51.00	b	50.4	b
35.71	c	31.41	c	49.01	c
35.7	c	28.00	d	39.23	d
29.46	d			29.5	e
F=3820.07	(p<0.001)	F=6780.55	(p<0.001)	F=1654.19	(p<0.001)

Treatments were found to be significantly different tested at 0.05 probability level.