

EVOLVING AND TESTING TECHNIQUES IN NEMATOLOGY  
WITH PARTICULAR REFERENCE TO SAMPLING, EXTRA-  
CTION, STORAGE OF SAMPLES, SEASONAL POPULATION  
FLUCTUATION AND NEMATOCIDAL SCREENING

BY  
HARI KISHUN SINGH

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Approved by :

Chairman		..... (S.K. PRASAD)
Members	1.	..... (E. KHAN)
	2.	..... (P. SARUP)
	3.	..... (S.L. MEHTA)

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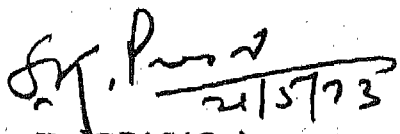


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C E R T I F I C A T E

I certify that the thesis entitled "Evolving and testing techniques in nematology with particular reference to sampling, extraction, storage of samples, seasonal population fluctuations and nematicidal screening" submitted in partial fulfilment of the requirement for the award of the degree of 'Doctor of Philosophy' in Nematology, is a faithful record of bonafide work done by Shri Hari Kishun Singh, under my supervision and guidance. No part of this thesis has been submitted for any degree or diploma. Any help or source of information, as has been availed of in this connection, is duly acknowledged.

Dated the 21<sup>st</sup> May, 1973

  
( S. K. PRASAD )  
Professor of Nematology  
Chairman, Advisory Committee

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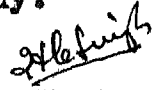
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## List of Abbreviations

Sq.m.	Square metre
m	metre
g	gram
ppm	parts per million
C.D.	Critical difference
temp.	Temperature
tech.	Technique
No.	Number
C.V.	Coefficient of variance
Fig.	Figure
rpm	Revolution per minute
min.	Minute
G	Gallon
he.	hectare
DD	1-2-Dichloropropane+1-3 Dichloropropene
DBCP	Dibromo-chloropropane
Dia.	Diameter
' <u>Rabi</u> '	Crops grown during winter time
' <u>Kharif</u> '	Crops grown during the monsoon (June-July to September-October)
<u>Khurpi</u>	A steel blade having flat sharp, anterior end and mounted in a wooden handle.
Aug.	August
Sept.	September
Oct.	October
Nov.	November
Dec.	December
Jan.	January

List of Abbreviations

Feb.	February
>	Greater than
M	Molar
DT	Direct soil placement over moistened facial tissue paper.
O.D.	Oostenbrink's elutriator with double layer of facial tissue paper.
CS	Cobb's modified decanting and sieving technique.
CSSSM	Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP <sub>7</sub> + 5 ppm methylene blue.
CSCF	Cobb's modified decanting and sieving + centrifugal flotation.
CSCFSM	Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP <sub>7</sub> + 5 ppm methylene blue.
cm	Centimetre (s)
ml	Millilitre (s)
lb	pound (s)
%	Percentage
R	Replication

## INTRODUCTION

Soil organisms occurring in enormous numbers and numerous forms, ranging in size from very small bacteria to comparatively large insects and earthworms are one of the many factors which influence the productivity of soil. About 80 to 90 per cent of all the multicellular animals in the soil are nematodes and perhaps due to their numerical strength, nematology owes its present recognition as an independent science. A nematode species, however, potentially injurious it may be, will cause the gravest concern to the economic nematologist only when its population rises to an epidemic proportion. Naturally, therefore, the study of the rise and fall of nematode population and the causes that govern the same is of paramount importance. For such studies accurate estimation of population in any given area or medium is the first basic necessity. In fact, accurate population determination is absolutely necessary in all fields of applied nematology, (i) scientific-research to investigate the nematode density prior to and following after experiment, (ii) investigations for advisory purposes to estimate critical nematode levels for damage to crops, (iii) establishing presence or absence of nematode for quarantine purposes and (iv) surveys. It is not possible to determine the success of any control operation unless one can accurately estimate the population before and after that operation.

Since anything like a complete enumeration of a population is impossible anywhere, sampling methods will have to be relied

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upon everywhere for estimating populations; naturally, the estimate should have the highest accuracy commensurate with the amount of work expended, and if this is to be so, a sampling technique which lays down the distribution, size and number of samples will need to be drawn up. There is little point in using accurate laboratory methods and spending a great deal of time to obtain laboratory accuracy greater than the error inherent in field sampling. There is no universal sampling method for a particular nematode population; it must be resolved about the distribution, life-cycle of the nematode involved and the cost (work involved) of sampling.

Since most plant parasitic nematodes have a soil phase, economic nematologists concentrate mainly on soil samples which are obtained by probing the soil with a sampling device which removes a core of soil. Some of the important considerations in sampling procedures are : (i) the number, the diameter, and depth of cores needed to provide a sample of appropriate size and (ii) a pattern of probing to cover the area and to obtain reliable data on population density means and estimates of variance.

Soil samples for nematode studies should be regarded as perishable because nematodes may or may not survive under all conditions of storage. Type of storage bags, temperature at which the samples are stored and duration of storage might affect their survival/viability.

In order to evolve a standard technique for accurate population we have to carry out what are known as 'precision' experiments. In this experiment a plot of suitable size is selected at random and is divided into very small units and the whole of it is examined unit by unit. Then the nature of distribution is ascertained by drawing dot-diagrams and the percentage of area to be sampled at a desired precision is determined.

There are a number of distinct extraction processes that can be used to separate nematode from the soil but their efficiency might depend on the size, structure, developmental stages and relative motility. Also, the effects of season on the stages present and their motility may affect the efficacy of extraction techniques.

Control of nematodes in the soil by chemical means is dependent upon bringing the nematicide into contact with the nematodes. Such contact can be accomplished by several means. Also, the efficiency of a nematicide could be evaluated by estimating the number of nematodes/certain reactions on the host plant.

The present investigation was, therefore, designed to study (i) the comparative efficacy of four soil samplers, (ii) distribution and sampling of nematodes, (iii) comparative efficiency of eleven nematode extraction techniques, (iv) effect of two storage bags, eight storage temperatures, six storage periods and three extraction techniques on recovery of nematodes

from the soil sample, (v) quantitative estimation of nematode population density by six extraction procedures as affected by season and seasonal fluctuation in nematode population, and (vi) evaluation of nematicidal efficiency with two techniques and timings of nematode inoculation.

## REVIEW OF LITERATURE

About 80 to 90 per cent of all multicellular animals in the soil are nematodes. Their main significance in energy flow cycle is connected with the widespread damage they cause as parasites of main crops. Population estimates for faunastic surveys, population studies and economic nematology require data on specific level of nematodes. The first requisite is to take a sample that truly represents the whole population. Most soil samples for nematode assay are obtained by probing the soil with a sampling device that removes a core of soil.

The soil samples are usually taken with an auger or a corer - golf-hole borer or metal tubing sharpened at one end make simple corer.

Cobb (1918) recommended the use of 1/10,000,000 acre tubes to be driven into the soil, dug out, and processed in the laboratory. These tubes were extensively used upto 1927, but rarely it was possible to process more than four sets of samples and more often only two were taken (Thorne, 1961).

Soil auger or wire worm - sampling tools of 4.0 cm diameter or larger were used formerly for Heterodera cysts.

For advisory purposes a conference of Advisory Entomologists held in 1946 (Anscombe, 1950) recommended that samples should be taken with a half-cylindrical sampler (diameter 2.54 cm) to a depth of 20 cm at a rate of 50 sampling units upto 4.05 hectare field. Chamberlain (1964) recommended that the soil should be

taken by 5.0 cm auger capable of taking borings upto a depth of 23 cm.

Cheese-sampler type with half cylindrical blade 20-30 cm long and 2.0 to 2.5 cm in diameter - now mostly used in U.K. and 50 cores usually provide about 2 kg soil.

It has been suggested that some animals may be killed by comparison when the core is forced from such instruments, and further more it is highly desirable to keep the core undisturbed, so more elaborate cores like O'Connor's split corer (O'Connors, 1957), Hust and Franklin corer (1937) and IISR soil sampler (Menon and Singh, 1963) have been developed.

In order to penetrate hard soils, it may be necessary to have equipments of the types described by Belfield (1956) and Potzgi (1955). In compost and soft humous rich situations, such as manure heaps, it is difficult to take an undisturbed sample; Von Torne (1962) has derived a sampler for these habitates consisting of two concentric tubes each with cutting teeth.

With the Dutch method generally used in several European countries, a much smaller, half cylindrical probe (diameter 1.3 cm attached to a long handle) is used to take 50 or more cores to fill a container having 250 or 500 ml soil. Modifications of the Dutch method are gaining favour particularly for detecting sampling because the smaller weight of field samplers to be carried, and less handling in the laboratory, make it practicable to take more samples from a given area.

Other tools like spade, digging forks, matlocks, garden travel and Khurpi etc. have also been used.

Chawla (1972) conducted a pilot trial to test the merits of O'Connor's split corer against Khurpi and reported that the former provided a lesser variation and therefore, it was more suitable for taking soil samples.

As it is normally impossible to count all the invertebrates in a habitat, it is necessary to estimate the population by sampling, naturally the estimate should have the highest accuracy commensurating with amount of work expended and if this is to be so a sampling programme which lays down the distribution, size and number of samples will need to be drawn up. There is no universal sampling method and although the statistical principles are given in several text-books, the sampling of a particular nematode population must be resolved about their distribution and life-cycle of the nematode involved. Assuming that the life-cycle is known, preliminary work will be necessary to gain some knowledge of the distribution of the nematode and the cost (work involved) of sampling. The first decision concerns the universe to be sampled; whether this is to be single habitate (e.g. a field) or representative of the habitate type from a wide geographical area will depend on whether an intensive or an extensive study is planned, and the second decision must determine the magnitude of population change it is to record.

Most nematodes have a soil phase and their distribution

is highly variable. Since individuals of many species occur in aggregates, sampling methods that assume random (poisson) distribution are inappropriate. Besides the experimental details of recovering the nematodes completely from soil, it is necessary to study as to how many cores should be taken and how should they be located. LeClerc (1967) characterised random stratified, and systematic approaches and recommended that a form of random sampling be used to avoid bias. For soil animals, occurring in aggregates, Hughe (1962) has suggested a combination approach in which the position of one sub-sample is fixed at random, and the other is taken at a fixed distance. Church *et al.* (1959) found that for Heterodera rostochiensis, sampling errors could be reduced by using systematic sampling.

Lewis and Taylor (1967) have suggested stratified sampling in which the whole area is divided into half as many sub-areas as the required sample units and two units are taken at random from each sub-area.

Sample size and number of probes comprising a sample vary widely. Generally, 20-100 cores of soil are taken from fields upto 2 hectares in size - 50 units from 5 acre or 100 units from 5-10 acres (Johnson and Thompson 1945 for Heterodera rostochiensis), 50 units for 10 acres (Anscombe, 1950 for H. rostochiensis), 180 acres/hectare (Gostenbrink, 1950 for H. rostochiensis), 60 samples from 1/3 acre (Kleijburg, 1960 for Ditylenchus dipsaci), 25-100 probes/hectare (Fenwick, 1961 for cyst forming nematodes), 15-20 units/hectare (Thunn, Herrmann

and Knickmann, 1955 for cyst forming nematodes), 120 cores/hectare (Chambalain, 1964 for cyst forming nematodes), 50 cores/hectare (Rasinya, 1967 for H. rostochiensis), Mukhopadhyay (1967) reported that collection of samples after dividing a field into a number of small areas (40 x 30') gave better results than sampling the field at random, 5 cores /150 sq. metre (Israel et al., 1969 for Hirschmanniella spp.), 10 cores/hectare for high population and 100 cores/hectare for low population (Merny and Dejardin, 1970 for cyst forming nematodes), 20 samples/1.6 hectare or 20-100 samples for 2 hectares (Barker and Nusbaum, 1971 - General).

Detection of low population is limited since less than  $1 \times 10^{-6}$  of the whole population is usually sampled (Jones, 1955). Although similar theoretical considerations apply when taking samples to count active soil nematodes; there is much less information about their heterogeneity in the field than for Heterodera species.

The sample population may consist of motile, or quiescent forms, cyst, individual eggs, egg masses or various combination of these. Portions of these may be in root fragments or debris. Methods of determining kinds and numbers of individuals in the sample population have been evaluated by Oostenbrink (1960), Cairns (1960), Thorne (1961), Seinhorst (1956, 1962) and Goodey (1963). The characters of nematodes on which techniques for extraction, concentration and enumeration are based, are: size and differential staining (hand sorting, direct microscopy; analysis of mixtures, identification), mechanical resistance (shedding blender preparation), activity (wet-funnel methods, use of filters/shape, use of sieves and

filters), specific weight (decanting, flotation, elutriation, centrifugation). The different techniques needed to evaluate the nematocoenosis various situations are conveniently headed under :

1. Direct microscopy (Seidenschwarz, 1923); Mindermann, 1956).
2. Centrifugal flotation (Caveness and Jenson, 1955; Mindermann, 1956; Jenkins, 1964).
3. Baermann funnel (Baermann, 1917; Overgaard Nielsen, 1949; Anderson and Yanagihara, 1955).
4. Modified Baermann funnel (Oostenbrink, 1960).
5. Direct cotton wool tray (Oostenbrink, 1954, 1960; Townshend, 1963).
6. Decantation + sieves (Cobb, 1918; Thorne, in : Goodey, 1951).
7. Decantation + sieves + filter (Christie and Perry, 1951; Tobar and Jimenez, 1963).
8. Decantation + cotton wool tray (Oostenbrink, 1960, 1964).
9. Inverted flask + sieves + filter (Seinhorst, 1955; Seinhorst, in : Murphy, 1962).
10. Split elutriator + sieves + filter (Seinhorst, 1962, 1965; Seinhorst, in : Murphy, 1962).
11. Funnel elutriator + sieves + filter (Oostenbrink, 1954, 1960; Tarjan, Simanton and Russel, 1956).
12. Sugar flotation sieving (Byard, Nusbaum and Barker, 1966).

Caveness and Jensen (1955) compared Baermann funnel with centrifugal flotation and found that the later method was 9 times more efficient. Oostenbrink (1955) tested 10 methods (1, 3, 4, 5, 6, 7, 8, 9, 10 and 11) and found that his elutriator and centrifugal flotation were equally good in time saving and quantitative recovery. Seinhorst (1956) compared Erlenmeyer flask method and funnel elutriator + sieves + filter and reported that with both types 85-90 per cent of the eelworms were recovered from soil samples of 500 g. Chapman (1958) compared Baermann funnel, Inverted Erlenmeyer flask method (IF) and Cobb's sieving (CF) and found that variability was too good and yields were too poor for quantitative studies with C.F. and B.F. I.F. provided good yield, lower variability and results were quite adequate for many quantitative purposes. Malo (1960) compared Oostenbrink's elutriator, Cobb's sieving and Seinhorst's split elutriator and reported that former extracted almost twice as many nematodes as the other two methods and was judged to be best adopted for processing numerous samples. Oostenbrink (1960) compared Baermann funnel, modified Baermann funnel, cotton wool filter and Oostenbrink's elutriator and reported that Baermann funnel gave 12 per cent of total population, Modified Baermann funnel 72 per cent and cotton wool filter and Oostenbrink's elutriator 100 per cent respectively. Townshend (1963) compared Oostenbrink's elutriator, Cobb's sieving and sugar flotation and recommended that these techniques were specific. With Oostenbrink's elutriator Pratylenchus penetrans was best

extracted, with Cobb's sieving Xiphinema and Criconemoides were best extracted and with sugar flotation Tylenchorhynchus and Rotylenchus were extracted in greater numbers than water control. Ayala et al. (1963) compared Oostenbrink's elutriator, Cobb's sieving and sugar flotation and recommended that sugar flotation was best for Hemicriconemoides and Criconemoides in all soil types and sample volume. Tobar (1963) compared Baermann funnel, Oostenbrink's elutriator, Erlenmeyer inverted flask method, Cobb's sieving and Seinhorst mistifier and recommended that Baermann funnel gave poor results, Oostenbrink's elutriator gave high recovery of nematodes of various sizes constant result between 50 g to 300 g of sample size. Erlenmeyer inverted flask method was best for 100 ml soil. Cobb's sieving was good for almost all nematodes except Pratylenchus, Seinhorst's mistifier was fairly good for Tylenchus. Whitehead and Hemming (1965) compared Seinhorst's split elutriator and centrifugation and recommended that when the centrifugation was replaced by separation through tissue paper, this method was superior to Seinhorst's split elutriator method. Sāvapalan (1967) compared Baermann funnel, centrifugal flotation, Oostenbrink's elutriator and Modified Baermann funnel by adding 200 Pratylenchus loosi in 100 g fumigated soil and remarked that these gave 50, 12, 60 and 19 per cent recovery respectively. Barker (1968) compared Baermann funnel, centrifugal flotation and sugar flotation sieving and reported that centrifugal flotation and sugar flotation sieving were specific. Barker, Nusbaum and Conglton (1968) compared Baermann funnel and sugar flotation sieving and reported

that sugar flotation sieving gave good yield of Belonolaimus longicaudatus, Xiphinema americanum and Tylenchorhynchus claytoni. Baermann funnel gave higher recovery of Pratylenchus zeae, Meloidogyne incognita and Helicotylenchus. Barker et al. (1969b) tested Baermann funnel, centrifugal flotation and sugar flotation sieving for extracting nematodes from soil in the field were shown to be specific selection in a study of nematode population dynamics over one year. Metlitski and Romanenko (1969) compared Baermann funnel, centrifugal flotation and combined sieving + chemical flotation and reported that later two methods recorded greatest number of nematodes. Elmiligy and DeGrisse (1970) compared centrifugal flotation technique and Oostenbrink's elutriator technique and found that centrifugal flotation was more efficient than the later in extraction of soil nematodes. DeGrisse (1970) compared extraction of nematodes from different soil types, using the centrifugal flotation and cotton wool filter method and reported that greater number of nematodes, particularly Cricconemoides were recovered by former technique from all soils. It was also good for Heterodera cysts. Oostenbrink (1970) compared 1, 2, 3, 5, 6, 7, 8, 9, 10 and 11 and recommended that 6 and 11 should be used for the extraction of active nematodes from small and large samples respectively. Dunn (1971) compared centrifugal flotation and Seinhorst's split elutriator and recommended that centrifugal flotation was least laborious and quickest method for nematode extraction. Kimpinski and Welch (1971) compared Baermann funnel and sugar flotation sieving and recommended that greater percentage was

recovered by sugar flotation sieving in sandy and clay soil. Szczygiel A. (1971) compared various methods along with sugar flotation and Inverted erlenmeyer flask method and former was quickest and labour saving. Chawla (1972) compared Cobb's sieving and gravity method, direct placement of soil, centrifugal flotation, Seinhorst erlenmeyer flask method, Oostenbrink's elutriator, and decantion and sieving method and recommended that maximum recovery was obtained with centrifugal flotation but the variation was least in respect of Oostenbrink's elutriator which could be preferred for studies involving relative estimates.

When the samples are brought to the laboratory it is not possible to process all the samples immediately. Therefore, it is often necessary to store the soil samples for varying lengths of time.

The number of nematodes recovered from any soil sample differs greatly with the extraction method, nematode species and the time and condition of storage. The number changes as the period between sampling and extraction increases. Therefore, in experimental programmes soil samples should be regarded as perishable and cared for accordingly.

Many plant parasitic nematodes can survive for months or years in soil stored in polythene bags. Longidorus elongatus survived at 18°C in moist soil for 29 months (Harrison and Hooper, 1963) Radenpholus similis, however lived less than six months when buried in such containers (Tarjan, 1961). The total number of nematodes remained at the same level for

18 weeks when the samples were stored at 6°C (Oostenbrink, 1960). Most species can be kept at 10-15°C for several months (Barker and Nusbaum, 1971). The adverse effects of high temperature have been shown by Bergeson (1959) and Barker *et al.* (1969b). Wallace (1963) concluded that low temperature range over which most plant-parasitic nematodes become inactive is about 5 to 15°C the optimum range 15-30°C and the high temperature range for inactivity is 30°C to 40°C. Outside this range temperatures are lethal.

Where sugar-flotation sieving technique is available soil samples can be stored at -15°C (Barker *et al.*, 1969b).

If extraction methods employing Baermann funnel or cotton wool filters are to be used, the optimum temperature (10-15°C) for maintaining nematodes in a physiologically active stage should be used for storage (Van Gundy *et al.*, 1967).

Barker, Nusbaum and Congleton (1968) reported that when no storage of samples were compared to long term storage of 4-16 weeks at the optimum temperatures of 13-24°C did not decrease the number of most species recovered by either extraction technique (3 and 12). Dasgupta and Raski (1968) worked on survival of Rotylenchulus parvus in soil at different temperatures reported that nematode population decreased with lower temperatures and longer period of exposure. Four weeks after storage at 1°C only one larvae was recovered from soil and only a few from the soil stored at 4°C.

Barker, Nusbaum and Nelson (1969) found that storage of

soil sample in plastic bags at  $-15^{\circ}\text{C}$  for 1-16 weeks greatly increased the nematode recovery.  $13^{\circ}\text{C}$  was optimum temperature for all nematode genera.

The soil phase plays a vital role in the life history of plant feeding nematodes. Being obligate parasite nematodes grow and reproduce in close association, with their hosts, but the soil is the principal arena for population studies. The structure of nematode population also varies greatly with regard to developmental stage of individuals present and their relative density, age, vitality, activity and distribution. Fluctuations in population of plant-parasitic nematodes in general reflect the state of balance between two opposing phenomena, birth rate and death rate. Reliable extraction procedures are essential for accurate estimation of seasonal fluctuation of nematode populations in soil. Rainfall, season and soil depth also influence on the nematode populations. Little work has been done, however, on the quantitative estimation of nematode population densities as affected by the season.

Micoletzky (1921) was the first man to present evidence of seasonal fluctuation in nematode number and species. He found that nematode population was maximum in the fall and minimum in the spring. The seasonal trends were attributed to changes in temperature, moisture, plant growth and its decomposition. Seidenschwarz (1923) found maximum number in August and minimum in November through January. Wehnt (1957) correlated seasonal population trends of Pratylenchus sp. and Tylenchorhynchus sp.

to the growth of white dutch clover. Hollis and Fielding (1958) studied the intra seasonal fluctuations in population of Pratylenchus, Tylenchorhynchus, Trichodorus, Tylenchus, Psilenchus and Ditylenchus. Report on seasonal fluctuation in population studies of Pratylenchus have been accumulated. P. zaeae on tobacco and corn (Graham, 1951), Pratylenchus spp. on cultivated brambles (Goheen and Williams 1955), P. coffee on strawberry (Reggs et al., 1956), P. penetrans in and around strawberry roots. (DiEdwardo, 1961), P. penetrans in tobacco roots (Olthof, 1967) and Pratylenchus spp. on peach (Fliegal and Golden, 1968).

Sasser and Nusbaum (1955) studied the seasonal fluctuation in the population of 3 spp. of Meloidogyne in 2 year tobacco rotation plots with cotton and corn. Kondo (1957) found a marked fluctuation in M. incognita population. He found only a few larvae in winter, but the number increased in May, declined in June and increased again in July. Norton (1959) correlated the population fluctuation of Tylenchorhynchus bravedans with rainfall. Ferris and Bernard (1961) reported that population of Paratylenchus, Helicotylenchus and Tylenchorhynchus build up during the growing season and reached a peak near the end of this period. Population of genus Paratylenchus in soil usually reach a peak early in the season and a decline followed. Barker (1968) and Barker et al. (1969b) studied seasonal fluctuation of nematode species by three extraction techniques and found that the method of extracting endo-parasitic nematodes from soil greatly influence the number recovered during

certain period of the year. Ducharme (1967) studied on Radopholus similis on citrus, grapes and always found maximum number of nematodes from October to December, the lowest always occurring during February to June. O'Bannon (1972) studied the seasonal changes in the population of 3 parasitic nematodes in Florida. He observed that Tylenchulus semipenetrans and Pratylenchus brachyurus had high and low population levels each year whereas the population of Pratylenchus coffea varied widely and were not related to season.

Control of nematodes in the soil by chemical means is dependent upon bringing the nematode-toxic chemical, termed as/nematicide, into contact with the nematodes in concentration high enough to kill them. Such contact can be accomplished by several means, including mechanical dispersal through the infested soil, percolation in water, or more commonly through a gaseous diffusion of a nematicidal fumigant through the pore spaces of the soil.

Klein and Allison (1957) prepared a soil mixture of sand, loam and peat which they infested with Meloidogyne, after which the soil was treated in sealed containers. After a desired test period, four days old cucumber seedlings were transplanted into treated and untreated soil and the root assayed for root-knot nematode one week later. Bishop (1958) has reported a simple procedure in which the nematodes are exposed to the materials, washed by aid of centrifugation and then tested for host penetration by adding to plants growing in vermiculite in

test tubes. Feldmesser and Feder (1955) discussed the use of various kind of test nematodes in vitro tests. They also covered the topics of indicator plants, formulations and dose rate, use of standard nematicides for comparison, interpretation of results and the correlation between the laboratory and field results.

## MATERIALS AND METHODS

Six experiments viz. (i) comparative efficiency of soil samplers, (ii) distribution and sampling of nematodes; (iii) comparative efficiency of nematode extraction techniques; (iv) effect of storage bag, storage temperature, storage period and extraction techniques on recovery of nematodes from the soil sample; (v) quantitative estimation of nematode population density by six extraction procedures as affected by season and seasonal fluctuation in nematode population; and (vi) evaluation of nematicidal efficiency with two techniques and timings of nematode inoculations, were carried out for evaluating some techniques in Nematology. The materials used and methods employed are presented below :

### I. Comparative efficiency of soil samplers.

This experiment was conducted in plot No. 13/3 of the Division of Vegetable Crops and Floriculture, Indian Agricultural Research Institute, New Delhi. A border of 10 m was left out and five sub-plots of 3 x 3 m were marked out - four of these were near the four corners and the fifth was in the centre. Khurpi, hollow tube type sampler with 1 cm diameter, O'Connor's split corer and hollow tube type sampler with 2.5 cm diameter were used for soil sampling. Three composite soil samples each consisting of 5 probes with the help of each sampler were collected from each plot. Similarly, composite samples with 15 and 30 probes were collected with the help of each sampler. Thus, there were 15 composite samples collected by means of each sampler. Similarly,

there were 15 composite samples for 15 and 30 sub-samples. The time taken in collecting one composite sample consisting of 5 or 15 or 30 sub-samples with the help of different samplers was also recorded.

Each composite soil sample was placed on a polythene sheet and mixed thoroughly by cone and corner method. Then the soil was spread on the sheet and 100 g was collected from different places for processing for which Cobb's modified decanting and sieving technique (Christie and Perry, 1951) was followed. The soil was placed in a bucket and about one litre of water was added to it. It was thoroughly mixed to bring it to the form of suspension. This suspension was passed through 20 mesh sieve to remove pieces of stones, debris etc. This suspension was allowed to settle for 30 seconds and was passed through 60 mesh sieve. The residue on 60 mesh was subsequently processed two times. Similarly, the process was repeated with 100, 200 and 325 mesh sieves. The final catch on 325 mesh sieve was poured on an aluminium wire support over a 10 cm petri-dish containing tap water touching the bottom of the wire support and was kept for 48 hours. The nematode suspension thus obtained from the petri-dish was made to 100 ml by adding tap water and an aliquot of 5 ml was pipetted out into a counting dish. While the aliquot was taken, the suspension was all the time kept homogenous by blowing through the mouth. Three such aliquots were processed for counting. The number of different genera of nematodes present were counted with the help of a stereoscopic binocular microscope

and their number in 100 g soil was calculated.

## II. Distribution and sampling of nematodes.

This experiment was conducted in Top Block-I of the Division of Agronomy, Indian Agricultural Research Institute, New Delhi. Five sub-plots (four from corners and one from centre) of 5 x 5 metre were selected as reported in the earlier experiment. Each sub-plot was further divided into 25 micro-plots of one sq. metre. Soil samples were drawn from the centre of each micro-plot with the help of a hollow tube type sampler with 2.5 cm diameter. The sampling was done upto a depth of 15 cm. The samples obtained from one micro-plot was mixed thoroughly and 50 g soil was processed for nematode extraction. The method of processing was similar to the one reported for Experiment 1.

## III. Comparative efficiency of nematodes extraction techniques (soil samples processed immediately after collection).

About 5 kg soil was collected with the help of a hollow tube type sampler with 2.5 cm diameter from a sugarcane field in the Top Block-I of the Division of Agronomy, Indian Agricultural Research Institute, New Delhi. The soil was thoroughly mixed and screened through 2 mm screen to remove stubbles, root debris etc. Fifty g soil sample was processed by each of the eleven techniques as presented below :

### 1) Direct soil placement over moistened facial tissue

paper : A thin layer of 50 g soil was kept on moistened facial tissue paper supported by a rectangular (20 x 15 cm size) aluminium wire support. It was placed over an enamel tray (20 x 15 cm size)

*How many layers*

containing tap water touching the wire support. After 48 hours the nematode suspension was concentrated by transferring the suspension into 1000 ml separating funnel. After four hours 100 ml suspension was taken in a beaker by opening the tap gently and the nematode counts were made as reported earlier.

ii) Oostenbrinks/elutriator : A constant water stream of 1000 ml/minute was allowed to enter the bottom of the can through a perforated pipe to fill the Oostenbrink's elutriator upto mark I. Soil sample (50 g) was placed in 1 mm pore size top sieve and was washed into the can via a funnel by means of a nozzle delivering about 750 ml/minute until the column was filled upto mark II. The top nozzle was then closed and the water stream of 1000 ml/minute was reduced to 750 ml/minute till the water level reached mark III. The suspension through opening tube was poured into a set of three 325 mesh sieves of 30 cm diameter, placed on top of one another. The catch was taken in a plastic pan and the nematode suspension was again passed through 325 mesh sieve of 16 cm diameter to reduce the amount of water. Nine such samples were processed and were further treated as follows :

- ii(a). Residue from 325 mesh sieve was poured on double layer of cotton wool filter (3 samples).
- (b). Residue from 325 mesh sieve was poured on single layer of cotton wool filter (3 samples).
- (c). Residue from 325 mesh sieve was poured on double layer of facial tissue paper (3 samples).

These were placed on extraction trays containing tap water touching the bottom of the support and were kept for 48 hours. The nematodes obtained were counted as reported earlier.

iii). Cobb's modified decanting and sieving technique :

The method adopted was similar to that as reported for Experiment I with the exception that here only 50 g soil sample was processed.

iv). Sugar flotation sieving technique with 12.5 ppm

Separan SP<sub>7</sub> : The soil sample (50 g) was placed in a bucket. About 350 ml of 1.0 M sugar solution (342 g sugar dissolved in one litre of water) containing 12.5 ppm Separan SP<sub>7</sub> was poured into the bucket. This was stirred for 20 seconds and allowed to settle for 4 minutes. The soil suspension was then poured through 20 mesh sieve set over 325 mesh sieve. The residue over 325 mesh sieve was rinsed and washed into a beaker. The contents were swirled, allowed to settle for a few seconds and then poured back on to the 325 mesh sieve leaving behind heavier particles. The residue on 325 mesh sieve was poured into a beaker and the nematode suspension was used for direct examination and counting which was done as in previous experiments.

v) Sugar flotation sieving technique with 12.5 ppm

Separan PG<sub>2</sub> : The method adopted was similar to the one reported for technique iv, with the exception that here only 12.5 ppm Separan PG<sub>2</sub> was used in place of 12.5 ppm Separan SP<sub>7</sub>.

vi) Cobb's modified decanting and sieving technique +

sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue : The soil sample (50 g) was processed through Cobb's modified

decanting and sieving technique. The catch on 325 mesh sieve was poured into a bucket. About 500 ml of 1.0 M sugar solution (342 g sugar dissolved in one litre of water with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue) was poured into the bucket. Other procedures were same as described for iv above.

vii) Cobb's modified decanting and sieving technique + sugar flotation sieving with 12.5 ppm Separan PG<sub>2</sub> + 5 ppm methylene blue : The method adopted was similar to the one reported for technique v with the exception that here 5 ppm methylene blue was also used besides 12.5 ppm Separan PG<sub>2</sub>.

viii) Centrifugal flotation : The soil sample (50 g) was distributed into four fifty ml centrifugal tubes with about 30 ml of water in each. After balancing for weight the soil was shaken well and was centrifuged for 5 minutes at 3000 rpm. The supernatant liquid was poured off and sugar solution of 1.18 specific gravity (484 g sugar dissolved in one litre of water) was added. The tubes were shaken well and were recentrifuged for 2 minutes at 3000 rpm. The supernatant liquid was poured off through 325 mesh sieve and the residue over the sieve was quickly rinsed with/tap water. The counting and identification of different nematode genera was done under stereoscopic binocular microscope as described earlier.

ix) Cobb's modified decanting and sieving technique + centrifugal flotation : The soil sample (50 g) was processed through Cobb's modified decanting and sieving technique. The residue over 325 mesh sieve was equally distributed in four

50 ml centrifugal tubes, leaving 1/4 part unfilled. The other procedures were the same as described for viii above.

x) Cobb's modified decanting and sieving technique + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> with 5 ppm methylene blue : The method adopted was similar to one adopted for ix above, with the exception that in the sugar solution, 12.5 ppm Separan SP<sub>7</sub> and 5 ppm methylene blue was also added.

xi) Seinhorst's (erlenmeyer) flask method : The soil sample (50 g) was placed in a bucket in which 500 ml water was poured and mixed thoroughly. The soil suspension was passed through 20 mesh sieve and was poured into one litre flask. The bucket was washed and the remaining water poured into one litre flask (A). A short plastic funnel, with an outlet aperture 12 mm in diameter, was attached with a rubber sleeve. By closing the funnel orifice with the finger, the flask was shaken thoroughly and inverted over a second similar flask (B) filled with fresh water. After 10 minutes, flask (A) was placed on similar flask (C) and flask (B) was placed on flask (D) which were also filled with fresh water. The final suspension of (A) and (B) was collected over 325 mesh sieve and the contents of (C) were collected over 200 mesh sieve. The residue of both the sieves was collected in a beaker. Counting and identification of different nematode genera was done under stereoscopic binocular microscope as described earlier.

IV. Effect of storage bag, storage temperature, storage period (week) and extraction techniques on the recovery of nematodes from soil samples.

About 32 kg soil was collected with the help of a hollow tube type sampler with 2.5 cm diameter from a sugarcane field in Top Block-I of the Division of Agronomy, Indian Agricultural Research Institute, New Delhi. The soil was thoroughly mixed and screened through 2 mm screen to remove pieces of stones, stubbles and root debris. Sixteen polythene (thickness 400 gauge) and sixteen paper bags were filled with soil samples containing one kg soil in each. Two bags of each unit were kept at eight temperatures viz. 0, 5, 10, 15, 20, 25, 30°C and at room temperature, for six periods viz. one, two, three, four, six and eight weeks. The room temperature during the period ranged from 15-20°C. One hundred and fifty g soil was taken out from each bag after the desired period. It was processed for nematode extraction by three nematode extraction techniques :

a) Cobb's modified decanting and sieving technique :

The method adopted was similar to those reported for (iii) of Experiment III.

b) Oostenbrink's elutriator : The method adopted was similar to those reported for (iic) of Experiment III.

c) Cobb's modified decanting and sieving technique + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> and 5 ppm methylene blue : The method adopted was similar to those reported for (x) of Experiment III.

V. Quantitative estimation of nematode population densities by six extraction procedures as affected by season and seasonal fluctuation in nematode population.

Two experiments were carried out to compare the efficiency of six nematode extraction procedures in measuring seasonal population shifts of plant-parasitic and free-living nematodes. The extraction techniques employed were :

- i) Direct soil placement over moistened facial tissue paper (DT).
- ii) Oostenbrink's elutriator with double layer of facial tissue paper (OD).
- iii) Cobb's modified decanting and sieving technique (CS).
- iv) Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue (CSSSM).
- v) Cobb's modified decanting and sieving + centrifugal flotation (CSCF).
- vi) Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue (CSCFSM).

The soil sample for the first experiment was collected from Block-II of the Division of Genetics, Indian Agricultural Research Institute, New Delhi. An area of 125 x 125 m was selected after leaving 10 m border. This area was sub-divided into 25 sub-plots of 25 x 25 m. These sub-plots were given to maize and wheat rotation. Beginning from August, 1971 the samples were taken at monthly intervals for one year. The

samples in the month of August, September and October were collected from maize. From November to April these were collected from wheat. Samples in May, June and July were taken from fallow. The soil temperature and rainfall during the period ranged from 13.0° to 33.8°C and from 0 to 35.8 cm respectively. These have been given graphically in Figure V-A. Fifteen probes from a 25 x 25 m area comprised one composite sample (about 1 kg). The soil samples were taken with a hollow tube type sampler with 2.5 cm diameter. Each sample was thoroughly mixed and 50 g was processed by each technique. This was done within 24 hours after collection. Further details about the processing by each technique were the same as reported for Experiment III on comparative efficiency of nematode extraction techniques.

The second experiment was conducted in Top Block-I of the Division of Agronomy, Indian Agricultural Research Institute, New Delhi. In this case also an area of 125 x 125 m was selected after leaving 10 m border and it was sub-divided into 25 sub-plots of 25 x 25 m. These sub-plots were given to eight crop rotations viz :

- i) Sugarcane-wheat-moong.
- ii) Jowar/baira-wheat.
- iii) Moong-wheat-lobia.
- iv) Maize (Cobb)-raddish-wheat.
- v) Moong + pigeon pea-wheat.
- vi) Maize-potato/toria-wheat-moong.
- vii) Maize-wheat-moong-baira/jowar.
- viii) Maize-oat/berseem/cowpea (for fodder).

Soil samples were collected in the same manner as reported for the first experiment. The details regarding the number of samples collected from various crops have been given in Table 63.

Twenty five samples in the month of August and September comprised 14 samples from maize, 4 samples from sugarcane, 2 samples from baira and one each from pigeon-pea, pigeon-pea + moong, lowar + pigeon-pea, lowar + moong and lowar. In October also the samples were collected from the same crops except in one case where in place of moong one more sample was taken from lowar. In November 25 samples were collected as follows : 2 samples from baira, 4 samples from sugarcane, 3 samples from pigeon-pea, one sample from lowar, 2 samples from raddish, 4 samples from potato + toria, 5 samples from wheat, 2 samples from oat and 2 samples from berseem. Maximum samples in December were collected from wheat (13). This was followed by sugarcane and potato + toria (4 in each). The remaining 4 samples were taken from oat and berseem (2 in each). In January, February and March maximum number of samples were collected from wheat (17 samples), followed by sugarcane (4 samples), oat (2 samples) and berseem (2 samples). In April the number of samples from wheat and sugarcane remained the same but in place of oat and berseem, 4 samples were collected from cowpea. In May and June maximum number of samples were collected from moong (10 samples), followed by sugarcane and cowpea (4 in each). In May, 2 samples were collected from lobia, 2 samples from lobia + moong and one sample from fallow. In

June, 3 samples were collected from lobia + moong and 3 samples from fallow. In July maximum samples were collected from maize (11 samples), followed by sugarcane (4 samples), baira (2 samples) pigeon-pea + moong (2 samples), cotton (2 samples) and pigeon-pea, jowar + pigeon-pea, moong and jowar (1 in each).

VI. Evaluation of nematicidal efficacy with two techniques and two timings of nematode inoculation.

This experiment was conducted in pots of 23 cm diameter each containing 3 kg of sterilised soil with tomato (Lycopersicum esculentus) variety Pusa Ruby. The seed was obtained from the Division of Vegetable Crops and Floriculture, Indian Agricultural Research Institute, New Delhi. The soil used for this experiment was collected from the Division of Agronomy, Indian Agricultural Research Institute, New Delhi.

For raising tomato nursery the field soil mixed with FYM in proportion of 4 : 1 by volume was passed through 16 mesh sieve to remove large stone, clods, and other materials. It was sterilised with Methyl Bromide @ 1 lb/100 cu.ft. This soil was filled in clean wooden trays and tomato seeds were sown in lines. The nursery was maintained in the glass house.

For maintaining a pure culture of Meloidogyne incognita (Kofoid and White 1919) Chitwood, 1949, in pots, soil was prepared by mixing field soil and FYM in the ratio of 4 : 1 by volume. It was used after Methyl Bromide fumigation. An examination carried out 48 hours after fumigation indicated that the fumigated soil was completely devoid of nematodes.

The sterilised soil was filled in washed 23 cm diameter earthen pots. Tomato seedlings (5 weeks old - variety Pusa Ruby) raised in sterilised soil were transplanted. M. incognita egg masses were obtained from the stock culture maintained in the Division of Nematology, Indian Agricultural Research Institute, New Delhi. The egg masses were incubated for hatching in 10 cm petri-dishes containing tap water. Inoculation was done @ 200 larvae/pot. For this purpose, 3 holes were made around the stem. These were 2.4 cm deep and 3-4 cm away from the stem. After inoculation the holes were closed. Periodical examinations were made to ensure proper gall development and egg production. Sub-culturing was also done to meet the requirement and altogether, about 100 culture pots were maintained for this experiment.

Two nematicides (DD and DBCP) were used at two dosages (DD @ 50 and 100 gallons and DBCP @ 5 and 10 gallons/hectare). Each treatment including control pots was replicated three times. The required amount was calculated on the area basis i.e. for 23 cm diameter earthen pots. The application was done 15 days before transplanting tomato seedlings. The required amount of each chemical was calculated and diluted in measured quantity of water in such a way that 5 ml and 10 ml solution/pot met the requirements of the treatments. Nematicides were injected by making two holes, with the help of a glass rod at a depth of 10 cm in the centre of each pot. After injection the holes were closed and sealed with water. Wherever, nematodes were inoculated three weeks after the nematicidal treatment, the chemicals were applied

one week earlier than the other treatments.

Nematode inoculation was done in two ways (i.e. one at the rate of 2000 larvae/pot and the other at the rate of 5 g chopped infested root galls/pot) and at two timings (i.e. one at the time of applying nematicides and other one week after transplanting/three weeks after nematicidal application). The details are furnished below :

a) Inoculation of 2000 larvae/pot at the time of nematicidal application : Seventy-two cleaned earthen pots were taken. These were filled with sterilised soil. Two thousand larvae were inoculated in each pot and the soil was thoroughly mixed to ensure homogeneous distribution of nematodes. Nematicides were applied as indicated above.

b) Inoculation of 5 g chopped infested root galls/pot at the time of nematicidal application : Seventy-two cleaned earthen pots were filled with sterilised soil. 5 g chopped infested root (ranging from 500-800 eggs and 1000-1500 larvae per 5 g root tissue) were added in each pot. The soil was well mixed to ensure uniform distribution of root galls in the soil. Nematicides were applied in the same manner as described earlier.

c) Inoculation of 2000 larvae/pot one week after transplanting/3 weeks after nematicidal application : Nematicides were applied in seventy-two pots in the same manner as described earlier. After fifteen days of nematicide application 5 weeks old, equal size tomato seedlings were transplanted. Two thousand larvae/pot were inoculated in the same manner as described above.

d) Inoculation of 5 g chopped infested root galls/pot one week after transplanting/3 weeks after nematicidal application: Fifteen days after nematicidal application, 5 weeks old equal size tomato seedlings were transplanted. Five g chopped infested root galls were inoculated in each pot near the root zone of tomato plants through 3 holes, 2-4 cm deep and 3 cm away from the stem.

Observations were recorded one, two, three, four, five, and six weeks after inoculation nematodes. Soil and root population of nematode were estimated.

- i) Estimation of nematode in soil : The soil collected from each treatment (3 replications) was mixed thoroughly and a sample (50 g) soil was taken for processing. The extraction of nematode population from soil was done with Cobb's modified decanting and sieving technique as reported earlier. The number of root-knot larvae present in the soil was counted.
- ii) Number of root galls : The root system of all the plants was examined after washing the roots and the number of galls was counted.
- iii) Estimation of nematode population in root tissue : The tops of plants were cut and the root system was removed carefully without any damage. Complete root system was washed with tap water and chopped into small pieces by means of a pair of fine scissors. Complete chopped root from each plant of each

treatment was placed in a Waring Blender 1/3rd of which was filled with tap water. The roots were blenderised for 15 seconds. The blenderised material was passed through 20 mesh sieve. The residue over the sieve was discarded. The suspension which was passed through 20 mesh sieve was passed through 325 and 400 mesh sieves. The catch on these sieves was collected in a beaker.

The blenderised suspension collected in beakers was equally distributed in 50 ml plastic centrifuge tubes. To facilitate the setting of nematodes about 5 mg of kaolin was added in each tube. This was centrifuged for 5 minutes at 3000 rpm. The tubes were taken out from the centrifuge and upper half of the material was removed from the tubes. These tubes were filled with sugar solution (454 g sugar/litre of water) and the material was thoroughly mixed with sugar solution so that the nematodes which settled with kaolin could also come in the suspension. The suspension was equally filled in the four tubes and again centrifuged for 3 minutes at 3000 rpm. The suspension from all the tubes was immediately passed through 325 and 400 mesh sieve and the catch was collected in petri-dishes. Nematodes were counted in the same way as described earlier. The total population of all the stages of M. incognita extracted from root tissue was estimated.

## EXPERIMENTAL RESULTS

Six experiments viz. (i) comparative efficiency of soil samplers, (ii) distribution and sampling of nematodes, (iii) comparative efficiency of nematode extraction techniques, (iv) effect of storage bag, storage temperature, storage period and extraction techniques on recovery of nematodes from the soil sample, (v) quantitative estimation of nematodes population density by six extraction procedures as affected by season and seasonal fluctuation in nematode population, and (vi) evaluation of nematicidal efficiency with two techniques and timings of nematode inoculations, were carried out for evaluating some techniques in Nematology. The results obtained in respect of each experiment are presented below :

### I. Comparative efficiency of soil samplers

Four types of soil sampling tools were used to test their efficiency in sampling soil for nematodes. These were : Khurpi, hollow tube type sampler with 1.0 cm diameter, O'Connor's split corer and hollow tube type sampler with 2.5 cm diameter. One composite soil sample consisted of 5 or 15 or 30 sub-samples.

An examination of soil samples indicated the prevalence of 8 genera of plant-parasitic nematodes (viz. Rotylenchulus sp., Tylenchorhynchus sp., Hoplolaimus sp., Pratylenchus sp., Meloidogyne larvae, Helicotylenchus sp., Trichodorus sp. and Tylenchus sp.), besides free-living ones. The data in respect of each as also for the total plant parasitic nematodes have

been furnished in Tables 1 and 2 and shown in histograms in Fig. I. It was seen that in general maximum number of nematodes were obtained when the soil samples were collected with the help of hollow tube type sampler with 2.5 cm diameter. Sampling with Khurpi and hollow tube type sampler with 1 cm diameter provided lower number of nematodes. There were few exceptions as in the case of Tylenchorhynchus in respect of which O'Connor's split corer was inferior to Khurpi/hollow tube type sampler with 1.0 cm diameter which were at par with hollow tube type sampler with 2.5 cm diameter.

It was seen that irrespective of sampling tools used 15 probes per sample provided maximum number of nematodes except in respect of Tylenchorhynchus, Hoplolaimus and Meloidogyne with O'Connor's split corer and in case of Trichodorus and Tylenchus with hollow tube type sampler with 1.0 cm and 2.5 cm diameter respectively in which case 5 or 30 probes resulted in higher number of nematodes.

In seven cases there was no significant difference due to difference in number of probes per sample in respect of hollow tube type sampler with 2.5 cm diameter. It was also true for 5, 3, and 4 cases in respect of O'Connor's split corer, hollow tube type sampler with 1.0 cm diameter and Khurpi respectively.

In case of Rotylenchulus there was no difference due to number of probes per sample irrespective of the sampling tool used. This was similar in respect of Meloidogyne except

TABLE - 1

Comparative efficiency of soil samplers based on the recovery of nematodes from composite soil samples consisting of 5, 15 or 30 sub-samples from 3x3 m. area

Nematode Genera	Soil samplers/nematode population in 100 g soil *																
	Khurpi			Hollow tube type sampler with 1 cm. dia.			O'Conner's split corer with 2.5 cm. dia.			Hollow tube type sampler with 2.5 cm. dia.							
	No. of sub-samples	5	15	30	No. of sub-samples	5	15	30	No. of sub-samples	5	15	30	C.D. 5% Sub-samples	5	15	30	Over-all samples
<u>Rotylenchulus</u>	80	164	65	75	74	67	142	141	103	165	183	140	29.4	39.8	36.9	42.7	
<u>Tylenchorhynchus</u>	370	450	336	332	428	384	281	264	376	394	446	416	60.6	50.3	202.3	64.6	
<u>Hoplolaimus</u>	115	228	104	120	188	132	215	145	166	290	328	284	32.3	208.6	31.4	56.6	
<u>Pratylenchus</u>	212	328	240	316	336	300	346	388	266	386	412	352	52.7	61.6	41.1	26.0	
<u>Meloidogyne</u> <u>larvae</u>	256	260	224	272	272	304	300	228	276	392	423	400	63.6	85.0	236.0	82.0	
<u>Helicotylenchus</u>	326	400	300	372	416	336	404	360	389	440	470	400	46.3	63.7	68.3	67.7	
<u>Trichodorus</u>	48	86	64	60	60	89	65	90	100	120	100	122	17.0	21.6	31.6	27.7	
<u>Tylenchus</u>	47	51	35	69	72	40	64	63	59	124	93	85	38.4	25.6	19.8	25.5	
Total plant parasitic	1516	1927	1438	1622	1834	1710	1854	1694	1730	2315	2467	2293	164.3	135.1	123.8	174.6	
Free-living nematodes	512	608	524	515	648	630	730	638	608	752	788	724	118.9	73.5	71.6	100.3	

\* Average of 5 replications.

TABLE - 2

Showing coefficient of variance for 5, 15 and 30 sub-samples

Nematode genera	Sub-samples		
	5	15	30
<u>Rotylenchulus</u>	21.0	28.4	19.1
<u>Tylenchorhynchus</u>	12.9	11.8	9.6
<u>Hoplolaimus</u>	12.8	21.7	10.3
<u>Pratylenchus</u>	12.0	12.5	10.3
<u>Meloidogyne</u> larvae	14.9	31.2	20.4
<u>Helicotylenchus</u>	8.5	11.3	13.0
<u>Trichodorus</u>	17.4	11.8	24.3
<u>Tylenchus</u>	36.1	26.8	105.6
Total plant parasitic nematodes	6.5	5.0	4.6
Free-living nematodes	13.7	8.5	8.3

TABLE - 3

Time required for collecting soil samples by different soil samplers (5, 15 and 30 sub-samples from 3 x 3 m area).

Samplers	Time in minutes		
	Sub-samples/plot (3 x 3 m)		
	5	15	30
<u>Khurpi</u>	3.3	7.3	15.8
Hollow tube type sampler with 1 cm diameter	3.5	9.5	17.8
O'Connor's split corer	7.7	12.5	36.3
Hollow tube type sampler with 2.5 cm diameter	5.2	8.8	19.9
C.D. 5%	2.1	4.5	15.9

in case of O'Connor's split corer, with 5 and 15 probes/sample. Also there was no difference due to number of probes/sample in respect of Helicotylenchus and Trichodorus when the samples were collected with the help of O'Connor's split corer or hollow tube type sampler with 2.5 cm diameter.

A perusal of C.V. values revealed that in seven cases it was lower when the composite samples consisted of 30 probes. It was maximum in case of Tylenchus which recorded the least numbers.

An examination of Table 3 revealed that the time taken in collecting one composite sample consisting of 5-30 probes was more in respect of O'Connor's split corer. It was also true in case of 15 probes except that in this case it was not different than hollow tube type sampler with 1 cm diameter. So far as this finding is concerned there was no real difference in respect of Khurpi, hollow tube type sampler with 2.5 cm diameter and hollow tube type sampler with 1.0 cm diameter.

## II. Distribution and sampling of nematodes.

In April 1971, 5 plots of 5 x 5 m were selected in a big field (150 x 150 m). The predominant nematode genera in the soil were Tylenchorhynchus, Hoplolaimus, Pratylenchus, Heterodera larvae, Helicotylenchus, Trichodorus, Hemicriconemoides and free-living nematodes. Each plot was divided into 25 micro plots of one sq. m. Starting from one corner of the plot the micro-plots were systematically numbered and one soil sample was collected

from each micro-plot. The soil samples were processed through Cobb's modified decanting and sieving techniques and the nematodes were counted. The sub-plot-wise data have been given in Tables 4-12.

The distribution of the nematodes in the subplots has been shown separately for each nematode as also for the total plant-parasitic nematodes by dot diagrams in Fig. II(A-J).

It was noticed that nematode population of some genera like Hemicriconemoides, Trichodorus, Heterodera larvae and Hoplolaimus were very sparse. The density in the plots in respect of Trichodorus ranged from 0-100, Heterodera 0-90 and Hoplolaimus 0-70. Further, it was noticed that the distribution of nematodes in the micro-plot was not uniform.

To evolve a suitable sampling technique for estimating the nematode population in the field at a desired level of accuracy, the data were examined after square-root transformation ( $\sqrt{n + 0.5}$ ). The idea was to determine the minimum number of rows and minimum number of samples (units) per row in order to have a steady estimation of the nematode population. Different hypothetical combinations of the rows x samples (units) have been examined to obtain the estimate with percentage error at 20 per cent and 10 per cent. These have been shown in Table 13.

Keeping the number of rows fixed at five, the number of units to be sampled per row has come out 16 to 24 per cent and 20 to 60 per cent for percentage error of 20 per cent and 10 per cent respectively in case of Tylenchorhynchus.

TABLE - 4

Showing the distribution of Tylenchorhynchus population in 50 g soil sample/sq. m. area

Sample No.	Replications				
	1	2	3	4	5
1	230	210	430	135	30
2	160	420	260	230	0
3	200	200	45	170	140
4	160	160	120	60	35
5	130	240	270	359	275
6	220	260	0	100	90
7	180	170	280	210	0
8	260	180	150	15	0
9	105	80	90	0	100
10	15	330	280	180	145
11	180	225	350	150	70
12	240	140	310	30	80
13	250	100	230	200	0
14	0	430	210	510	0
15	90	45	210	20	315
16	0	40	0	370	195
17	80	470	0	90	15
18	180	360	380	190	0
19	275	380	210	190	30
20	190	270	225	100	15
21	210	0	0	0	140
22	200	0	620	190	0
23	0	320	280	0	140
24	150	230	210	320	120
25	0	80	0	0	150

TABLE - 5

Showing the distribution of Hoplolaimus population in 50 g soil samples/sq. m area.

Sample No.	Replications				
	1	2	3	4	5
1	20	0	20	0	0
2	0	0	0	0	0
3	0	0	0	30	0
4	0	0	30	15	0
5	30	30	0	10	0
6	10	10	0	15	15
7	20	45	45	20	60
8	10	40	45	40	0
9	0	110	0	4	0
10	0	90	23	10	10
11	0	0	0	0	0
12	0	0	0	0	0
13	50	40	30	0	30
14	60	30	45	0	75
15	90	0	25	0	0
16	0	60	0	0	0
17	0	20	0	0	0
18	70	50	0	0	0
19	40	30	0	0	0
20	10	15	0	70	0
21	0	30	15	0	0
22	40	60	15	0	40
23	0	0	10	0	25
24	0	0	0	10	10
25	0	0	0	30	20

TABLE - 6

Showing distribution of *Pratylenchus* population in  
50 g soil sample/sq. m. area

Sample No.	Replications				
	1	2	3	4	5
1	0	90	240	130	20
2	0	60	70	0	30
3	20	120	0	0	0
4	10	0	270	190	120
5	30	130	210	75	180
6	40	70	220	120	110
7	60	20	120	60	30
8	30	180	165	0	0
9	20	40	180	190	5
10	0	90	215	40	0
11	40	0	0	0	0
12	30	165	0	0	0
13	50	50	420	170	205
14	30	0	0	40	0
15	15	30	0	0	40
16	40	40	130	70	200
17	30	50	180	10	0
18	0	70	120	80	0
19	100	20	290	100	120
20	45	0	100	0	100
21	0	160	235	80	30
22	30	80	120	270	70
23	10	20	0	120	210
24	20	120	20	0	0
25	0	80	15	0	215

TABLE - 7

Showing distribution of Heterodera larvae  
population in 50 g soil sample/sq. m. area

Sample No.	Replications				
	1	2	3	4	5
1	0	20	60	45	30
2	45	15	0	40	40
3	50	105	50	60	0
4	30	0	0	0	0
5	0	30	0	0	0
6	30	60	10	35	40
7	0	30	15	40	45
8	0	20	10	10	25
9	40	0	20	10	15
10	0	10	0	60	30
11	0	0	0	0	0
12	60	15	15	5	20
13	50	50	50	170	100
14	40	90	90	45	45
15	25	0	240	40	90
16	30	40	10	35	75
17	0	0	0	0	60
18	0	0	60	60	0
19	80	120	60	70	30
20	0	30	105	75	0
21	0	30	45	10	0
22	0	20	210	0	0
23	0	0	0	0	90
24	80	30	60	80	60
25	60	80	0	5	65

TABLE - 8

Showing distribution of Helicotylenchus  
population in 50 g soil sample/sq.m. area

Sample No.	Replications				
	1	2	3	4	5
1	20	110	160	0	30
2	120	45	20	120	20
3	0	90	230	170	35
4	50	20	240	0	0
5	80	110	70	15	45
6	0	80	40	15	60
7	10	50	60	40	25
8	0	90	180	80	0
9	90	60	40	70	35
10	0	40	40	40	25
11	100	175	60	70	0
12	0	90	135	100	170
13	25	30	180	0	35
14	40	90	90	45	10
15	30	75	0	60	65
16	0	10	15	0	100
17	0	20	110	30	50
18	15	0	170	50	65
19	0	100	0	0	0
20	30	40	0	100	70
21	0	0	105	60	40
22	0	115	0	0	80
23	70	60	30	0	0
24	0	50	100	0	0
25	0	0	0	150	190

TABLE - 9

Showing distribution of Trichodorus population  
in 50 g soil sample/sq. m. area

Sample No.	Replications				
	1	2	3	4	5
1	30	10	45	60	15
2	10	45	10	30	30
3	10	45	0	100	15
4	30	0	0	0	0
5	0	30	0	0	0
6	40	10	60	0	0
7	10	0	0	0	0
8	30	20	80	0	15
9	30	10	0	15	35
10	20	15	0	0	0
11	30	0	0	0	0
12	0	0	0	10	30
13	0	0	0	0	0
14	10	10	40	10	0
15	35	0	0	0	0
16	100	20	0	40	35
17	0	30	10	0	0
18	0	0	30	45	0
19	0	10	50	50	0
20	10	0	45	0	0
21	0	20	0	0	0
22	0	10	10	0	15
23	0	0	0	0	45
24	0	0	0	0	30
25	60	0	10	0	70

TABLE - 10

Showing distribution of Hemicriconemoides  
population in 50 g soil sample/sq.m. area

Sample No.	Replications				
	1	2	3	4	5
1	0	20	10	0	0
2	0	0	30	0	0
3	10	0	0	0	0
4	10	20	45	0	0
5	0	30	20	5	0
6	0	60	30	0	0
7	0	15	10	10	0
8	60	0	10	10	0
9	0	0	0	170	0
10	0	20	30	0	0
11	0	0	0	200	0
12	10	25	15	150	0
13	30	30	30	40	0
14	0	30	50	0	0
15	0	80	0	0	0
16	0	20	40	0	0
17	0	45	30	40	0
18	20	15	45	30	0
19	20	0	30	0	0
20	0	0	0	60	0
21	0	0	40	0	0
22	0	0	0	15	0
23	0	0	60	0	0
24	0	0	0	40	0
25	0	60	70	130	0

TABLE - 11

Showing distribution of total plant parasitic  
nematodes population in 50 g soil sample/sq.m.  
area

Sample No.	Replications				
	1	2	3	4	5
1	300	35	350	285	255
2	340	285	425	380	270
3	350	195	455	285	10
4	320	380	180	110	250
5	300	420	375	170	365
6	460	595	400	325	355
7	660	300	435	600	550
8	560	540	300	595	310
9	210	340	680	615	230
10	600	550	330	240	220
11	965	595	470	475	440
12	415	330	515	630	975
13	325	640	930	805	380
14	705	400	525	330	390
15	570	360	475	195	95
16	370	330	430	435	345
17	340	640	260	410	285
18	535	400	580	265	350
19	265	360	710	170	420
20	465	330	120	515	315
21	125	500	70	365	210
22	120	360	270	195	205
23	190	370	340	125	520
24	520	285	260	130	170
25	500	15	510	425	710

TABLE - 12

Showing distribution of free-living nematodes  
population in 50 g soil sample/sq.m. area

Sample No.	Replications				
	1	2	3	4	5
1	170	190	230	165	110
2	110	290	90	160	240
3	140	135	180	310	200
4	160	110	240	160	150
5	80	160	105	170	290
6	115	180	170	195	305
7	75	185	160	350	275
8	130	120	145	210	350
9	105	140	120	390	180
10	475	130	215	200	230
11	195	225	180	150	270
12	90	120	85	190	300
13	200	145	190	250	235
14	120	310	180	290	290
15	125	190	120	190	270
16	310	230	190	170	180
17	150	110	70	170	240
18	180	240	190	180	170
19	170	200	180	280	270
20	105	310	180	460	280
21	195	240	175	160	260
22	275	180	195	100	315
23	250	160	80	240	200
24	265	710	250	270	630
25	130	610	200	510	245

TABLE - 13

Hypothetical combination of rows x units

Nematode genera	C.V.	Row No.	Units	Total No. of units	% of total units
<u>Tylenchorhynchus</u>	20%	1	4.0	4	16
		2	3.0	6	24
		3	2.0	6	24
		4	1.0	4	16
	10%	1	5.0	5	20
		2	4.0	8	32
		3	4.0	12	48
		4	3.0	12	48
		5	3.0	15	60
	<u>Hoplolaimus</u>	20%	1	5.3*	6
2			3.9	8	32
3			3.0	9	36
4			2.6	12	48
10%		1	6.7*	7	-
		2	5.6*	12	-
		3	4.8	15	60
		4	4.3	20	80
		5	3.9	20	80
<u>Pratylenchus</u>		20%	1	3.2	4
	2		2.8	6	24
	3		2.2	9	36
	4		1.8	8	32
	5		1.5	10	40
	10%	1	5.3*	6	-
		2	4.6	10	40
		3	4.0	12	48
		4	3.6	16	64
		5	3.2	20	80
<u>Heterodera</u> larvae	20%	1	4.0	4	16
		2	3.0	6	24
		3	2.5	9	36
		4	2.0	8	32
		5	1.8	10	40
	10%	1	5.0	5	20
		2	4.5	10	40
		3	4.0	12	48
		4	3.8	16	64
		5	3.2	20	80

Contd.

Nematode genera	C.V.	Row No.	Units	Total No. of units	% of total units
<u>Helicotylenchus</u>	20%	1	3.8	4	16
		2	2.9	6	24
		3	2.5	9	36
		4	2.0	8	32
		5	1.6	10	40
	10%	1	5.4*	6	-
		2	4.8	10	40
		3	4.2	15	60
		4	3.7	16	64
		5	3.5	20	80
<u>Trichodorus</u>	20%	1	5.5*	6	-
		2	3.9	8	32
		3	3.0	9	36
		4	2.5	12	48
		5	1.7	10	40
	10%	1	7.0*	7	-
		2	5.8*	12	-
		3	4.9	15	60
		4	4.0	16	64
		5	3.7	20	80
<u>Hemicriconemoides</u>	20%	1	5.2*	6	-
		2	3.9	8	32
		3	2.9	9	36
		4	2.6	12	48
		5	2.1	15	60
	10%	1	6.6*	7	-
		2	5.5*	12	-
		3	4.5	15	60
		4	4.2	20	80
		5	3.8	20	80
Total plant parasitic nematodes	20%	1	3.0	3	12
		2	1.6	4	16
		3	0.6	3	12
	10%	1	3.9	4	16
		2	2.9	6	24
		3	1.6	6	24
Free-living nematodes	20%	1	3.0	3	12
		2	2.2	6	24
		3	1.0	3	12
	10%	1	5.5*	6	24
		2	4.5	10	40
		3	3.8	12	48

\*Values are more than the required units.

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The number of units to be sampled per row has come out 32 to 48 per cent and 60 to 80 per cent for percentage error of 20 per cent and 10 per cent respectively in case of Hoplolaimus.

The number of units to be sampled per row has come out 16 to 40 per cent and 40 to 80 per cent for percentage error of 20 per cent and 10 per cent respectively in case of Pratylenchus.

The number of units to be sampled per row has come out 16 to 40 per cent and 20 to 80 per cent for percentage error of 20 per cent and 10 per cent respectively in case of Heterodera larvae.

The number of units to be sampled per row has come out 16 to 40 per cent and 40 to 80 per cent for percentage error of 20 per cent and 10 per cent respectively in case of Helicotylenchus.

The number of units to be sampled per row has come out 32 to 60 per cent and 60 to 80 per cent for percentage error of 20 per cent and 10 per cent respectively in case of Trichodorus.

The number of units to be sampled per row has come out 32 to 60 per cent and 60 to 80 per cent for percentage error of 20 and 10 per cent respectively in case of Hemicriciconemoides.

The number of units to be sampled per row has come out 12 to 16 per cent and 16 to 20 per cent for percentage error of 20 and 10 per cent respectively in case of total plant-parasitic nematodes.

The number of units to be sampled per row has come out

12 to 24 per cent and 40 to 43 per cent for percentage error of 20 and 10 per cent respectively for free-living nematodes.

III. Comparative efficiency of nematode extraction techniques (soil samples processed immediately after collection).

Eleven techniques viz :

- i) Direct soil placement over moistened facial tissue paper.
- ia) Oostenbrink's elutriator with double layer of cotton wool filter.
- ib) Oostenbrink's elutriator with single layer of cotton wool filter.
- ic) Oostenbrink's elutriator with double layer of facial tissue paper.
- ii) Cobb's modified decanting and sieving technique with double layer of facial tissue paper.
- iv) Sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub>.
- v) Sugar flotation sieving with 12.5 ppm Separan PG<sub>2</sub>.
- vi) Cobb's modified decanting and sieving + sugar flotation<sup>sieving</sup> with 12.5 ppm Separan PG<sub>2</sub> + 5 ppm methylene blue.
- vii) Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue.
- viii) Centrifugal flotation.
- ix) Cobb's modified decanting and sieving technique + centrifugal/flotation.

x) Cobb's modified decanting and sieving technique + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue, and

xi) Seinhorst's erlenmeyer flask method, have been compared to evaluate their efficiency in nematode extraction from sandy loam soil which harboured Tylenchorhynchus, Hoplolaimus, Pratylenchus, Helicotylenchus, Trichodorus, Hemicriconemoides and free-living nematodes. Nematode counts were made and the data have been furnished in Table 14. These have also been shown in histograms in Fig. III.

According to number of nematodes obtained by the use of a particular technique, the techniques have been grouped into three categories viz : efficient, moderately efficient and poor.

In general, Cobb's modified decanting and sieving technique with double layer of facial tissue paper, Cobb's modified decanting and sieving technique + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> and 5 ppm methylene blue, Cobb's modified decanting and sieving technique + centrifugal flotation and Cobb's modified decanting and sieving technique + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> and 5 ppm methylene blue were found to be efficient except in a few cases like Cobb's modified decanting and sieving technique + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> and 5 ppm methylene blue in respect of Helicotylenchus and free-living nematodes, and Cobb's modified decanting and sieving technique + centrifugal flotation and Cobb's modified decanting and sieving technique + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> and 5 ppm methylene blue for Trichodorus.

TABLE - 14

Comparative efficiency of nematode extraction techniques  
(processed immediately after collection soil sample)

Extraction techniques	Nematode population in 50 g soil *			
	Plant-parasitic nematodes			
	Tylenchorhynchus	Hoplolaimus	Pratylenchus	Helicotylenchus
1. Direct soil placement over moistened facial tissue paper.	62.0	30.0	102.0	82.0
2a. Oostenbrink's elutriator with double layer cotton wool filter.	146.0	40.0	154.0	142.0
2b. Oostenbrink's elutriator with single layer cotton wool filter.	184.0	62.0	206.0	148.0
2c. Oostenbrink's elutriator with double layer facial tissue paper.	198.0	72.0	238.0	180.0
3. Cobb's modified decanting and sieving tech. with double layer of facial tissue paper.	256.0	136.0	450.0	408.0
4. Sugar flotation sieving with 12.5 ppm Separan SP7	220.0	76.0	354.0	335.0
5. Sugar flotation sieving with 12.5 ppm Separan PG2	241.0	81.0	390.0	410.0
6. Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan PG2 + 5 ppm methylene blue.	200.0	45.0	294.0	200.0
7. Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue.	306.0	160.0	459.0	110.0
8. Centrifugal flotation	192.0	82.0	338.0	306.0
9. Cobb's modified decanting and sieving + centrifugal flotation.	380.0	110.0	448.0	490.0
10. Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue.	436.0	138.0	534.0	564.0
11. Seinhorst's erlenmeyer flask method.	150.0	51.0	160.0	178.0
C.D. 5%	27.1	12.5	45.4	31.4

Cont'd

Nematode population in 50g soil		Plant-parasitic nematodes				Free-living nematodes
Extraction techniques		Trichodorus	Hemicricoides	Total	Total	
1.	Direct soil placement over moistened facial tissue paper.	35.0	27.0	338.0	314.0	
2a.	Oostenbrink's elutriator with double layer cotton wool filter.	59.0	38.0	583.0	436.0	
2b.	Oostenbrink's elutriator with single layer cotton wool filter.	72.0	48.0	720.0	648.0	
2c.	Oostenbrink's elutriator with double layer facial tissue paper.	90.0	54.0	830.0	530.0	
3.	Cobb's modified decanting and sieving tech. with double layer of facial tissue paper.	112.0	68.0	1470.0	624.0	
4.	Sugar flotation sieving with 12.5 ppm Separan SP7	74.0	88.0	1147.0	475.0	
5.	Sugar flotation sieving with 12.5 ppm Separan PG2	64.0	76.0	1263.0	468.0	
6.	Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan PG2 + 5 ppm methylene blue.	60.0	64.0	863.0	392.0	
7.	Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue.	105.0	92.0	1516.0	550.0	
8.	Centrifugal flotation.	46.0	88.0	1052.0	658.0	
9.	Cobb's modified decanting and sieving + centrifugal flotation.	54.0	84.0	1546.0	584.0	
10.	Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue.	50.0	108.0	1848.0	820.0	
11.	Seinhorst's erlenmeyer flask method.	71.0	39.0	649.0	448.0	
C.D. 5%		5.2	6.2	28.2	26.2	

\* Average of 5 replications.

Name of extraction techniques.

1. Direct soil placement over moistened facial tissue paper.
- 2a. Oostenbrink's elutriator with double layer of cotton wool filter.
- 2b. Oostenbrink's elutriator with single layer of cotton wool filter.
- 2c. Oostenbrink's elutriator with double layer of facial tissue paper.
3. Cobb's modified decanting and sieving technique with double layer of facial tissue paper.
4. Sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub>.
5. Sugar flotation sieving with 12.5 ppm Separan PG<sub>2</sub>.
6. Cobb's modified decanting and sieving technique + sugar flotation sieving with 12.5 ppm Separan PG<sub>2</sub> + 5 ppm methylene blue.
7. Cobb's modified decanting and sieving technique + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue.
8. Centrifugal flotation.
9. Cobb's modified decanting and sieving technique + centrifugal flotation.
10. Cobb's modified decanting and sieving technique + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue.
11. Seinhorst's erlenmeyer flask method.

Also, Cobb's modified decanting and sieving technique with double layer of facial tissue paper and Cobb's modified decanting and sieving technique + centrifugal flotation were moderately efficient in respect of Hemicriconemoides.

Sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> and sugar flotation sieving with 12.5 ppm Separan PG<sub>2</sub> were moderately efficient. Oostenbrink's elutriator with double layer of facial tissue paper, Cobb's modified decanting and sieving technique + sugar flotation sieving with 12.5 ppm Separan PG<sub>2</sub> + 5 ppm methylene blue, and centrifugal flotation were generally moderately efficient except in case of Trichodorus and free-living nematodes, where Oostenbrink's elutriator with double layer of facial tissue paper was towards efficient side. Centrifugal flotation was very poor in case of Trichodorus.

Direct soil placement over moistened facial tissue paper, Oostenbrink's elutriator with double layer of cotton wool filter, Oostenbrink's elutriator with single layer of cotton wool filter and Seinhorst's erlenmeyer flask method were poor except in case of Trichodorus where Seinhorst's erlenmeyer flask method was moderately efficient.

#### IV. Effect of storage bag, storage temperature, storage period (weeks) and extraction techniques on the recovery of nematodes from the soil sample.

These studies were carried out with two types of storage bags (Polythene and paper), eight storage temperatures (0, 5, 10, 15, 20, 25, 30°C and room temperature (15-20°C), six storage periods (1, 2, 3, 4, 6 and 8 weeks) and three

extraction techniques (Oostenbrink's elutriator, Cobb's modified decanting and sieving technique and Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP<sub>7</sub> with 5 ppm methylene blue).

The nematodes observed were Tylenchorhynchus, Hoplolaimus, Pratylenchus, Helicotylenchus, Trichodorus and free-living. The number of nematodes processed by three extraction techniques immediately after soil sampling have been furnished in Table 15. The pooled data in respect of each genera as also for free-living nematodes and total plant-parasitic nematodes are furnished in Tables 16 to 56. The original data are given in Appendix I to VI. The histogram drawn on the basis of the original data have been shown in Fig. IV (A-E).

An examination of pooled data indicated that in general, polythene bag was superior to paper bag except in respect of Hoplolaimus in which case there was no significant difference between polythene and paper bags. With few exceptions 15°C was found to be the best storage temperature for all the nematodes under observation except Hoplolaimus for which the best storage temperature was 10°C. There was no real difference amongst 5, 10, 15, 20 and room temperature (15-20°C) in respect of Tylenchorhynchus. Also, there was no significant difference between 10, 15 or 20°C and between 15°C and room temperature (15-20°C) in respect of Pratylenchus and Helicotylenchus respectively.

TABLE - 15

No. of nematodes recovered by three extraction techniques immediately after soil sampling (50 g soil)

Nematode genera	Extraction techniques *		
	Oostenbrink's elutriator with double layer of facial tissue paper	Cobb's modified decanting and sieving technique	
<u>Tylenchorhynchus</u>	160	190	210
<u>Hoplolaimus</u>	20	30	75
<u>Pratylenchus</u>	60	90	170
<u>Heterodera</u> larvae	40	60	90
<u>Helicotylenchus</u>	320	370	410
<u>Trichodorus</u>	60	50	35
Free-living nematodes	210	240	310
Total nematode population	860	1040	1320

\* Average of 5 replications.

TABLE - 16

Effect of storage temperature, storage bag, and their interaction on the recovery of Tylenchorhynchus\*

Storage Temperature (°C)	Storage Bag		C.D. 5%
	Polythene Bag	Paper Bag	
0	56.5	41.7	49.1
5	134.7	74.6	104.7
10	130.9	95.9	113.4
15	147.3	80.1	116.7
20	138.9	98.9	118.9
25	95.3	74.4	84.9
30	82.6	65.8	74.2
Room temp. (15-20°C)	136.1	92.3	114.2
Pooled	115.3	78.7	

For Storage Bag = 20.6  
 For Storage Temp. = 41.2  
 For Storage Bag x Storage temp. = 58.3

\* Average of 3 replications.

TABLE - 16

Effect of storage temperature, extraction technique and their interaction on the recovery of Tylenchorhynchus

Storage temperature (°C)	Extraction techniques		C.D.5%
	Oostenbrink's elutriator	Cobb's modified decanting and sieving technique	
0	43.1	46.7	49.1
5	87.5	101.8	104.7
10	96.3	116.0	113.4
15	99.2	115.7	116.7
20	94.3	123.6	118.8
25	64.2	86.1	84.9
30	58.9	77.5	74.2
Room temp. (15-20 °C)	96.5	116.3	114.2
Pooled	80.1	97.9	113.1
			For tech. = 25.2
			For temp. = 58.3
			For temp. x tech. = 60.3

\* Average of 3 replications.

TABLE - 17

Effect of storage bag, extraction technique and their interaction on the recovery of Tylenchorhynchus

Storage bag	Extraction technique	Interaction
Polythene	98.2	115.1
Paper	61.7	80.8
Pooled	80.1	97.9
		132.6
		93.6
		113.1
		115.3
		78.7
		For bags = 20.6
		For tech. = 25.2
		For tech. x bag = 30.6

\* Average of 3 replications.

TABLE - 18

Effect of storage temperature, storage period (week) and their interaction on the recovery of Tylenchothynchus\*

Storage temperature (°C)	Storage period					C.D. 5%		
	One week	Two weeks	Three weeks	Four weeks	Six weeks		Eight weeks	Pooled
0	151.1	82.2	60.6	0	0	0	49.1	
5	158.3	154.2	121.7	85.0	81.7	27.2	104.7	For temp. = 41.2
10	146.7	146.9	125.6	113.8	88.9	58.6	113.4	For storage period = 35.6
15	138.9	145.0	137.2	109.4	94.2	75.6	116.7	For temp. x storage period = 58.3
20	165.7	152.2	143.6	117.2	84.7	50.0	118.9	
25	137.8	122.5	118.9	65.8	40.8	23.3	84.9	
30	131.7	98.3	88.9	50.6	41.4	34.4	74.2	
Room temp. (15-20°C)	133.6	135.3	133.9	116.7	96.4	69.4	114.2	
Pooled	145.5	129.7	116.3	82.3	66.0	42.3		

\*Average of 3 replications.

TABLE - 19

Effect of storage period (week), extraction technique and their interaction on the recovery of Tylenchorhynchus\*

Storage period (weeks)	Extraction techniques*				C.D. 5%
	Oostenbrink's elutriator	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP <sub>7</sub> with 5 ppm methylene blue.	Pooled	
1	122.5	144.3	169.6	145.5	For storage period = 35.6
2	109.7	127.9	151.4	129.7	
3	99.2	118.2	131.0	116.3	For extraction tech. = 25.2
4	69.4	85.6	92.0	82.3	
6	50.4	67.0	80.6	66.0	For storage period x extraction techniques = 60.8
8	28.8	44.1	51.2	42.3	
Pooled	80.1	97.9	113.1		

\* Average of 3 replications.

TABLE - 20

Effect of storage period (week), storage bag and their interaction on the recovery of Tylenchorhynchus\*

Storage period (weeks)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
1	159.1	131.9	145.5	For storage bag = 20.6
2	145.9	113.4	129.7	
3	142.9	89.6	116.3	For storage period (week) = 35.6
4	101.3	63.4	82.3	
6	83.5	48.5	66.0	For storage bag x storage period(weeks) = 50.5
8	59.2	25.5	42.3	
Pooled	115.3	78.7		

\* Average of 3 replications.

TABLE - 21

Effect of storage temperature, storage bag, and their interaction on the recovery of Hoplolaimus\*

Storage temp. (°C)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
0	12.3	10.0	11.1	For storage temp. = 6.8
5	21.7	12.7	17.2	
10	29.5	17.6	23.6	
15	22.3	12.7	17.4	For storage bag = 8.8
20	18.1	15.1	16.6	
25	10.3	9.4	8.9	
30	7.8	6.4	5.7	For storage temp. x storage bag = 11.6
Room temp. (15-20°C)	19.1	6.8	11.5	
Pooled	17.4	10.6		

\*Average of 3 replications

TABLE - 22

Effect of storage temperature, extraction technique and their interaction on the recovery of Hoplolaimus\*

Storage temp. (°C)	Extraction techniques				C.D. 5%
	Oostenbrink's elutria-tor.	Cobb's modified decanting and sieving.	Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP <sub>7</sub> and 5 ppm methylene blue.	Pooled	
0	7.5	10.6	15.4	11.1	For storage temp. = 6.8
5	13.1	17.2	21.4	17.2	
10	14.1	24.0	36.2	30.1	
15	11.9	16.4	23.8	17.4	For extraction tech. = 10.3
20	11.7	17.9	20.3	16.7	
25	6.9	8.2	11.5	8.9	
30	3.8	6.5	6.5	5.6	For storage temp. x extraction technique = 13.7
Room temp. (15-20°C)	10.4	11.8	12.2	11.5	
Pooled	9.9	14.1	19.8		

\*Average of 3 replications.

TABLE - 23

Effect of storage bag, extraction technique and their interaction on the recovery of Hoplolaimus\*

Storage bag	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator.	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP <sub>w</sub> and 5 ppm methylene blue.	Pooled	
Polythene	13.0	18.2	21.7	17.4	For storage bag = 8.8
Paper	6.8	10.1	16.5	10.6	For extra- ction tech. = 10.3
Pooled	9.9	14.1	19.8		For storage bag x extra- ction tech. = 12.3

\*Average of 3 replications

TABLE - 24

Effect of storage temperature, storage period (week) and their interaction on the recovery of Hoplolaimus\*

Storage temp. (°C)	Storage period						Pooled	C.D. 5%
	One week	Two weeks	Three weeks	Four weeks	Six weeks	Eight weeks		
0	26.7	21.4	18.9	0	0	0	11.2	For storage temp. = 6.8
5	17.2	17.2	14.2	18.3	23.8	12.5	17.2	For storage period = 9.4
10	31.6	27.2	25.8	24.7	17.5	15.0	23.6	For storage temp. x storage period = 15.3
15	27.5	27.5	15.8	14.4	13.9	5.5	17.4	
20	34.7	19.7	17.9	15.9	11.9	0	16.6	
25	25.5	15.5	12.5	0	0	0	8.9	
30	18.1	13.6	0	0	0	0	5.6	
Room temp. (15-20°C)	20.3	16.4	11.4	11.4	6.4	5.3	11.5	
Pooled	25.2	19.8	13.5	9.9	8.6	4.8		

\*Average of 3 replications.

TABLE - 25

Effect of storage period (week), extraction technique and their interaction on the recovery of Hoplolaimus

Storage period (weeks)	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator.	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving tech. + centrifugal flotation + 12.5 ppm Separan SP <sub>7</sub> with 5 ppm methylene blue.	Pooled	
1	16.8	26.7	31.9	25.2	For storage period = 9.4
2	14.6	19.5	25.4	19.8	
3	10.0	13.2	17.2	13.5	For extraction tech. = 10.3
4	7.9	9.2	12.3	9.9	
6	3.2	4.8	6.3	8.6	For storage period x extraction tech. = 15.9
8	6.9	10.9	14.7	4.8	
Pooled	9.9	14.1	19.8		

\*Average of 3 replications

TABLE - 26

Effect of storage period (week), storage bag and their interaction on the recovery of Hoplolaimus\*

Storage period (weeks)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
1	29.1	22.4	25.2	For storage period = 9.4
2	23.6	16.1	19.8	
3	18.7	8.2	13.5	For storage bag = 8.8
4	12.8	6.9	9.9	
6	12.5	9.2	8.6	For storage period x storage bag = 14.4
8	8.5	1.4	4.8	
Pooled	17.4	10.6		

\*Average of 3 replications.

TABLE - 27

Effect of storage temperature, storage bag and their interaction on the recovery of Pratylenchus\*

Storage temp. (°C)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
0	17.2	12.2	14.7	For storage temp. = 11.1
5	29.2	19.6	24.4	
10	40.9	23.3	36.5	For storage bag = 5.5
15	39.4	23.8	31.7	
20	34.4	33.1	33.8	For storage temp. x storage bag = 12.3
25	14.4	15.3	14.9	
30	27.3	16.4	21.8	
Room temp. (15-20°C)	28.3	15.5	21.9	
Pooled	28.9	20.6		

\*Average of 3 replications.

TABLE - 28

Effect of storage temperature, extraction technique and their interactions on the recovery of Pratylenchus\*

Storage temp. (°C)	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator.	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving tech. with 12.5 ppm Separan SP7 + 5 ppm methylene blue.	Pooled	
0	12.1	15.9	16.1	14.7	For storage temp. = 11.1
5	16.4	23.2	33.6	24.4	
10	23.2	35.3	45.0	36.5	For extraction tech. = 6.8
15	19.6	32.8	42.6	31.7	
20	27.1	32.4	42.0	33.8	For storage temp. x extraction tech. = 13.4
25	11.8	14.9	19.7	14.9	
30	11.8	18.2	26.6	21.8	
Room temp. (15-20°C)	16.8	22.5	35.6	21.9	
Pooled	17.3	24.4	32.4		

\*Average of 3 replications.

TABLE - 29

Effect of storage bag, extraction technique and their interaction on the recovery of Pratylenchus\*

Storage bag	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator.	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP <sub>7</sub> with 5 ppm methylene blue.	Pooled	
Polythene	17.8	28.9	39.7	28.9	For storage bag = 5.5
Paper	16.8	19.9	25.1	20.6	For extra- ction tech. = 6.8
Pooled	17.3	24.4	32.4		For extraction tech. x storage bag = 9.8

\*Average of 3 replications

TABLE - 30

Effect of storage temperature, storage period (week) and their interaction on the recovery of Pratylenchus\*

Storage temp. (°C)	Storage period						Pooled	C.D. 5%
	One week	Two weeks	Three weeks	Four weeks	Six weeks	Eight weeks		
0	41.3	24.2	22.8	0	0	0	14.7	For storage temp. = 11.1
5	52.2	22.5	38.3	22.8	10.6	0	24.4	
10	52.2	36.4	30.8	30.6	24.2	22.8	36.5	For storage period = 9.6
15	45.8	38.6	21.1	21.4	17.2	10.2	31.7	
20	49.7	40.3	30.0	24.4	23.4	17.8	33.8	For storage temp. x storage period = 14.2
25	43.6	27.0	12.5	5.8	0	0	14.9	
30	24.3	20.6	17.3	13.3	0	0	21.8	
Room temp. (15-20°C)	48.9	28.3	24.4	37.0	26.8	13.6	21.9	
Pooled	44.9	28.1	25.3	21.5	14.7	13.8		

\*Average of 3 replications.

TABLE -31

Effect of storage period (week), extraction technique and their interaction on the recovery of Pratylenchus\*

Storage period (weeks)	Extraction techniques				C.D. 5%
	Oostenbrink's elutria-tor.	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving tech. with 12.5 ppm Separans SP <sub>7</sub> and 5 ppm methylene blue	Pooled	
1	33.0	45.4	56.3	44.9	For storage period = 9.6
2	20.9	25.9	37.4	28.1	
3	19.8	26.0	30.2	25.3	For extraction tech. = 6.8
4	11.9	17.9	34.6	21.5	
6	10.3	16.5	17.3	14.7	For storage period x extraction tech. = 10.5
8	7.8	14.6	18.9	13.8	
Pooled	17.3	24.4	32.4		

\*Average of 3 replications.

TABLE - 32

Effect of storage period (week), storage bag and their interaction on the recovery of Pratylenchus\*

Storage period (weeks)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
1	44.9	30.4	44.9	For storage period = 9.6
2	33.5	22.6	28.1	
3	33.3	18.3	25.3	For storage bag = 5.5
4	29.0	14.0	21.5	
6	18.0	11.4	14.7	For storage period x storage bag = 11.3
8	15.1	12.4	13.8	
Pooled	28.9	20.6		

\*Average of 3 replications.

TABLE - 33

Effect of storage temperature, storage bag and their interaction on the recovery of Helicotylenchus\*

Storage temp. (°C)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
0	113.1	72.2	92.7	For storage bag = 38.5
5	228.4	102.0	165.2	
10	196.1	114.3	155.2	For storage temp. = 60.1
15	285.9	193.8	264.9	
20	224.6	160.8	192.7	For storage temp. x storage bag = 90.5
25	196.9	167.8	182.3	
30	153.7	117.9	135.8	
Room temp. (15-20°C)	235.8	196.7	216.3	
Pooled	203.1	140.7		

\*Average of 3 replications

TABLE - 34

Effect of storage temperature, extraction technique and their interaction on the recovery of Helicotylenchus\*

Storage temp. (°C)	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator.	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue.	Pooled	
0	69.6	99.7	108.8	92.7	For storage temp. = 60.1
5	127.8	164.6	203.3	165.2	
10	119.4	162.4	183.8	165.2	For extraction tech. = 42.5
15	170.4	250.7	283.6	264.9	
20	151.3	204.4	222.5	192.7	For storage temp. x extraction tech. = 103.4
25	128.2	191.9	226.8	182.3	
30	99.4	139.6	168.3	135.8	
Room temp. (15-20°C)	159.2	220.1	269.4	216.3	
Pooled	128.2	179.2	208.3		

\*Average of 3 replications.

TABLE - 35

Effect of storage bag, extraction technique and their interaction on the recovery of Helicotylenchus\*

Storage bag	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator.	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving and centrifugal flotation + 12.5 ppm Separan SP <sub>7</sub> with 5 ppm methylene blue	Pooled	
Polythene	153.9	214.1	241.3	203.1	For storage bag = 35.5
Paper	102.3	144.3	175.3	140.7	For extraction tech. = 42.5
Pooled	128.2	179.2	208.3		For storage bag x extraction tech. = 55.6

\*Average of 3 replications.

TABLE - 36

Effect of storage temperature, storage period (week) and their interaction on the recovery of Helicotylenchus\*

Storage temp. (°C)	Storage period							C.D. 5%
	One week	Two weeks	Three weeks	Four weeks	Six weeks	Eight weeks	Pooled	
0	208.9	213.1	134.2	0	0	0	92.7	For storage temp. = 60.1
5	235.6	220.3	195.6	145.0	119.4	75.6	165.2	
10	207.2	195.8	188.9	144.2	119.2	75.8	155.2	For storage period = 60.5
15	367.5	278.6	252.7	199.4	145.8	125.3	264.9	
20	301.4	267.8	203.4	173.3	126.7	83.9	192.7	
25	311.7	283.1	215.3	156.1	102.8	25.0	182.3	For storage temp. x storage period = 74.4
30	295.6	206.9	108.8	102.3	80.0	21.1	135.8	
Room temp. (15-20°C)	311.7	235.6	229.4	212.2	167.5	141.1	216.3	
Pooled	279.9	237.6	183.6	149.0	107.7	73.5		

\*Average of 3 replications.

TABLE - 37

Effect of storage period (week), extraction technique and their interaction on the recovery of Helicotylenchus\*

Storage period (weeks)	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator.	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP <sub>7</sub> and 5 ppm methylene blue.	Pooled	
1	214.3	289.8	335.7	279.9	For storage period = 60.5
2	181.4	246.4	285.2	237.6	
3	142.3	190.6	217.9	183.6	For extraction tech. = 42.5
4	98.5	161.4	187.4	149.0	
6	76.3	113.2	133.5	107.7	For storage period x extraction tech. = 59.6
8	56.3	74.1	90.1	73.5	
Pooled	128.2	179.2	208.3		

\*Average of 3 replications.

TABLE - 38

Effect of storage period (week), storage bag and their interaction on the recovery of Helicotylenchus\*

Storage period (week)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
1	295.3	264.5	279.9	For storage period = 60.5
2	273.8	201.0	237.6	
3	226.2	143.0	183.6	For storage bag = 35.5
4	182.4	115.6	149.0	
6	148.6	66.7	107.7	For storage period x storage bag = 80.0
8	94.2	52.7	73.5	
Pooled	203.1	140.7		

\*Average of 3 replications.

TABLE - 39

Effect of storage temperature, storage bag and their interaction on the recovery of Trichodorus\*

Storage temperature (°C)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
0	7.3	7.9	7.6	For storage temperature = 20.1
5	12.8	8.9	10.9	
10	19.1	13.1	16.1	For storage bag = 8.6
15	78.6	18.9	43.8	
20	14.4	10.5	12.4	For storage temp. x storage bag = 39.6
25	9.6	8.4	9.0	
30	14.6	9.5	12.1	
Room temp. (15-20°C)	15.6	10.4	12.9	
Pooled	21.5	9.7		

\*Average of 3 replications

TABLE - 40

Effect of storage temperature, extraction technique and their interaction on the recovery of Trichodorus\*

Storage temp. (°C)	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP <sub>7</sub> with 5 ppm methylene blue	Pooled	
0	4.5	8.3	10.1	7.6	For storage temp. = 20.1
5	7.6	13.3	11.6	10.9	
10	11.8	21.4	14.7	16.1	For extraction tech. = 13.0
15	49.4	63.6	28.3	43.8	
20	9.5	15.2	10.4	12.4	For storage temp. x extraction tech. = 30.2
25	6.7	18.1	12.2	9.0	
30	10.5	11.6	9.6	12.1	
Room temp. (15-20°C)	9.1	17.3	12.6	12.9	
Pooled	13.6	16.7	12.1		

\*Average of 3 replications.

TABLE - 41

Effect of storage bag, extraction technique and their interaction on the recovery of Trichodorus\*

Storage bag	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP <sub>7</sub> with 5 ppm methylene blue	Pooled	
Polythene	20.4	28.6	20.2	21.5	For storage bag = 8.6
Paper	6.9	12.5	9.2	9.7	For extraction tech. = 13.0
Pooled	13.6	16.7	12.1		For storage bag x extraction tech. = 19.6

\*Average of 3 replications.

TABLE - 42

Effect of storage temperature, storage period (week) and their interaction on the recovery of Trichodorus\*

Storage temp. (°C)	Storage period						Pooled	C.D. 5%
	One week	Two weeks	Three weeks	Four weeks	Six weeks	Eight weeks		
0	19.4	19.2	7.2	0	0	0	7.6	For storage temp. = 20.1
5	20.8	20.3	15.8	8.1	0	0	10.8	
10	37.7	23.6	22.8	8.9	3.8	0	16.1	For storage period = 6.1
15	39.7	72.3	51.8	32.9	27.2	20.7	43.8	
20	29.7	18.1	16.7	0	0	0	12.1	For storage temp. x storage period = 28.6
25	22.5	17.2	14.4	0	0	0	9.0	
30	32.5	14.7	16.4	0	0	0	12.1	
Room temp. (15-20°C)	32.2	20.3	18.6	6.7	0	0	13.0	
Pooled	30.1	23.1	23.0	8.2	7.6	3.4		

\*Average of 3 replications.

TABLE - 43

Effect of storage period (week), extraction technique and their interaction on the recovery of Trichodorus\*

Storage period (weeks)	Extraction techniques			C.D. 5%	
	Oostenbrink's elutriator	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP7 with 5 ppm methylene blue		Pooled
1	19.9	39.4	35.1	28.1	For storage period = 6.1
2	24.5	28.8	18.8	23.1	
3	23.3	25.9	19.9	23.0	For extra- ction tech. = 13.0
4	3.5	14.8	6.3	8.2	
6	4.2	11.2	9.4	7.6	For storage period x extraction tech. = 18.6
8	10.4	0	0	3.4	
Pooled	13.7	16.7	12.1		

\*Average of 3 replications.

TABLE - 44

Effect of storage period (week), storage bag and their interaction on the recovery of Trichodorus\*

Storage period (weeks)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
1	34.0	26.3	30.1	For storage period = 6.1
2	27.5	18.8	23.1	
3	32.8	13.3	23.0	For storage bag = 8.6
4	16.4	0	8.2	
6	15.3	0	7.6	For storage period x storage bag = 12.7
8	6.9	0	3.4	
Pooled	20.2	12.1		

\*Average of 3 replications.

TABLE - 45

Effect of storage temperature, storage bag and their interaction on the recovery of free living nematodes\*

Storage temp. (°C)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
0	216.1	147.9	182.0	For storage temp. = 66.4
5	436.0	224.3	330.1	
10	425.6	278.9	352.2	For storage bag = 54.3
15	510.6	336.4	423.6	
20	436.8	325.9	381.2	For storage temp. x storage bag = 150.8
25	328.4	277.9	303.1	
30	280.7	220.6	250.6	
Room temp. (15-20°C)	443.9	324.7	384.3	
Pooled	384.7	267.1		

\*Average of 3 replications.

TABLE - 46

Effect of storage temperature, extraction technique and their interaction on the recovery of free-living nematodes\*

Storage temp. (°C)	Extraction techniques				C.D. 5%
	Oostenbrink's elutria-tor	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP <sub>7</sub> with 5 ppm methylene blue	Pooled	
0	143.5	189.0	213.6	182.0	For storage temp. = 66.4
5	258.5	327.5	404.4	330.1	
10	271.5	362.9	422.2	352.2	For extraction tech. = 60.3
15	317.9	439.7	513.0	423.6	
20	301.1	396.1	446.2	382.1	For storage temp. x extraction tech. = 127.1
25	217.4	315.4	376.7	303.1	
30	188.3	261.1	302.6	250.7	
Room temp. (15-20°C)	300.6	391.1	461.3	384.3	
Pooled	249.8	325.3	392.5		

\*Average of 3 replications.

TABLE - 47

Effect of storage bag, extraction technique and their interaction on the recovery of free living nematodes\*

Storage bag	Extraction techniques				C.D. 5%
	Oostenbrink's elutria-tor	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP <sub>7</sub> with 5 ppm methylene blue	Pooled	
Polythene	300.5	399.3	454.4	384.7	For storage bag = 54.3
Paper	199.1	271.4	300.8	267.1	For extra- ction tech. = 60.3
Pooled	249.8	335.3	392.5		For storage bag x extrac- tion tech. = 153.5

\*Average of 3 replications.

TABLE - 48

Effect of storage temperature, storage period (week) and their interaction on the recovery of free living nematodes\*

Storage temp. (°C)	Storage period						C.D. 5%	
	One week	Two weeks	Three weeks	Four weeks	Six weeks	Eight weeks		
0	473.1	375.3	243.9	0	0	0	182.0	For storage temp. = 66.4
5	507.9	447.2	395.3	281.7	235.0	113.9	330.1	For storage period = 93.0
10	512.2	438.1	404.4	315.3	271.4	171.9	352.7	For storage temp. x storage period = 165.8
15	686.1	538.1	392.2	409.2	273.6	292.2	423.6	
20	611.9	514.2	405.6	328.1	265.3	161.7	382.1	
25	551.7	474.2	373.3	227.8	143.6	48.3	303.1	
30	543.8	272.8	232.8	177.5	121.4	55.6	250.7	
Room temp. (15-20°C)	540.3	442.8	410.6	376.4	294.7	241.1	384.3	
Pooled	547.1	450.3	357.3	264.5	200.7	135.6		

\*Average of 3 replications.

TABLE - 49

Effect of storage period (week), extraction technique and their interaction on the recovery of free-living nematodes\*

Storage period (weeks)	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP <sub>7</sub> with 5 ppm methylene blue	Pooled	
1	424.4	562.5	654.6	547.1	For storage period = 93.0
2	352.9	456.9	541.1	450.3	
3	285.7	367.3	418.8	357.3	For extraction tech. = 60.3
4	191.1	279.5	322.8	264.5	
6	144.6	208.2	249.3	200.7	For storage period x extraction tech. = 102.8
8	100.2	133.7	168.8	135.6	
Pooled	249.8	335.3	392.5		

\*Average of 3 replications.

TABLE - 50

Effect of storage period (week), storage bag and their interaction on the recovery of free-living nematodes\*

Storage period (weeks)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
1	584.0	510.1	547.1	For storage period = 93.0
2	511.3	389.4	450.3	
3	439.8	274.7	357.3	For storage bag = 54.3
4	329.2	199.8	264.5	
6	264.3	137.2	200.7	For storage period x storage bag = 110.2
8	179.8	91.4	135.6	
Pooled	384.7	267.1		

\*Average of 3 replications.

TABLE - 51

Effect of storage temperature, storage bag and their interaction on the recovery of total plant parasitic nematodes\*

Storage temp. (°C)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
0	103.8	41.4	72.5	For storage temp. = 54.3
5	147.7	113.9	130.7	
10	132.1	103.4	117.7	For storage bag = 27.1
15	168.7	114.1	141.4	For storage temp. x storage bag = 66.8
20	152.6	116.5	134.5	
25	159.1	110.2	134.6	
30	109.3	94.1	101.7	
Room temp. (15-20°C)	177.3	118.7	148.0	
Pooled	143.8	101.5		

\*Average of 3 replications.

TABLE - 52

Effect of storage temperature, extraction technique and their interaction on the recovery of total plant parasitic nematodes\*

Storage temp. (°C)	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator	Cobb's modified and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP <sub>7</sub> with 5 ppm methylene blue	Pooled	
0	55.4	68.3	94.0	72.5	For storage temp. = 54.3
5	108.6	127.8	155.9	130.7	
10	90.6	124.3	138.5	117.7	For extraction tech. = 33.8
15	116.7	145.7	161.9	141.4	
20	100.8	138.4	164.4	134.5	For storage temp. x extraction tech. = 84.1
25	90.9	138.3	174.7	134.6	
30	86.2	108.8	109.6	101.6	
Room Temp. (15-20°C)	107.2	151.5	185.2	148.0	
Pooled	94.5	125.4	148.0		

\*Average of 3 replications.

TABLE - 53

Effect of storage bag, extraction technique and their interaction on the recovery of total plant parasitic nematodes\*

Storage bag	Extraction techniques				C.D. 5%
	Oostenbrink's elutriator	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP7 with 5 ppm methylene blue	Pooled	
Polythene	110.8	148.7	171.9	143.8	For storage bag = 27.1
Paper	78.3	102.0	124.2	101.5	For extra- ction tech. 33.3
Pooled	94.5	125.4	148.0		For storage bag x extra- ction tech. = 47.1

\*Average of 3 replications.

TABLE - 54

Effect of storage temperature, storage period (week) and their interaction on the recovery of total plant parasitic nematodes\*

Storage temp. (°C)	Storage period						Pooled	C.D. 5%
	One week	Two weeks	Three weeks	Four weeks	Six weeks	Eight weeks		
0	173.6	141.9	102.2	177.7	0	0	72.5	For storage temp. = 54.3
5	231.7	161.7	153.3	84.4	82.2	71.3	130.7	For storage period = 47.0
10	146.4	135.5	133.9	112.2	91.3	87.2	117.7	For storage temp. x storage period = 123.1
15	159.7	146.1	137.7	130.7	124.4	115.0	141.4	
20	180.0	161.9	135.8	120.2	107.5	101.9	134.5	
25	181.9	155.0	126.7	128.8	113.8	101.6	134.6	
30	195.8	168.3	103.3	78.5	64.4	0	101.6	
Room temp. (15-20°C)	204.4	204.4	155.5	118.3	105.5	99.7	148.0	
Pooled	184.2	158.1	129.4	94.0	88.5	81.8		

\*Average of 3 replications.

TABLE - 55

Effect of storage period (week), extraction technique and their interaction on the recovery of total plant parasitic nematodes\*

Storage period (weeks)	Extraction techniques				C.D. 5%
	Oostenbrink's elutria-tor	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP <sub>7</sub> with 5 ppm methylene blue	Pooled	
1	143.2	186.7	222.6	184.2	For storage period = 47.0
2	118.0	164.2	192.0	158.1	
3	103.6	129.0	155.5	129.4	For extraction tech = 33.3
4	75.7	92.5	113.8	94.0	
6	64.0	94.8	106.5	88.5	For storage period x extraction tech. = 71.4
8	62.7	85.0	97.9	81.8	
Pooled	94.5	125.4	148.0		

\*Average of 3 replications.

TABLE - 56

Effect of storage period (week), storage bag and their interaction on the recovery of total plant parasitic nematodes\*

Storage period (weeks)	Storage bag			C.D. 5%
	Polythene bag	Paper bag	Pooled	
1	200.7	167.6	184.2	For storage period = 47.0
2	176.4	139.7	158.1	
3	153.2	105.6	129.4	For storage bag = 27.1
4	119.5	68.4	94.0	
6	117.5	59.5	88.5	For storage period x storage bag = 56.5
8	95.4	68.2	81.8	
Pooled	143.8	101.5		

\*Average of 3 replications.

Storage period for one week was superior to other storage periods for all nematodes but there was no significant difference between one, two and three weeks storage.

Oostenbrink's elutriator was found to be the least efficient method of nematode extraction, while Cobb's modified decanting and sieving + centrifugal flotation + 12.5 ppm Separan SP<sub>7</sub> with 5 ppm methylene blue was superior in respect of Tylenchorhynchus and free-living nematodes and it was similar to Cobb's modified decanting and sieving technique in case of Pratylenchus, Helicotylenchus and total plant-parasitic nematodes. For Hoplolaimus and Trichodorus there were no real differences among the techniques.

i) Storage temperature x storage bag : Polythene bag was superior at 0°C in respect of total plant-parasitic nematodes; at 5°C in respect of Helicotylenchus and free-living nematodes; at 10°C in respect of Hoplolaimus, Pratylenchus, Trichodorus and free-living nematodes; at 15°C in respect of Tylenchorhynchus, Pratylenchus, Helicotylenchus and free-living nematodes; at 20°C in respect of Helicotylenchus; at 25°C in respect of free-living nematodes and at room temperature (15-20°C) in respect of Pratylenchus. For the rest there was no real difference due to polythene and paper bags.

ii) Storage temperature x storage period (weeks) : Storage period for one week was significantly superior at 0°C in respect of Tylenchorhynchus and Pratylenchus; at 5°C in respect of Helicotylenchus and free-living nematodes; at 25°C

in respect of Pratylenchus; at 30°C in respect of Helicotylenchus and free-living nematodes and at room temperature (15-20°C) in respect of Pratylenchus and Helicotylenchus.

Comparative study of the effect of storage temperature x storage period revealed that at 5°C there was no significant difference among one, two and three weeks of storage but were superior to four, six and eight weeks. At 15°C, two weeks storage was superior to eight. At 20°C, one week storage was superior to six and eight weeks; at 25°C, one week was superior to four, six and eight week and at room temperature two weeks was superior to eight weeks storage in respect of Tylenchorhynchus.

The effect of storage temperature storage period in respect of Hoplolaimus revealed that at 10°C one week storage was significantly superior to eight weeks storage; at 15°C one and two weeks storage were superior to eight weeks and at 20°C one week was superior to three weeks storage.

The comparative study of the effect of storage temperature x storage period revealed that at 5, 10, 15, 20°C and at room temperature (15-20°C), three weeks storage was superior to one week storage in respect of Pratylenchus.

The comparative study of the effect of storage temperature x storage period revealed that at 5°C two weeks storage was superior to three weeks, at 5°C one week storage was superior to four weeks storage, and at 20 and 25°C one week was significantly superior to three weeks storage in respect of Helicotylenchus.

The comparative study of storage temperature x storage period in respect of free-living nematodes revealed that at 15°C one week storage was superior to all; at 20°C, 25°C and at room temperature (15-20°C) one week storage was significantly superior to three weeks. Other temperatures showed no real differences.

iii) Storage temperature x extraction technique : Cobb's modified decanting and sieving + centrifugal flotation was superior to Oostenbrink's elutriator at 5°C in-respect of Pratylenchus, and free-living nematodes; at 10°C in respect of Hoplolaimus, Pratylenchus and free-living nematodes; at 15°C in respect of Pratylenchus, Helicotylenchus and free-living nematodes; at 20°C in respect of Pratylenchus and free-living nematodes; at 25°C in respect of free-living nematodes and at room temperature (15-20°C) in respect of Pratylenchus, Helicotylenchus and free-living nematodes.

At 15°C, Cobb's modified decanting and sieving technique was superior to Oostenbrink's elutriator and Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> and 5 ppm methylene blue in respect of Trichodorus.

iv) Extraction technique x storage bag : Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> with 5 ppm methylene blue was found to be the best technique when the samples were stored in polythene bag in respect of Tylenchorhynchus, Pratylenchus, Helicotylenchus, free-living nematodes and total plant-parasitic nematodes,

but it showed no significant differences in respect of Hoplolaimus and Trichodorus.

Oostenbrink's elutriator was found to be inferior in respect of Hoplolaimus, Pratylenchus, Trichodorus and total plant-parasitic nematodes.

Cobb's modified decanting and sieving technique was the best in respect of Tylenchorhynchus, Pratylenchus, Helicotylenchus and Trichodorus when the samples were stored in polythene bag.

v) Extraction technique x storage period (weeks) :

Comparative study of extraction technique x storage period revealed that Oostenbrink's elutriator gave good results in respect of Tylenchorhynchus, when the samples were stored for one week; in respect of Pratylenchus, one, two and three weeks storage were superior to six and eight weeks storage; in respect of Helicotylenchus one week storage was superior to three weeks storage and in respect of free-living nematodes one week storage was superior to all. Cobb's modified decanting and sieving technique revealed that in respect of Tylenchorhynchus one and two weeks storage showed no significant difference and were superior to three weeks storage; in respect of Hoplolaimus one week storage was superior to all; in respect of Pratylenchus, one, two and three weeks storage were superior to all; in respect of Helicotylenchus one and two weeks storage showed no real difference; in respect of free-living nematodes one and two weeks storage were superior and in respect of total plant-parasitic nematodes there

was no significant difference amongst one, two and three weeks storage. In case of Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> and 5 ppm methylene blue, one, two and three weeks storage were significantly superior to four, six and eight weeks storage in respect of Tylenchorhynchus; in respect of Hoplolaimus one week storage was superior; in respect of Pratylenchus, one, two, three and four weeks storage were superior to six and eight weeks of storage; in respect of Helicotylenchus and total plant-parasitic nematodes there was no significant differences amongst one, two and three weeks of storage; in respect of free-living nematodes one and two weeks storage was superior.

In respect of Trichodorus all the techniques were equally good for one, two and three weeks storage.

vi) Storage period (week) x storage bag : Comparative study of storage period x storage bag revealed that polythene bag was superior when the samples were stored for one week in respect of Pratylenchus; two weeks in respect of Pratylenchus and free-living nematodes; three weeks in respect of Tylenchorhynchus, Pratylenchus, Helicotylenchus, Trichodorus and free-living nematodes; and four weeks in respect of Pratylenchus, Helicotylenchus, Trichodorus and free-living nematodes.

V. Quantitative estimation of nematode population densities by six extraction procedures as affected by season and seasonal fluctuation in nematode population.

Two experiments were designed to compare the efficiency

of six nematode extraction methods (viz. Direct soil placement over moistened facial tissue paper; Costenbrink's elutriator with double layer of facial tissue paper; Cobb's modified decanting and sieving technique; Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue; Cobb's modified decanting sieving + centrifugal flotation; and Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue) in measuring seasonal population shifts of plant parasitic and free-living nematodes.

The first experiment was carried out in a field given to maize-wheat rotation. Samples during August or September and October were taken from maize. From November to April these were collected from wheat. Samples during May, June and July were taken from fallow. Soil samples were processed by each technique and the data in respect of plant-parasitic and free-living nematodes have been furnished in Tables 57 to 6<sup>7</sup>. These have been graphically represented in Fig. V-A. The plant-parasitic nematodes observed and studies were Tylenchorhynchus, Hoplolaimus, Pratylenchus and Helicotylenchus.

The other experiment carried out in Top Block-I of the Division of Agronomy comprised 8 crop rotations viz :

- i) Sugarcane-wheat-moong.
- ii) Jowar/bajra-wheat.
- iii) Moong-wheat-lobia.

TABLE - 57

Population fluctuation and efficiency of extraction techniques during different periods for obtaining Tylenchorhynchus

		Mean number of nematodes from 50 g soil							
		Extraction techniques							
Year/ Month	Crop	Direct placement of soil over moistened facial tissue paper	Oosteh- brink's elutria- tor	Cobb's modified decanting and siev- ing tech.	Cobb's modified decanting and siev- ing + cen- trifugal flotation.	Cobb's modified decanting and siev- ing + cen- trifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methyle- ne blue	Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	C.D. 5%	C.D. 1%
<u>1971</u>									
August	Maize	12.5	41.1	55.6	66.4	68.3	86.1	15.6	17.7
Sept.	Maize	18.3	28.1	42.2	43.6	52.6	70.2	9.6	10.8
Oct.	Maize	48.4	64.2	105.3	116.8	131.0	185.3	25.7	28.9
Nov.	Wheat	33.1	47.8	60.8	84.2	97.2	129.2	20.6	23.2
Dec.	Wheat	41.5	56.2	58.5	69.5	78.3	101.0	13.4	15.1
<u>1972</u>									
Jan.	Wheat	49.5	67.0	83.5	100.5	120.0	177.5	22.9	25.7
Feb.	Wheat	26.8	58.9	81.4	106.3	110.5	169.7	23.4	26.4
March	Wheat	35.8	56.1	82.6	116.8	102.4	159.7	20.3	22.8
April	Wheat	60.0	105.2	115.0	130.0	136.0	203.1	20.6	23.2
May	Fallow	38.5	45.5	68.3	89.3	110.3	141.3	18.4	20.7
June	Fallow	30.8	37.6	46.3	61.1	80.5	102.1	10.7	12.0
July	Fallow	15.3	34.2	43.9	50.0	52.9	70.3	11.7	13.2

TABLE - 58

Population fluctuation and efficiency of extraction techniques during different periods for obtaining *Hoplolaimus*

Mean number of nematodes from 50 g soil

Year/ Month	Crop	Extraction techniques						C.D. 5%	C.D. 1%
		Direct place- ment of soil over moistened facial tissue paper	Oosten- brink's elutria- tor	Cobb's modified decanting and siev- ing tech.	Cobb's modified decanting and sieving+ sugar flota- tion sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue	Cobb's modified decanting and siev- ing+centri- fugal flo- tation	Cobb's modified decanting and sieving+ centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue		
<u>1971</u>									
August	Maize	15.2	41.6	50.8	65.3	70.3	90.5	15.7	17.6
Sept.	Maize	13.5	26.0	35.8	41.5	55.8	72.7	10.3	11.6
Oct.	Maize	19.0	30.5	47.3	55.6	72.5	116.5	18.4	20.6
Nov.	Wheat	29.7	42.8	65.3	74.2	93.9	134.4	25.6	28.7
Dec.	Wheat	20.8	42.8	53.0	66.0	80.8	113.0	15.1	16.9
<u>1972</u>									
Jan.	Wheat	16.0	25.5	34.8	37.0	48.0	75.0	9.6	10.8
Feb.	Wheat	18.8	22.0	31.3	32.5	40.5	49.8	6.3	7.4
March	Wheat	26.9	56.3	59.8	75.2	87.9	112.6	21.7	24.4
April	Wheat	41.3	79.3	77.8	106.7	118.3	155.8	26.0	29.3
May	Fallow	52.3	73.0	86.0	100.8	109.5	146.5	20.9	23.5
June	Fallow	21.7	41.3	54.3	59.7	66.0	87.7	12.7	14.5
July	Fallow	17.6	34.4	62.6	95.8	89.1	149.4	10.3	11.6

TABLE - 59

Population fluctuation and efficiency of extraction techniques during different periods for obtaining *Pratylenchus*

Mean number of nematodes from 50 g soil

Year/ Month	Crop	Extraction techniques						C.D. 1%
		Oostenbrink's elutriator	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving+ sugar flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	Cobb's modified decanting and sieving+fugal flotation	Cobb's modified decanting and sieving+ centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	C.D. 5%	
<b>1971</b>								
August	Maize	31.5	75.5	99.5	102.5	125.0	18.1	20.3
Sept.	Maize	18.3	53.8	65.8	69.0	92.5	11.1	12.5
Oct.	Maize	30.0	72.0	86.5	100.0	157.3	18.7	21.0
Nov.	Wheat	32.0	71.8	93.5	109.5	151.8	20.6	23.0
Dec.	Wheat	30.5	62.3	94.3	104.0	130.8	19.9	22.4
<b>1972</b>								
Jan.	Wheat	35.2	81.4	106.7	118.8	141.9	18.2	20.5
Feb.	Wheat	25.7	90.7	113.2	133.6	188.2	21.7	24.3
March	Wheat	42.9	102.7	115.7	131.4	189.8	21.8	24.5
April	Wheat	50.2	111.2	132.6	139.7	195.1	18.3	20.5
May	Fallow	26.4	74.2	103.9	105.6	126.9	18.9	21.3
June	Fallow	17.9	50.0	60.6	71.8	97.4	13.4	15.6
July	Fallow	15.3	36.3	46.1	60.6	88.7	10.1	11.2

TABLE - 60

Population fluctuation and efficiency of extraction techniques during different periods for obtaining Helicotylenchus

Mean number of nematodes from 50 g soil

Year/ Month	Crop	Extraction techniques						C.D. 5%	C.D. 1%
		Direct placement of soil over moistened facial tissue paper	Costen- brink's elutria- tor	Cobb's modified decanting and siev- ing tech.	Cobb's modified decanting and sieving+ sugar flota- tion sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue.	Cobb's modified decanting and siev- ing+centri- fugal flo- tation	Cobb's modified decanting and sieving+ centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue		
<u>1971</u>									
August	Maize	18.7	37.6	50.0	61.3	66.1	90.5	13.6	15.3
Sept.	Maize	16.2	25.7	33.1	40.2	42.9	62.4	10.7	12.0
Oct.	Maize	28.3	47.4	62.9	83.8	107.1	178.9	21.6	24.3
Nov.	Wheat	52.7	64.5	74.7	86.4	97.7	140.7	15.7	17.6
Dec.	Wheat	60.4	81.1	100.2	123.9	151.1	225.9	20.1	23.4
<u>1972</u>									
Jan.	Wheat	76.3	93.4	114.3	139.9	166.9	244.6	23.6	26.5
Feb.	Wheat	69.3	98.3	128.3	159.4	200.5	307.2	29.8	33.4
March	Wheat	34.8	45.0	63.3	95.5	146.0	245.0	33.4	37.5
April	Wheat	27.8	54.8	70.8	95.3	103.5	144.3	19.5	21.8
May	Fallow	18.0	53.0	99.0	146.0	149.3	227.0	35.1	40.0
June	Fallow	19.5	46.8	71.3	93.3	107.3	148.8	11.6	13.1
July	Fallow	21.9	27.7	37.2	55.3	62.8	97.5	14.2	15.4

TABLE - 61

Population fluctuation and efficiency of extraction techniques during different periods for obtaining free-living nematodes

Mean number of nematodes from 50 g soil

Year/ Month	Extraction techniques							C.D. 5%	C.D. 1%
	Oosten- brink's elutriator	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving sugar flota- tion sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue.	Cobb's modified decanting and sieving fugal flo- tation.	Cobb's modified decanting and sieving centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue				
1971									
August	81.8	140.4	169.4	177.2	196.8	227.4	18.2	20.4	
September	78.8	107.4	164.0	171.0	209.1	215.3	15.5	17.4	
October	86.0	148.8	158.6	168.0	200.2	237.4	21.7	24.4	
November	100.0	150.0	168.3	175.5	230.3	275.2	22.6	25.6	
December	120.0	130.0	105.6	190.0	210.5	300.5	20.3	21.6	
1972									
January	130.0	175.5	185.7	190.0	230.0	340.7	22.6	25.3	
February	145.6	201.4	242.4	280.4	310.8	374.3	16.6	18.7	
March	148.0	196.3	281.0	310.2	349.9	395.4	16.5	18.5	
April	131.9	176.9	180.9	197.8	219.8	327.3	14.2	15.9	
May	173.6	216.4	272.4	389.0	345.0	477.9	18.8	21.2	
June	155.4	177.5	184.4	189.9	203.2	255.3	16.0	18.0	
July	166.8	198.2	216.8	241.6	271.6	357.6	12.6	14.2	

TABLE - 62

Population fluctuation and efficiency of extraction techniques during different periods for obtaining total plant-parasitic nematodes

		Mean number of nematodes from 50 g soil						
		Extraction techniques						
Year/ Month	Dirrec	Oosten- brink's elutria- tor.	Cobb's modified decanting and siev- ing tech.	Cobb's modified decanting and siev- ing+centri- fugal flota- tion sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue.	Cobb's modified decanting and siev- ing+centri- fugal flota- tion. 12.5 ppm Separan SP7 + 5 ppm methylene blue	Cobb's modified decanting and sieving+ centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	C.D. 5%	C.D. 1%
1971								
August	77.9	184.3	232.1	292.5	307.2	392.1	23.2	25.4
September	66.3	126.6	164.9	191.1	220.3	297.8	19.5	22.4
October	125.7	202.9	287.5	342.7	410.6	608.0	26.7	28.5
November	147.5	210.1	272.6	308.2	398.3	556.1	26.6	30.7
December	153.2	233.4	274.0	353.7	384.2	570.7	24.3	26.4
1972								
January	177.0	250.2	288.9	384.1	453.7	638.0	26.7	29.4
February	140.5	244.0	331.7	411.9	475.1	704.9	20.4	22.7
March	140.4	223.4	308.4	423.2	477.7	716.1	21.7	25.3
April	179.3	315.4	374.8	464.6	497.7	728.2	17.5	22.6
May	135.2	220.9	327.5	410.3	474.7	642.1	19.4	25.4
June	89.9	168.2	221.9	274.7	325.8	433.0	18.5	21.4
July	70.1	128.4	180.0	247.1	265.4	405.9	21.6	25.7

- iv) Maize (Cobs) - raddish-wheat.
- v) Moong + pigeon-pea-wheat.
- vi) Maize-potato/toria-wheat-moong.
- vii) Maize-wheat-moong-baira/lowar.
- viii) Maize-oat/berseem/cowpea (for fodder).

The plant-parasitic nematodes observed and studied were Tylenchorhynchus, Hoplolaimus, Pratylenchus, Helicotylenchus, Heterodera larvae and Trichodorus.

The details regarding the number of samples collected from various crops have been given in Table 63.

Twenty-five samples in the month of August and September comprised 14 samples from maize, 4 samples from sugarcane, 2 samples from baira and one each from pigeon-pea, pigeon-pea + moong, lowar + pigeon-pea, lowar + moong and lowar. In October also the samples were collected from the same crops except in one case where in place of moong one more sample was taken from lowar. In November 25 samples were collected as follows : 2 samples from baira, 4 samples from sugarcane, 3 samples from pigeon-pea, one sample from lowar, 2 samples from raddish, 4 samples from potato + toria, 5 samples from wheat, 2 samples from oat and 2 samples from berseem. Maximum samples in December were collected from wheat (13). This was followed by sugarcane and potato + toria (4 in each). The remaining 4 samples were taken from oat and berseem (2 in each). In January, February and March maximum number of samples were collected from wheat (17 samples), followed by sugarcane (4 samples), oat (2 samples) and berseem (2 samples).

TABLE - 63

Number of soil samples collected from various crops in eight crop rotations from August, 1971 to July, 1972

Crops	Number of samples											
	Aug- ust	Sept- ember	Octo- ber	Nov- ember	Dece- mber	Jan- uary	Feb- ruary	Mar- ch	Ap- ril	May	June	July
Bajra	2	2	2	2	-	-	-	-	-	-	-	2
Sugarcane	4	4	4	4	4	4	4	4	4	4	4	4
Maize	14	14	14	-	-	-	-	-	-	-	-	11
Pigeon-pea	1	1	1	3	-	-	-	-	-	-	1	1
Pigeon-pea+MOONG	1	1	1	-	-	-	-	-	-	-	-	2
Jowar + pigeon-pea	1	1	1	-	-	-	-	-	-	-	-	1
Jowar	1	1	2	1	-	-	-	-	-	10	10	1
MOONG	1	1	-	-	-	-	-	-	-	-	-	1
Raddish	-	-	-	2	-	-	-	-	-	-	-	-
Potato + toria	-	-	-	4	4	17	17	17	17	2	2	-
Wheat	-	-	-	5	13	17	17	17	4	4	4	-
Cowpea	-	-	-	2	2	2	2	2	-	-	-	-
Oat	-	-	-	2	2	-	-	-	-	2	-	-
Lobia	-	-	-	-	-	-	-	-	-	-	-	2
Cotton	-	-	-	-	-	2	2	2	-	-	-	-
Berseem	-	-	-	2	2	-	-	-	-	2	-	-
Lobia + MOONG	-	-	-	-	-	-	-	-	-	1	3	-
Fallow	-	-	-	-	-	-	-	-	-	-	3	-
Total	25	25	25	25	25	25	25	25	25	25	25	25

In April the number of samples from wheat and sugarcane remained the same but in place of oat and berseem, 4 samples were collected from cowpea. In May and June maximum number of samples were collected from moong (10 samples), followed by sugarcane and cowpea (4 in each). In May, 2 samples were collected from lobia, 2 samples from lobia + moong and one sample from fallow. In June, 3 samples were collected from lobia + moong and 3 samples from fallow. In July maximum samples were collected from maize (11 samples), followed by sugarcane (4 samples), haira (2 samples), pigeon-pea + moong (2 samples), cotton (2 samples) and pigeon-pea, lowar + pigeon-pea, moong and lowar (1 in each).

The data for 8 crop rotations have been furnished in Tables 64 to 71. These have also been shown graphically in Fig. V-B.

An examination of these Tables and Figures V-A indicated that maximum number of nematodes (Tylenchorhynchus, Hoplolaimus, Pratylenchus, Helicotylenchus, free-living nematodes, and total plant-parasitic nematodes) were extracted by Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> and 5 ppm methylene blue.

Cobb's modified decanting and sieving + centrifugal flotation technique was 3, 6, 10 and 11 times superior to Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue in case of Tylenchorhynchus, Helicotylenchus, free-living and total plant-parasitic nematodes. It was only one time superior in respect of

TABLE - 64

Population fluctuation and efficiency of extraction techniques during different periods for obtaining Tylenchothynchus (8 crop rotations)

Mean number of nematodes from 50 g soil		Extraction techniques						C.D.
Year/ Month	Direct placement of soil over moistened faecal tissue paper	Oostenbrink's elutriator	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving+ sugar flotation sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue.	Cobb's modified decanting and sieving+ centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	Cobb's modified decanting and sieving+ centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	C.D.	
1971								
August	17.1	40.2	52.9	54.8	63.1	89.3	13.8	
September	15.9	68.1	87.6	89.3	102.9	132.7	21.3	
October	38.5	75.3	138.6	95.3	148.3	194.7	21.4	
November	24.4	43.3	56.4	80.4	93.4	120.7	26.6	
December	20.4	43.0	47.3	60.3	80.5	100.6	21.3	
1972								
January	48.4	60.4	75.6	90.3	109.4	120.6	25.7	
February	55.0	73.3	104.5	109.5	134.3	177.5	7.4	
March	56.0	93.3	109.4	116.3	140.5	189.8	16.8	
April	64.1	113.6	126.8	131.6	245.0	269.0	15.4	
May	61.5	81.3	96.3	103.6	112.5	125.5	12.0	
June	46.8	69.8	81.6	90.3	94.3	117.5	10.5	
July	30.4	52.6	56.2	64.2	63.8	78.7	8.2	

TABLE - 65

Population fluctuation and efficiency of extraction techniques during different periods for obtaining Hoplolaimus (8 crop rotations)

		Mean number of nematodes from 50 g soil						
		Extraction techniques						
Year/ Month	Direct place- ment of soil over moistened facial tissue paper	Oosten- brink's elutria- tor	Cobb's modified decanting and siev- ing tech.	Cobb's modified decanting and sieving+ sugar flota- tion sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue	Cobb's modified decanting and siev- ing+centri- fugal flota- tion	Cobb's modified decanting and sieving+ centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	C.D. 5%	C.D. 1%
1971	August	15.4	44.1	45.4	49.3	58.9	8.5	9.6
	September	26.5	92.6	78.0	82.8	128.6	23.3	26.2
	October	10.9	47.5	54.1	65.9	87.5	12.6	14.2
	November	9.6	24.3	32.6	38.6	48.4	3.6	4.5
	December	12.3	27.3	43.4	48.4	60.5	8.9	9.8
1972	January	14.3	29.4	45.0	54.4	68.6	4.7	5.8
	February	17.6	31.7	47.1	63.3	77.2	9.5	10.7
	March	20.9	36.3	56.9	65.3	91.3	10.6	12.0
	April	36.5	65.3	80.4	123.6	168.6	28.3	31.7
	May	15.4	48.1	75.4	109.3	138.4	9.3	10.5
	June	14.4	39.8	69.3	80.8	90.5	2.4	4.3
	July	13.5	28.6	32.6	34.3	48.4	6.6	7.4

TABLE - 66

Population fluctuation and efficiency of extraction techniques during different periods for obtaining *Pratylenchus* (8 crop rotations)

Year/ Month	Mean number of nematodes from 50 g soil						C.D. 5%	C.D. 1%
	Direct placement soil over moistened facial tissue paper	Cobb's modified decanting and siev- ing tech.	Cobb's modified decanting and siev- ing tech. sugar flota- tion sieving with 12.5 ppm Separan SP7 † 5 ppmmethylene blue	Cobb's modified decanting and siev- ing+centri- fugal flota- tion	Cobb's modified decanting and sieving† centrifugal flotation with 12.5 ppm Separan SP7 † 5 ppm methylene blue			
<u>1971</u>								
August	21.0	40.3	54.0	65.3	85.0	98.7	8.3	9.4
September	24.5	83.3	88.3	85.4	106.2	141.9	26.2	29.5
October	28.4	88.2	99.4	97.9	122.3	145.9	21.7	24.4
November	23.5	68.5	90.4	97.5	100.3	135.9	20.6	23.0
December	20.4	58.3	65.4	75.3	87.3	130.9	19.9	22.4
<u>1972</u>								
January	21.3	60.4	68.4	80.4	97.4	131.9	18.4	20.5
February	61.2	86.5	105.5	110.7	112.1	138.7	14.6	16.4
March	63.5	65.2	89.5	103.4	162.7	175.5	10.2	11.4
April	64.7	87.0	93.6	108.4	177.3	193.2	14.2	16.0
May	41.8	52.5	64.8	89.5	167.5	182.9	11.1	12.5
June	27.3	42.4	57.2	64.3	74.4	96.9	11.9	12.6
July	21.6	34.7	41.0	43.4	50.8	62.6	7.9	8.9

TABLE - 67

Population fluctuation and efficiency of extraction techniques during different periods for obtaining *Heterodera* larvae (8 crop rotations)

		Mean number of nematodes from 50 g soil						
		Extraction techniques						
Year/ Month	Direct place- ment of soil over moistened facial tissue paper	Dosten- brink's elutria- tor	Cobb's modified decanting and siev- ing tech.	Cobb's modified decanting and siev- ing tech. sugar flota- tion sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue	Cobb's modified decanting and siev- ing+centri- fugal flota- tion	Cobb's modified decanting and sieving+ centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	C.D. 5%	C.D. 1%
<b>1971</b>								
August	10.0	15.2	15.5	20.5	30.4	40.6	5.6	7.3
September	15.3	30.4	30.6	40.5	45.6	47.5	7.0	8.2
October	25.3	31.4	35.6	45.6	52.7	55.6	6.8	7.9
November	20.0	10.5	30.4	40.7	50.3	52.3	5.5	6.1
December	20.4	25.0	31.4	42.6	44.3	54.4	7.5	8.9
<b>1972</b>								
January	20.8	30.4	41.4	44.3	46.7	58.1	9.6	10.8
February	21.22	36.5	40.8	45.8	47.9	58.9	8.0	9.1
March	13.3	18.0	19.0	21.7	25.3	34.0	5.7	6.4
April	22.1	29.6	30.7	30.7	32.1	42.5	7.4	8.3
May	37.9	41.4	49.3	49.5	55.3	78.5	11.5	12.9
June	20.5	30.8	47.5	57.5	63.4	75.8	6.8	7.7
July	20.0	25.5	39.2	40.4	43.7	51.7	5.5	6.1

TABLE - 68

Population fluctuation and efficiency of extraction techniques during different periods for obtaining Helicotylenchus (8 crop rotations)

		Mean number of nematodes from 50 g soil							
		Extraction techniques							
Year/ Month	Direct place- ment of soil over moistened facial tissue paper	Oosten- brink's elutria- tor	Cobb's modified decanting and siev- ing tech.	Cobb's modified decanting and sieving+ sugar flota- tion sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue	Cobb's modified decanting and siev- ing+centri- fugal flota- tion	Cobb's modified decanting and sieving+ centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	C.D. 1%	C.D. 5%	C.D. 1%
<b>1971</b>									
August	13.3	29.8	34.8	45.0	68.9	78.5	9.2	10.3	
September	21.0	51.3	62.4	70.8	84.5	113.7	19.1	21.5	
October	28.5	53.5	67.3	93.8	117.3	130.7	21.5	24.2	
November	42.3	74.6	84.6	96.4	127.4	164.3	16.7	18.7	
December	46.4	82.6	100.3	113.4	140.3	150.4	21.6	24.7	
<b>1972</b>									
January	50.8	90.4	104.3	125.6	145.7	174.4	20.6	23.7	
February	56.4	94.3	109.4	129.4	150.7	178.4	12.9	14.5	
March	81.8	132.3	146.4	169.6	255.3	292.6	10.1	11.4	
April	89.5	117.3	135.4	148.4	195.9	289.6	15.1	17.8	
May	30.0	49.6	68.6	88.6	98.8	139.4	8.4	9.5	
June	21.3	33.4	38.4	64.5	73.6	83.4	9.2	10.3	
July	43.2	55.0	65.7	78.3	89.7	98.4	9.4	10.5	

TABLE - 69

Population fluctuation and efficiency of extraction techniques during different periods for obtaining *Trichostrongylus* (8 crop rotations)

		Mean number of nematodes from 50 g soil						
		Extraction techniques						
Year/ Month	Direct place- ment of soil over moistened facial tissue paper	Oosten- brink's elutria- tor	Cobb's modified decanting and siev- ing tech.	Cobb's modified decanting and sieving+ sugar flota- tion+sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue	Cobb's modified decanting and siev- ing+centri- fugal flota- tion	Cobb's modified decanting and sieving+ centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	C.D. 5%	C.D. 1%
<b>1971</b>								
August	14.7	20.9	29.7	35.6	20.0	15.9	8.5	9.6
September	15.4	23.2	35.4	39.6	12.5	14.4	10.8	12.1
October	15.8	24.6	21.9	20.8	12.2	10.7	7.5	8.5
November	13.4	22.6	21.7	20.4	15.3	10.4	8.6	9.5
December	11.4	20.4	19.7	20.4	16.4	11.4	7.6	9.0
<b>1972</b>								
January	10.8	21.4	19.7	20.4	14.3	10.4	8.5	9.6
February	10.5	16.1	15.5	17.2	10.5	13.5	3.6	4.5
March	19.2	19.6	15.0	14.2	12.5	10.8	6.2	7.0
April	16.4	18.6	18.7	16.8	12.3	8.2	5.8	6.5
May	14.5	27.3	30.0	27.3	16.4	14.5	8.3	9.4
June	16.7	17.7	20.0	21.0	15.7	11.0	5.4	6.1
July	20.4	21.7	22.1	21.6	18.8	18.8	7.1	8.3

TABLE - 70

Population fluctuation and efficiency of extraction techniques during different periods for obtaining free-living nematodes (8 crop rotations)

Mean number of nematodes from 50 g soil		Extraction techniques						C.D. 5%	C.D. 1%
Year/ Month	Direct place- ment of soil over moistened facial tissue paper	Oosten- brink's elutriator	Cobb's modified decanting and sieving tech.	Cobb's modified decanting and sieving sugar flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	Cobb's modified decanting and sieving centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	Cobb's modified decanting and sieving centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue			
1971									
August	194.5	241.3	258.0	280.2	298.3	315.9	15.9	17.9	
September	214.8	267.2	291.8	293.4	317.5	357.1	21.5	24.2	
October	244.3	248.4	268.4	278.3	380.5	399.5	21.7	24.5	
November	276.3	360.0	378.3	275.6	340.5	495.5	25.6	29.0	
December	230.5	230.6	256.4	295.5	310.5	485.5	20.4	22.3	
1972									
January	330.6	475.5	585.7	590.0	670.5	850.0	36.7	39.8	
February	340.5	461.5	515.3	610.5	680.5	950.3	16.7	18.6	
March	235.0	277.0	288.4	399.4	413.5	430.6	16.4	18.3	
April	248.3	286.3	318.0	430.4	449.5	570.2	14.2	15.9	
May	273.6	336.4	327.4	338.4	341.4	579.5	33.3	37.5	
June	355.6	476.5	485.5	513.4	495.6	740.3	26.5	29.3	
July	266.8	398.2	399.4	431.5	441.6	675.4	23.5	25.9	

TABLE - 71

Population fluctuation and efficiency of extraction techniques during different periods for obtaining total plant-parasitic nematodes (8 crop rotations)

Mean number of nematodes from 50 g soil

Year/ Month	Extraction techniques								C.D. 1%	
	Direct place- ment of soil over moistened facial tissue paper	Oosten- brink's elutria- tor	Cobb's modified decanting and siev- ing tech.	Cobb's modified decanting and sieving sugar flota- tion sieving with 12.5 ppm Separan SP7 + 5 ppm methylene blue	Cobb's modified decanting and siev- ing+centri- fugal flota- tion	Cobb's modified decanting and sieving centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue	C.D. 5%	C.D. 1%		
<u>1971</u>										
August	91.5	181.0	231.0	266.6	316.7	381.9	20.9	26.1		
September	118.6	334.2	396.9	403.6	434.5	578.8	26.7	31.9		
October	147.4	357.0	410.3	407.5	519.2	625.1	28.9	33.6		
November	133.2	263.8	307.8	367.9	425.2	532.0	33.6	39.4		
December	129.4	253.6	291.4	355.4	417.2	508.2	26.7	29.5		
<u>1972</u>										
January	166.4	289.3	338.8	406.0	469.1	564.0	41.7	47.3		
February	221.9	336.2	407.4	429.4	518.8	644.2	18.3	24.6		
March	254.7	359.0	415.6	482.8	661.6	794.0	17.7	22.2		
April	293.3	409.6	450.9	553.9	585.2	671.3	19.6	25.7		
May	201.1	330.2	353.3	433.9	559.9	631.3	39.6	45.6		
June	147.0	221.5	284.5	366.4	402.2	474.3	31.7	36.7		
July	129.1	213.3	252.8	280.5	301.1	358.6	23.3	31.3		

Pratylenchus and Hoplolaimus.

Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue was significantly better than Cobb's modified decanting and sieving technique, one, five, eight, six, five and eleven times in case of Hoplolaimus, Tylenchorhynchus, Pratylenchus, Helicotylenchus, free-living nematodes and total plant-parasitic nematodes respectively.

Cobb's modified decanting and sieving technique was significantly superior to Costenbrink's elutriator six, two, six, four, seven and twelve times in respect of Tylenchorhynchus, Hoplolaimus, Pratylenchus, Helicotylenchus, free-living nematodes and total plant-parasitic nematodes in the same order.

Costenbrink's elutriator was significantly better than direct placement of soil over moistened facial tissue paper in respect of Pratylenchus, free-living nematodes and total plant-parasitic nematodes <sup>except</sup> /once in December in case of free-living nematodes. It was superior seven, eight and seven times in case of Tylenchorhynchus, Hoplolaimus and Helicotylenchus respectively.

Seasonal fluctuations of different nematodes as determined by six extraction methods have been shown in Fig. V-A. An examination of these data revealed significant interactions between extraction procedures and time with Tylenchorhynchus, the peaks were observed in October, January and April. The

highest peak was in April. From April to July there was a rapid fall in the population. There were marked differences due to techniques.

Hoplolaimus population was increasing from September to November and again from February to April. There was a gradual fall from November to February. The population was maximum in April. In May and June there was a fall in the population, which showed an increase in July. The differences due to extraction techniques were not very much marked.

In September Pratylenchus population was low. It increased in October and from October to December there was a gradual fall. From January there was a gradual increase upto April. It was maximum in April. From April to September there was a fall in the population. There were differences due to extraction techniques.

Helicotylenchus population increased from September to March. The population was maximum in March. From April to September there was a gradual fall in the population. There were marked differences due to extraction techniques.

The population of free-living nematodes increased from September to March. In April there was a rapid fall and again in May there was rapid increase in the population. Again, there was a rapid fall in June. There were marked differences due to extraction techniques.

Further, it was seen that there were marked variations

in the number of total plant-parasitic nematodes. From July-August to October there was an increase in the population. There was a gradual fall from October to December. From December to April the population increased and in April it was maximum. From April to July there was a rapid fall. However, there was a marked peak in April only in respect of 8 crop rotations. There were marked differences due to extraction techniques.

An examination of these data indicated that maximum number of nematodes (Tylenchorhynchus, Hoplolaimus, Pratylenchus, Helicotylenchus, free-living nematodes and total plant-parasitic nematodes) were extracted by Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue except in case of Trichodorus.

Cobb's modified decanting and sieving + centrifugal flotation technique was superior to Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue, four, six, six, four, nine, seven and eleven times in respect of Tylenchorhynchus, Hoplolaimus, Pratylenchus, Heterodera larvae, Helicotylenchus free-living and total plant-parasitic nematodes respectively.

Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue was only once significantly better than Cobb's modified decanting and sieving technique in case of Tylenchorhynchus and Trichodorus. This was eight, four, five, seven, seven and ten times superior in respect of Hoplolaimus, Pratylenchus, Heterodera larvae,

Helicotylenchus, free-living and total plant-parasitic nematodes respectively.

Cobb's modified decanting and sieving technique was significantly superior to Oostenbrink's elutriator, four, six, six, four, four, two, seven and nine times in respect of Tylenchorhynchus, Hoplolaimus, Pratylenchus, Heterodera larvae Helicotylenchus, Trichodorus, free-living nematodes and total plant-parasitic nematodes respectively.

Oostenbrink's elutriator was significantly superior to direct soil placement over moistened facial tissue paper in respect of Pratylenchus, Helicotylenchus and total plant-parasitic nematodes except once in December in case of Pratylenchus. It was superior ten, ten, seven and nine times in case of Tylenchorhynchus, Hoplolaimus, Heterodera larvae and free-living nematodes respectively.

In case of Trichodorus there was no significant difference due to extraction techniques except in a few cases when Cobb's modified decanting and sieving technique was better than Oostenbrink's elutriator in August and September and direct soil placement over moistened facial tissue paper and Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue were superior to Cobb's modified decanting and sieving + centrifugal flotation technique in April and May respectively. Further, it was seen that the population fluctuations in respect of Tylenchorhynchus, Hoplolaimus and free-living nematodes were pronounced than in

case of Pratylenchus, Heterodera larvae, Helicotylenchus and Trichodorus.

Seasonal fluctuations of different nematodes as determined by six extraction methods have been shown in Fig. V-B, revealed that in the case of Tylenchorhynchus there were two peaks - one in the month of October and the other in April. The population increased from August to October and again from January to April. It was maximum in April. From April onward there was a drastic reduction. The pattern was same in respect of all the extraction techniques employed. In respect of Hoplolaimus also there were two peaks - one in the month of September and the other in April. There was a gradual decrease from September to November, a gradual increase from November to March, a rapid build up from March to April-May, and a rapid fall again from May to July. Here also the pattern was the same in respect of all the extraction methods.

In case of Pratylenchus there was only one peak in the month of April. From September to March there was not much fluctuation but from March to May the population increased and there was a rapid fall during May-July. There was not much difference in the population fluctuations due to extraction procedures.

In respect of Helicotylenchus also there was only one peak during March-April. There was a gradual increase in the population from August to February. It rapidly increased during March-April. There was not much fluctuation upto May after

which there was a gradual increase in the population. The pattern remained the same in respect of all the extraction techniques.

There was not much fluctuation in case of Trichodorus population, except in respect of August and September. Also, there was not much variation due to extraction procedures.

There was not much fluctuation in the Heterodera larval population. Only one peak was observed in May. From July to February the population was generally the same. From February to April there was a drastic fall in the population. The variation due to extraction techniques was not much.

Only one peak was observed in February in case of free-living nematode population. From August to January the population was gradually increasing and it was maximum in February. In March there was a drastic fall and from March to July the population increased. Cobb's modified decanting and sieving centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue was found to be the best. There was not much difference due to other techniques. From December to April there was a gradual increase in the population of the plant-parasitic nematodes. Only one peak was observed in April. There was a marked decline from April to July-August. A slight increase was observed from September to November. There were marked differences due to extraction methods.

VI. Evaluation of nematicidal efficacy with two techniques and two timings of nematode inoculation :

Pot studies were carried out to evaluate the nematicidal efficacy with two techniques and two timings of nematode inoculation. Observations on soil population, root galls/plant and root tissue populations were recorded at different intervals (i.e. one, two, three, four, five and six weeks after transplanting of 5 weeks old tomato seedlings). The results furnished in Tables 72 to 74 and Fig. VI are presented hereunder :

a) Inoculation of 2000 larvae/pot at the time of nematicidal application : An examination of data collected during the first week after transplanting tomato seedlings indicated that when 2000 larvae were inoculated at the time of nematicidal application, DBCP @ 10 gallons/hectare was at par with DBCP @ 5 gallons/hectare which was superior to control. In case of DD treatments nematodes could be obtained from the soil but not from the root-tissue. Also, there was no gall formation.

Similar observations were recorded during the later five weeks but DD treatments did not show nematode populations even in the soil as reported for the first week. Also, DBCP @ 10 gallons/hectare was later found to be superior to DBCP @ 5 gallons/hectare which in turn was superior to control. However, during the sixth week there was no difference between DBCP @ 5 gallons/hectare and control in case of females and larval population in root-tissue.

b) Inoculation of 5 g chopped infested roots/pot at the time of nematicidal application : In general, irrespective of

TABLE - 72

Evaluation of nematicidal efficacy with two techniques and two timings of nematode inoculation - on the basis of soil population

A. 2000 larvae inoculated at the time of nematicidal application (November 1, 1972).

Treatments	Number of nematodes obtained from 50 g soil*					
	1/2/3/4/5/6 weeks after transplanting (15.11.72)					
	1	2	3	4	5	6
DD @ 50 G/he. <sup>a</sup>	30.6	0	0	0	0	0
DD @ 100 G/he.	20.6	0	0	0	0	0
DBCP @ 5 G/he.	40.3	0	0	0	0	0
DBCP @ 10 G/he.	20.3	0	0	0	0	0
Control	60.3	40.3	15.3	0	0	0
C.D. 5%	10.5					

B. 5 g chopped infested root-gall inoculated at the time of nematicidal application (November 1, 1972)

DD @ 50 G/he.	20.3	30.3	10.6	10.3	0	0
DD @ 100 G/he.	10.3	10.6	10.3	0	0	0
DBCP @ 5 G/he.	30.3	60.3	45.5	20.3	0	0
DBCP @ 10 G/he.	20.3	35.5	30.7	10.3	0	0
Control	90.3	80.6	50.3	40.3	0	0
C.D. 5%	7.8	8.6	3.5	4.3		

Cont'd

C, 2000 larvae inoculated (15.11.72)/3 weeks after nematicidal application (24.10.72) and one week after transplanting (7.11.72).

Treatments	Number of nematodes obtained from 50 g soil*					
	1/2/3/4/5/6 weeks after nematode inoculation					
	Weeks					
	1	2	3	4	5	6
DD @ 50 G/he.	60.6	25.5	20.3	0	0	0
DD @ 100 G/he.	30.3	15.6	10.3	0	0	0
DBCP @ 5 G/he.	80.3	45.6	30.3	0	0	0
DBCP @ 10 G/he.	40.3	20.3	20.3	0	0	0
Control	115.3	90.3	60.3	0	0	0
C.D. 5%	16.8	10.3	10.4			

D. 5 g chopped infested root-gall inoculated (15.11.72)/3 weeks after nematicidal application (24.10.72) and one week after transplanting (7.11.72).

DD @ 50 G/he.	30.3	40.4	60.3	30.4	0	0
DD @ 100 G/he.	20.3	30.3	20.6	10.3	0	0
DBCP @ 5 G/he.	65.3	60.3	70.3	50.5	0	0
DBCP @ 10 G/he.	30.3	40.4	55.3	25.4	0	0
Control	100.3	120.0	85.3	70.4	0	0
C.D. 5%	9.7	8.5	18.3	7.4		

\*Average of 3 replications.

TABLE - 73

Evaluation of nematicidal efficacy with two techniques and two timings of nematode inoculation - on the basis of root-gall/plant.

A. 2000 larvae inoculated at the time of nematicidal application (November 1, 1972).

Treatments	Number of root-galls/plant					
	1/2/3/4/5/6 weeks after transplanting(15.11.72)					
	Weeks					
	1	2	3	4	5	6
DD @ 50 G/he.	0	0	0	0	0	0
DD @ 100 G/he.	0	0	0	0	0	0
DBCP @ 5 G/he.	5.7	8.3	11.8	18.3	21.1	23.3
DBCP @ 10 G/he.	4.0	7.0	10.6	12.7	14.6	18.7
Control	8.3	12.3	18.6	22.4	26.5	28.9
C.D. 5%	1.4	1.0	0.8	2.1	3.1	4.3

B. 5 g chopped infested root-gall inoculated at the time of nematicidal application (November 1, 1972)

DD @ 50 G/he.	3.0	5.0	11.3	13.3	14.0	16.3
DD @ 100 G/he.	0	0	4.3	7.0	8.6	11.0
DBCP @ 5 G/he.	11.0	13.3	18.0	19.0	22.6	23.6
DBCP @ 10 G/he.	8.6	9.6	10.7	14.3	15.7	18.7
Control	15.3	21.4	25.6	27.8	30.5	33.5
C.D. 5%	2.5	3.8	5.3	6.8	7.5	6.5

Cont'd

C. 2000 larvae inoculated (15.11.72)/3 weeks after nematocidal application (24.10.72) and one week after transplanting (7.11.72).

Treatments	Number of root-galls/plant*					
	1/2/3/4/5/6 weeks after nematode inoculation					
	Weeks					
	1	2	3	4	5	6
DD @ 50 G/he.	15.6	18.0	26.0	30.6	35.6	35.3
DD @ 100 G/he.	8.6	11.3	14.6	18.6	23.7	26.2
DBCP @ 5 G/he.	22.6	23.3	29.4	33.3	35.3	38.6
DBCP @ 10 G/he.	18.6	19.0	27.1	30.3	31.1	31.3
Control	25.0	30.5	32.6	36.5	39.5	46.3
C.D. 5%	6.3	5.7	6.8	10.5	9.5	6.8

D. 5 g chopped infested root-gall inoculated (15.11.72)/3 weeks after nematocidal application (24.10.72) and one week after transplanting (7.11.72).

DD @ 50 G/he.	6.6	20.0	20.3	23.1	26.3	29.6
DD @ 100 G/he.	0	10.3	13.3	15.0	18.6	21.3
DBCP @ 5 G/he.	14.1	18.3	21.0	23.6	29.3	33.6
DBCP @ 10 G/he.	9.0	11.3	19.3	21.5	25.6	27.7
Control	20.5	25.3	27.3	29.4	36.5	42.7
C.D. 5%	4.3	5.4	6.6	8.7	9.5	10.6

\*Average of 3 replications.

TABLE - 74

Evaluation of nematicidal efficacy with two techniques and two timings of nematode inoculation - on the basis of root-tissue population

A. 2000 larvae inoculated at the time of nematicidal application (November 1, 1972).

Treatments	Number of nematodes obtained from root-tissue*							
	1/2/3/4/5/6 weeks after transplanting (15.11.72)							
	Weeks						6	
	1	2	3	4	5	Female	Larvae	Eggs
DD @ 50 G/he.	0	0	0	0	0	0	0	0
DD @ 100 G/he.	0	0	0	0	0	0	0	0
DBCP @ 5 G/he.	7.3	10.5	18.5	21.7	25.5	33.3	57.5	193.4
DBCP @ 10 G/he.	6.5	9.3	12.6	14.6	16.7	21.5	43.3	126.3
Control	10.3	13.5	26.0	29.3	20.0	26.1	60.5	215.6
C.D. 5%	1.6	1.1	3.5	5.4	6.7	5.8	4.3	10.8

B. 5 g chopped infested root-gall inoculated at the time of nematicidal application (November 1, 1972)

DD @ 50 G/he.	5.6	8.5	17.3	20.5	25.3	29.4	34.3	81.3
DD @ 100 G/he.	0	3.5	6.6	10.3	15.3	19.6	27.3	40.4
DBCP @ 5 G/he.	13.3	16.4	23.4	27.9	30.7	35.6	64.3	225.4
DBCP @ 10 G/he.	10.6	15.3	20.5	23.6	26.7	28.9	49.3	165.3
Control	20.3	27.3	30.6	31.5	33.5	39.4	69.3	259.3
C.D. 5%	2.6	2.8	3.5	4.8	5.7	6.1	5.8	22.5

Cont'd

C. 2000 larvae inoculated (15.11.72)/3 weeks after nematicidal application (24.10.72) and one week after transplanting (7.11.72).

Treatments	No. of nematodes obtained from root-tissue*							
	1/2/3/4/5/6 weeks after nematode inoculation							
	Weeks						6	
	1	2	3	4	5	Female	Larvae	Eggs
DD @ 50 G/he.	18.8	21.2	29.3	35.4	36.9	41.4	60.3	116.5
DD @ 100 G/he.	10.3	15.4	16.3	27.3	29.4	35.4	39.3	49.5
DBCP @ 5 G/he.	25.3	27.4	36.5	39.5	42.3	46.4	82.3	292.3
DBCP @ 10 G/he.	20.4	22.3	26.4	31.3	33.4	36.6	50.4	167.3
Control	27.3	33.5	38.4	42.3	46.5	49.6	73.4	315.6
C.D. 5%	6.5	8.5	10.3	5.6	7.6	10.4	12.3	14.7

D. 5 g chopped infested root-gall inoculated (15.11.72)/3 weeks after nematicidal application (24.10.72) and one week after transplanting (7.11.72).

DD @ 50 G/he.	10.3	23.4	24.6	26.7	31.4	39.6	46.7	73.4
DD @ 100 G/he.	3.4	13.4	15.3	18.4	21.5	29.3	34.6	46.5
DBCP @ 5 G/he.	17.3	25.5	29.4	35.6	38.6	46.4	62.3	168.4
DBCP @ 10 G/he.	12.3	18.3	26.3	31.6	33.9	36.7	42.6	138.5
Control	23.5	29.4	33.5	39.4	43.6	48.5	80.5	235.6
C.D. 5%	3.4	4.7	5.3	4.9	5.7	4.8	11.3	26.7

nematode population in soil or in root tissue or number of root galls/plant and time observations, DD @ 100 gallons/hectare was superior to DD @ 50 gallons/hectare, which in turn was better than DBCP @ 10 gallons/hectare. DBCP @ 10 gallons/hectare was superior to DBCP @ 5 gallons/hectare, which was better than control. There were a few exceptions when DD @ 100 gallons/hectare was at par with DD @ 50 gallons/hectare and DD @ 50 gallons/hectare was similar to DBCP @ 10 gallons/hectare.

In a few cases DBCP @ 5 gallons/hectare was at par with control. This was always the case in respect of nematode population in the soil.

After fourth week, nematodes were not recorded from the soil but these were found in root tissue. The root galls were also noticed.

c) Inoculation of 2000 larvae/pot one week after transplanting/three weeks after nematicidal application : When 2000 larvae were inoculated one week after transplanting/three weeks after the nematicidal application, DD @ 100 gallons/hectare was found to be most effective irrespective of the time of observation. But in general, there was no significant difference between DD @ 50 gallons/hectare, DBCP @ 10 gallons/hectare, DBCP @ 5 gallons/hectare or control except in case of soil population during the first three weeks, number of root-galls/plant during the one or two weeks and number of eggs and total population in root during the sixth week when DBCP @ 5 gallons/hectare was better than control. Also, DBCP @ 10 gallons/hectare was better

than DD @ 50 gallons/hectare in case of soil population during the first week and number of eggs and total nematode population during the sixth week.

d) Inoculation of 5 g chopped infested root/pot one week after transplanting/three weeks after nematicidal application:

When 5 g chopped infested roots were added to the soil one week after transplanting/3 weeks after nematicidal application DD @ 100 gallons/hectare was mostly better than either DD @ 50 gallons/hectare or DBCP @ 10 gallons/hectare. It was superior to DBCP @ 10 gallons/hectare in respect of number of root galls observed during second, fourth and sixth weeks. It was also true for number of larvae in root tissue recorded during the sixth week. Similar observations were recorded for DD @ 50 gallons/hectare, except that during the second week it was inferior to DBCP @ 5 gallons/hectare, which in turn was inferior to DBCP @ 10 gallons/hectare, which was at par with DD @ 100 gallons/hectare.

DBCP @ 10 gallons/hectare was at par with DD @ 50 gallons/hectare with a few exceptions where either one was superior to other. DBCP @ 10 gallons/hectare was either better or similar to DBCP @ 5 gallons/hectare. This was also the case in respect of DBCP @ 5 gallons/hectare and control and DD @ 50 gallons/hectare and DBCP @ 5 gallons/hectare.

## DISCUSSION

Six experiments viz. (i) comparative efficiency of soil samplers, (ii) distribution and sampling of nematodes, (iii) comparative efficiency of nematode extraction techniques, (iv) effect of storage bag, storage temperature, storage period and extraction techniques on recovery of nematodes from the soil sample, (v) quantitative estimation of nematode population density by six extraction procedures as affected by season and seasonal fluctuation in nematode population, and (vi) evaluation of nematicidal efficiency with two techniques and timings of nematode inoculations, were carried out for evaluating some techniques in Nematology. The results of each experiment have been discussed separately and are presented below :

1) Comparative efficiency of soil samplers : Four types of soil sampling tools (Khurpi, hollow tube-type sampler with 1.0 cm diameter, O'Connor's split corer and hollow tube-type sampler with 2.5 cm diameter) were used to test their efficiency. One composite soil sample consisted of 5 or 15 or 30 sub-samples. Besides the free-living nematodes the plant-parasitic nematodes encountered were : Rotylenchulus, Tylenchorhynchus, Hoplolaimus, Pratylenchus, Meloidogyne larvae, Trichodorus and Tylenchus.

In general, maximum number of nematodes were obtained when the soil samples were collected with the help of a hollow tube-type sampler with 2.5 cm diameter. This was followed by O'Connor's split corer. Sampling with Khurpi and hollow

tube-type sampler with 1.0 cm diameter provided lower number of nematodes. Chawla (1972) reported that there was no significant difference in the number of nematodes extracted when the soil samples were collected either by Khurpi or O'Connor's split corer but the variation between samples was very high in respect of Khurpi. Yates and Finney (1942) showed that although 10 and 15 cm diameter area were equally efficient at low densities, at high densities the comparative efficiency of the 15 cm sampler falls off. In 1946 the conference of Advisory Entomologists recommended that samples should be taken with a half-cylindrical sampler (diameter 2.54 cm) or soil auger (diameter 3.8 cm) to a depth of 20 cm and at a rate of 50 sampling units for fields upto 4.05 hectare (Anscombe, 1950). This was not based on any experiment. Also, it was suggested for the collection of soil samples for estimating the potato root-eelworm cyst population.

It was surprising that irrespective of sampling tool used, 15 probes per sample provided maximum number of nematodes except in respect of Tylenchorhynchus, Hoplolaimus and Meloidogyne larvae in case of O'Connor's split corer, and in case of Trichodorus and Tylenchus in respect of hollow tube-type sampler with 1.0 or 2.5 cm diameter respectively in which case 5 or 30 probes resulted in higher number of nematodes. However, a perusal of C.V. values revealed that in seven cases it was lower when the composite samples consisted of 30 probes. It was maximum in case of Tylenchus which recorded the least numbers. The higher number of nematodes from 15 cores in a few cases in comparison to

30 cores could be due to the patchy distribution of nematodes (dot diagrammes Figs. 2A-I) indicating that if there is a wide range in the possible results of our sample, we are the more likely to happen upon 'exceptional' cores which will throw our average off the true value.

Observations regarding the time taken in collecting one composite sample consisting of 5 or 15 or 30 probes revealed that there was no real difference in respect of Khurpi and hollow tube-type sampler with 1.0 cm and 2.5 cm diameter. However, more time was taken in respect of O'Connor's split corer but probably the fixed diameter of this split corer could be helpful in taking homogeneous soil samples with little personal error. Also, with the O'Connor's split corer, the risk of compressing the sample by forcing it out of the corer is avoided. After the core has been taken, the clamping band can be loosened, the two halves of the corer separated and the sample exposed. Further more, sample can then be easily divided into different soil layers.

ii) Distribution and sampling of nematodes : The data of the precision experiment were collected on the basis of micro-plot (1 sq. metres) as the smallest practicable unit. The spatial distribution of each nematode genus, total plant-parasitic nematodes and free-living nematodes represented in dot diagrams showed that nematodes were not uniformly distributed over different portion of the plot, but these were distributed in patches of varying densities. The density in micro-plot ranged from 0-625, 0-110, 0-420, 0-240, 0-250, 0-100, 0-200, 0-975 and 0-630 in

respect of Tylenchorhynchus, Hoplolaimus, Pratylenchus, Heterodera larvae, Helicotylenchus, Trichodorus, Hemicriciconemoides, total plant-parasitic nematodes and free-living nematodes in the same order.

The method of stratified sampling was adopted for the estimation of minimum percentage of the sample for a dependable estimate of the population with row and column densities in the successive zones for Tylenchorhynchus, Hoplolaimus, Pratylenchus, Heterodera larvae, Helicotylenchus, Trichodorus, Hemicriciconemoides, total plant-parasitic nematodes and free-living nematodes. The percentage of population to be sampled have been obtained for the various nematodes in order to have the estimate of the population at 20 per cent and 10 per cent level of accuracy. These have been shown in the following table :

<u>Genera</u>	<u>Percentage estimate of population at</u>	
	<u>20 per cent</u>	<u>10 per cent</u>
<u>Tylenchorhynchus</u>	16-40	20-60
<u>Hoplolaimus</u>	32-48	60-80
<u>Pratylenchus</u>	16-40	40-80
<u>Heterodera</u> larvae	16-40	20-80
<u>Helicotylenchus</u>	16-40	40-80
<u>Trichodorus</u>	32-60	40-80
<u>Hemicriciconemoides</u>	32-60	60-80
Total plant-parasitic nematodes	12-16	16-24
Free-living nematodes	12-24	40-48

It may be argued that a sample of 4 units taken from one random row or one unit taken from 4 random rows consisting of 16 per cent of the total number of units would give the estimate with the error of 20 per cent of the mean. The population was so predominantly heterogenous that we cannot expect to get the estimate with a percentage error below 20 per cent.

Israel *et al.* (1969) reported that in the paddy nursery area, one sample from 5 soil cores drawn at random in an area of 50 sq.m was sufficient. In the fields, strata were marked to comprise 5m x 5 m squares and one sample for every 25 sq.m area was found to be essential.

iii) Comparative efficiency of nematode extraction techniques (soil samples processed immediately after collection) :  
 Eleven techniques viz. (i) direct soil placement over moistened facial tissue paper; (iia) Oostenbrink's elutriator with double layer of cotton wool filter; (iib) Oostenbrink's elutriator with single layer of cotton wool filter; (iic) Oostenbrink's elutriator with double layer of facial tissue paper; (iii) Cobb's modified decanting and sieving technique with double layer of facial tissue paper; (iv) sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub>; (v) sugar flotation sieving with 12.5 ppm Separan PG<sub>2</sub>; (vi) Cobb's modified decanting and sieving technique + sugar flotation sieving with 12.5 ppm Separan PG<sub>2</sub> + 5 ppm methylene blue; (vii) Cobb's modified decanting and sieving technique + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue; (viii) centrifugal flotation; (ix) Cobb's modified decanting

and sieving technique + centrifugal flotation; (x) Cobb's modified decanting and sieving technique + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue and (xi) Seinhorst's erlenmeyer flask method have been compared to evaluate their efficiency in nematode extraction from soil samples. The soil was sandy loam and harboured Tylenchorhynchus, Hoplolaimus, Pratylenchus, Helicotylenchus; Trichodorus; Hemicriconemoides and free-living nematodes.

The average number of nematodes obtained by the use of various techniques ranged from 27 to 1848. Nematode-wise these ranged as follows : Tylenchorhynchus : 62 to 436, Hoplolaimus : 30 to 160, Pratylenchus : 102-534, Helicotylenchus: 82 to 564, Trichodorus : 35 to 112, Hemicriconemoides : 27 to 108, total plant parasitic nematodes : 338 to 1848 and free-living nematodes : 314 to 820.

On the basis of number of nematodes obtained, the extraction techniques could be grouped into three categories, namely : efficient, moderately efficient and poor. These have been generally based on the following criteria:

Genera	Number of nematodes		
	Efficient	Moderately efficient	Poor
<u>Tylenchorhynchus</u>	250	151-249	150
<u>Hoplolaimus</u>	100	51-99	50
<u>Pratylenchus</u>	450	201-449	200
<u>Helicotylenchus</u>	400	151-399	150
<u>Trichodorus</u>	90	61-89	60
<u>Hemicriconemoides</u>	90	51-89	50
Total plant-parasitic nematodes	1470	601-1469	600
Free-living nematodes	600	401-599	400

Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue, Cobb's modified decanting and sieving + centrifugal flotation, Cobb's modified decanting and sieving + sugar flotations sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue and Cobb's modified decanting and sieving technique might be placed in the efficient category and amongst these Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue was superior to others except in respect of Trichodorus, in which case this technique could be categorised as moderately efficient to poor.

Cobb's modified decanting and sieving + centrifugal flotation was inferior to Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene

blue and superior to Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue only in respect of Tylenchorhynchus, Helicotylenchus and free-living nematodes. It was inferior in respect of others. Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue was generally inferior to Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue and superior to Cobb's modified decanting and sieving technique with two kinds of exceptions - (i) when Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue was superior to Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue and (ii) when it was inferior to Cobb's modified decanting and sieving technique as reported in respect of Helicotylenchus, Trichodorus and free-living nematodes. Cobb's modified decanting and sieving was generally inferior to Cobb's modified decanting and sieving + centrifugal flotation, Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue and Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue, but it was found to be the most efficient technique for extraction of Trichodorus. Also, in respect of Hemicriconemoides it was inferior to four other techniques, besides Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue and Cobb's modified decanting

and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue.

The techniques which could be placed in moderately efficient category are Oostenbrink's elutriator with double layer of facial tissue paper, sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub>, sugar flotation sieving with 12.5 ppm Separan PG<sub>2</sub> and centrifugal flotation.

The techniques which could be placed in poor category are direct soil placement over moistened facial tissue paper, Oostenbrink's elutriator with double layer of cotton wool filter, Oostenbrink's elutriator with single layer of cotton wool filter, Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan PG<sub>2</sub> + 5 ppm methylene blue and Seinhorst's erlenmeyer flask method. There were few exceptions when Oostenbrink's elutriator with double layer of cotton wool filter and Oostenbrink's elutriator with single layer of cotton wool filter were superior in respect of Trichodorus.

Chawla (1972) has also reported that centrifugal flotation method was found to give maximum recovery of nematodes but the extraction was not significantly different either from decanting and sieving method or Cobb's gravity and sieving method but in present studies the Cobb's modified decanting and sieving + centrifugal flotation and centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue were significantly superior to all the techniques used.

Caveness and Jensen (1955), Oostenbrink (1956) Whitehead

and Hemming (1965), MetClitski and Ramanenko (1969), Elmiligy and DeGrisse (1970), DeGrisse (1970) have also recommended that centrifugal flotation technique was <sup>The</sup> best. Barker et al. (1969) reported that Baermann funnel, centrifugal flotation and sugar flotation sieving were species selective in a study of nematode population dynamics over one year. Under <sup>The</sup> present investigations Cobb's modified decanting and sieving + centrifugal flotation with 12.5 Separan SP<sub>7</sub> + 5 ppm methylene blue has proved to be the best for all the nematodes except Trichodorus in which case Cobb's modified decanting and sieving technique was best.

iv) Effect of storage bag, storage temperature, storage period (week) and extraction techniques on the recovery of nematodes from soil samples : These studies were carried out with two types of storage bags (polythene and paper), eight storage temperatures (0, 5, 10, 15, 20, 25, 30°C and room temperature 15-20°C), six storage periods (one, two, three, four, six and eight weeks) and three extraction techniques (Oostenbrink's elutriator, Cobb's modified decanting and sieving technique and Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue). Observations were made in respect of Tylenchorhynchus, Hoplolaimus, Pratylenchus, Helicotylenchus, Trichodorus and free-living nematodes.

An examination of pooled data indicated that, in general, polythene bag was superior to paper bag except in respect of Hoplolaimus, in which case there was no difference between the two types of storage bags. With few exceptions 15°C was the best

storage temperature for all the nematodes, except Hoplolaimus for which 10°C was found better than others. Storage period for the one week was most suitable for all the nematodes. Amongst all the extraction techniques used Oostenbrink's elutriator was least efficient, Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue was superior in respect of Tylenchorhynchus and free-living nematodes. It was similar to Cobb's modified decanting and sieving technique in case of Pratylenchus, Helicotylenchus and total plant parasitic nematodes. Similar results were observed in case of experiment III.

An examination of the pooled data pertaining to storage temperature x storage bag revealed that polythene bag was superior at 0°C in respect of total plant-parasitic nematodes; at 5°C in respect of Helicotylenchus and free-living nematodes; at 10°C in respect of Hoplolaimus, Pratylenchus, Trichodorus and free-living nematodes; at 15°C in respect of Tylenchorhynchus, Pratylenchus, Helicotylenchus and free-living nematodes; at 20°C in respect of Helicotylenchus; at 25°C in respect of free-living nematodes and at room temperature (15-20°C) in respect of Pratylenchus.

The comparative study of storage temperature x storage period revealed that at 5°C there was no significant difference amongst one, two and three weeks storage but these were superior to four, six and eight weeks storage. At 15°C, two weeks storage was superior to eight weeks. At 20°C one week storage was

superior to six and eight weeks; at 25°C one week was superior to four, six and eight weeks and at room temperature two weeks storage was superior to eight weeks storage in respect of Tylenchorhynchus; at 10°C one week storage was significantly superior to eight weeks storage; at 15°C one and two weeks storage were superior to eight weeks and at 20°C one week was superior to three weeks storage in respect of Hoplolaimus; at 5, 10, 15, 20°C and at room temperature (15-20°C), three weeks storage was superior to one week storage in respect of Pratylenchus, at 0°C two weeks storage was superior to three weeks; at 5°C one week storage was superior to four weeks storage, and at 20 and 25°C one week was significantly superior to three weeks storage in respect of Helicotylenchus; at 15°C one week of storage was superior to all; at 20°C, 25°C and at room temperature (15-20°C) one week storage was significantly superior to three weeks in respect of free-living nematodes.

The comparative study of storage temperature x extraction technique revealed that Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue was superior to Oostenbrink's elutriator at 5°C in respect of Hoplolaimus, Pratylenchus and free-living nematodes; at 15°C in respect of Pratylenchus, Helicotylenchus and free-living nematodes; at 25°C in respect of free-living nematodes and at room temperature (15-20°C) in respect of Helicotylenchus and free-living nematodes.

A comparative study of extraction techniques x storage

bag revealed that Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue was found to be the best technique when the samples were stored in polythene bag in respect of Tylenchorhynchus, Pratylenchus, Helicotylenchus, free-living and total plant-parasitic nematodes. Oostenbrink's elutriator was inferior in respect of Hoplolaimus, Pratylenchus and total plant-parasitic nematodes. Cobb's modified decanting and sieving technique was best in respect of Tylenchorhynchus, Pratylenchus, Helicotylenchus and Trichodorus when the samples were stored in polythene bag. This finding also supports the findings reported in experiment III.

Comparative study of extraction technique x storage period revealed that in case of Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue, one, two and three weeks storage was significantly superior to four, six and eight weeks storage in respect of Tylenchorhynchus. In case of Hoplolaimus, however, one week storage was superior. With Pratylenchus, one, two, three and four weeks storage were superior to six and eight weeks storage. In case of free-living nematodes, one and two weeks storage were superior to others. With Cobb's modified decanting and sieving technique one and two weeks storage was superior to all in respect of Tylenchorhynchus. With Helicotylenchus there was no real difference between one and two weeks storage but in respect of free-living nematodes one and two weeks storage were superior to all storage periods.

Comparative study of storage period x storage bag

revealed that polythene bag was superior when the samples were stored for one week in respect of Pratylenchus; two weeks in respect of Pratylenchus and free-living nematodes; three weeks in respect of Tylenchorhynchus, Pratylenchus, Helicotylenchus, Trichodorus and free-living nematodes and four weeks in respect of Pratylenchus, Helicotylenchus, Trichodorus and free-living nematodes. Oostenbrink (1960) has reported that the population of Pratylenchus pretensis increased whereas that of Tylenchorhynchus dubius gradually decreased in soil stored for 18 weeks at 4-6°C in plastic bags. Number of both species declined when soil was kept in paper bags. Griffin and Barker (1966)<sup>and</sup> Barker et al. (1969) obtained a higher recovery of ectoparasites when the soil was stored at freezing temperature prior to extraction. They demonstrated that storage temperatures affect the efficiency of nematode extraction procedures. It was suggested that when sugar flotation sieving methods of extraction were employed, soil samples might be stored at -15 or 13°C but when methods which depend on nematode motility such as Baermann funnel procedure were to be used. Samples might be stored at 13°C. Dasgupta and Raski (1968) worked on survival of Rotylenchulus parvus in soil at different temperatures<sup>and</sup> reported that nematode population decreased with lower temperatures and longer period of exposure. Four weeks after storage at 1°C only one larva was recovered from soil and only a few from the soil stored at 4°C.

v) Quantitative estimation of nematode population densities by six extraction procedures as affected by season and seasonal fluctuation in nematode population: Two experiments were designed to compare the efficiency of six nematode extraction methods: direct soil placement over moistened facial tissue paper (DT); Oostenbrink's elutriator with double layer of facial tissue paper (OD), Cobb's modified decanting and sieving technique (CS); Cobb's modified decanting and sieving + sugar flotation sieving with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue (CSSSM); Cobb's modified decanting and sieving + centrifugal flotation (CSCF); and Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue (CSCFSM) in measuring seasonal population shifts of plant-parasitic and free-living nematodes.

The first experiment was carried out in a field given to maize-wheat rotation. Samples during August, September and October were taken from maize. From November to April these were collected from wheat. Samples during May, June and July were taken from fallow. These have been graphically represented in Fig. V-A. The plant-parasitic nematodes observed and studied were Tylenchorhynchus, Hoplolaimus, Pratylenchus and Helicotylenchus.

The other experiment carried out in Top Block-I of the Division of Agronomy comprised 8 crop rotations viz :

- i) Sugarcane-wheat-moong.
- ii) Jowar/baira-wheat.
- iii) Moong-wheat-lobia.

- iv) Maize (Cobs) -raddish-wheat.
- v) Moong + pigeon-pea-wheat.
- vi) Maize-potato/toria-wheat-moong.
- vii) Maize-wheat-moong-baira/jowar.
- viii) Maize-oat/berseem/cowpea (for fodder).

The plant-parasitic nematodes observed and studied were Tylenchorhynchus, Hoplolaimus, Pratylenchus, Helicotylenchus, Heterodera larvae and Trichodorus.

The details regarding the number of samples collected from various crops have been given in Table 63.

Twenty-five samples in the month of August and September comprised 14 samples from maize, 4 samples from sugarcane, 2 samples from baira and one each from pigeon-pea, pigeon-pea + moong, jowar+ pigeon-pea, jowar + moong and jowar. In October also, the samples were collected from the same crops except in one case where in place of moong one more sample was taken from jowar. In November, 25 samples were collected as follows : 3 samples from pigeon-pea, one sample from jowar, 2 samples from raddish, 4 samples from potato + toria, 5 samples from wheat, 2 samples from oat and 2 samples from berseem. Maximum samples in December were collected from wheat (13). This was followed by sugarcane and potato + toria (4 in each). The remaining 4 samples were taken from oat and berseem (2 in each). In January, February and March maximum number of samples were collected from wheat (17 samples), followed by sugarcane (4 samples), oat (2 samples) and berseem (2 samples). In April, the number of samples from wheat and sugarcane remained

the same but in place of oat and berseem, 4 samples were collected from cowpea. In May and June, maximum number of samples were collected from moong (10 samples) followed by sugarcane and cowpea (4 in each). In May, 2 samples were collected from lobia, 2 samples from lobia + moong and one sample from fallow. In June, 3 samples were collected from lobia + moong and 3 samples from fallow. In July, maximum samples were collected from maize (11 samples), followed by sugarcane (4 samples), bajra (2 samples) pigeon-pea + moong (2 samples), cotton (2 samples) and pigeon-pea, lowar + pigeon-pea, moong and lowar (1 in each).

The data for 8 crop rotations have been presented in Tables 64 to 71. These have also been shown in Fig. V-B.

The results pertaining to the two experiments have been discussed below.

Combined data for the two experiments with different nematodes showed highly significant interactions between each extraction method and time. Irrespective of crop rotations - two or eight, the maximum variation in the number of nematodes obtained by different methods was 5-6 times, except in respect of Trichodorus and Heterodera larvae in which case the variation was only upto 2-3 times.

The data have been further summarised to show as to when there was no significant difference between the extraction procedures (Table 75).

TABLE - 75

Summarised data showing the number of times there was no significant difference between the various extraction techniques

Relation- ship between different techniques	Nematodes									
	Tylenchorhynchus	Hoplolaimus	Pratylenchus	Helicotylenchus	Heterodera larvae	Trichogonus	Free-living	Total plant-parasitic		
	2	8	2	8	2	8	2	8	2	8
1=2-3=4=5=6	1	3	1	1	1	1	1	1	1	1
1=2-3=4=5	1	3	1	1	1	1	1	1	1	1
1=2-3=4	1	1	1	1	1	1	1	1	1	1
1=2-3	1	1	1	1	1	1	1	1	1	1
1=2	1	1	1	1	1	1	1	1	1	1
1=2-5=6	1	1	1	1	1	1	1	1	1	1
1=5=6	1	1	1	1	1	1	1	1	1	1
1=4	1	1	1	1	1	1	1	1	1	1
2=3=4=5	2	6	1	4	1	2	1	1	1	1
2=3=4	3	1	2	2	1	1	1	1	1	1
2=3	1	1	1	1	1	1	1	1	1	1
2=4	1	1	1	1	1	1	1	1	1	1
3=4=5	1	1	1	1	1	1	1	1	1	1
3=4	1	1	1	1	1	1	1	1	1	1
3=5	1	1	1	1	1	1	1	1	1	1
4=5=6	1	1	1	1	1	1	1	1	1	1
4=5	3	1	1	1	1	1	1	1	1	1
5=2	1	1	1	1	1	1	1	1	1	1
5=6	1	1	1	1	1	1	1	1	1	1

1 = DT; 2 = OD; 3 = CS; 4 = CSSSM; 5 = CSCF and 6 = CSCFSM.

In general, irrespective of nematode and time of sampling, CSCFSM was superior to CSSF, except in case of Trichodorus which was found only in respect of 8 crop rotations.

CSSF was either superior or equal to CSSSM except in a few cases in respect of Trichodorus. In respect of plant-parasitic nematodes, however, it was mostly superior. With Hoplolaimus and Pratylenchus while in case of maize-wheat rotation it was superior as in respect of September and July respectively, in case of 8 crop rotations it was better than CSSSM in 50 per cent cases. In case of Tylenchorhynchus, however, irrespective of crop rotations it was only 3-4 times superior to CSSSM. With Helicotylenchus and free-living nematodes it was 6-10 times better than CSSSM.

DI was always inferior to CSCFSM except in the case of Trichodorus and once in the case of Tylenchorhynchus in respect of 8 crop rotation. In several cases there was no significant difference between OD and CS (14), CS and CSSSM (7), CSSSM and CSCF (19), OD, CS and CSSSM (13), OD, CS, CSSM and CSCF (22) and CS, CSSM and CSCF (15).

Combined data for the two experiments showed that with Tylenchorhynchus there were three peaks (October, January and April) in case of maize-wheat rotations and 2 peaks (October and April) in respect of 8 crop rotations. Low numbers were found during April to July. There was a marked variation due to extraction procedures. The peaks were appreciable in case of CSCFSM and CSCF and to some extent <sup>in</sup> CS, in respect of 8 crop

rotations. This was not very appreciable in case of maize-wheat rotation except in case of CSCFSM. The peaks were noticed at the harvest time of maize and wheat.

The number of Hoplolaimus was increasing from September to November and again from February to April. In April one peak was observed in maize-wheat rotation. In 8 crop rotations there were two peaks : one in September and the other in April. From November to March-April there was an increase in the population which showed a fall during May and June.

With Pratylenchus there was one peak in April and minimum numbers were recorded during September to March. This was found in both the crop rotations studied.

With Helicotylenchus also there was only one peak which was noticed during March-April.

Heterodera larvae were found in respect of 8 crop rotations. One peak was observed in May. There was not much variation during July to February. From February to April there was a marked fall in the population.

With Trichodorus there was not much fluctuation in the population except in August and September.

There was one peak in February in case of free-living nematodes in respect of 8 crop rotations. In case of maize-wheat rotation the maximum population was recorded in May. The population increased from September to March.

With the total plant-parasitic nematodes there was a marked fall from April to July-August. From December to April

there was a gradual increase in the population. However, there was a marked peak in April only in respect of 8 crop rotations.

Host crops seem to affect the prevalence of nematode genera because seasonal peaks of the nematodes corresponded to the maximum growth of the host crop (the rabi (winter) crops are at maturity during March-April and Kharif (summer) crops during October). Thus, seasonal effects were confounded with the stages of crop growth. In October and April the average soil temperature was 21 and 26°C respectively which helped in crop growth. The rainfall during these months was 2.5 cm.

Ferris and Barnard (1967) also reported that in soil of soybean field, population of Paratylenchus, Helicotylenchus and Tylenchorhynchus built up during the growing season and reached a peak near the end of the crop. Paratylenchus population in the soil usually reached a peak early in the season and a decline followed. There were drops in the population of most nematodes during very dry periods.

vi) Evaluation of nematicidal efficacy with two techniques and two timings of nematode inoculation : Pot studies were carried out to evaluate the nematicidal efficacy with two techniques and two timings of nematode inoculation. Nematode inoculation was done in two ways viz. 2000 larvae per pot and 5 g chopped infested root galls/pot. These were introduced three weeks after or at the time of nematicidal application. Observations on nematode population in soil and root-tissue and number of root-knot galls/plant were recorded at one, two,

three, four, five and six week after transplanting. The results in respect of two techniques and two timings of nematode inoculation are discussed below :

There were four situations viz : (i) inoculation of 2000 larvae/pot at the time of nematicidal application, (ii) inoculation of 5 g chopped infested root-galls/pot at the time of nematicidal application, (iii) inoculation of 2000 larvae/pot, one week after transplanting/3 weeks after nematicidal application, and (iv) inoculation of 5 g chopped root-galls/pot, one week after transplanting/3 weeks after nematicidal application, under which the efficacy of DD and DBCP at two dosages have been studied.

On the basis of nematode population in soil/root-tissue and number of galls/plant, the four situations could be placed in the following order : inoculation of 2000 larvae/pot one week after transplanting/3 weeks after nematicidal application > inoculation of 5 g chopped infested root-gall/pot one week after transplanting/3 weeks after nematicidal application > inoculation of 5 g chopped root galls/plant at the time of nematicidal application > inoculation of 2000 larvae/pot at the time of nematicidal application.

It may be argued that when the nematodes were inoculated at the time of nematicidal application, the recovery of nematode was poor indicating that the nematodes were affected by all the treatments and it was difficult to differentiate between the treatments. Also, when nematode larvae were inoculated, nematodes could not be seen even after one week but when

infested chopped roots were added, they could be found upto four weeks, indicating that addition of 5 g infested chopped root galls/pot at the time of nematicidal application might show some differentiation in the nematicidal efficacy which was not possible in the former case. The nematode population in soil was either nil or only a few to give any significant difference due to nematicides. Also, even when the nematodes/chopped infested roots were inoculated 3 weeks after the nematicidal application, the trends were not the same in respect of soil population after nematicidal application.

Both the methods of inoculation at the time of nematicidal application recorded either none or only a few nematodes either in the soil or in the root tissue. Also, the number of galls were few. However, this was not true when nematodes/infested chopped roots were inoculated three weeks after the nematicidal application indicating that this timing of inoculation might provide better differentiation in nematicidal efficacy. Further, in comparison to chopped root inoculation, the nematode population in root tissue and the number of galls/plant was more when the nematode larvae were inoculated. While it was increasing with the time in the case of inoculation of 5 g chopped root galls/pot, it showed a fall in respect of inoculation of 2000 larvae. These results showed that nematode population in soil would not be a reliable criteria for evaluating the efficacy of nematicides. Since the root tissue observation was more complicated and time consuming, it would be better to count the number of galls/plant. Also, since there was not much

difference in the results after 3 weeks, the observations upto three weeks after transplanting would be sufficient to give a reliable estimate of the nematicidal efficacy.

## SUMMARY

Six experiments viz. (i) comparative efficiency of soil samplers, (ii) distribution and sampling of nematodes, (iii) comparative efficiency of nematode extraction techniques, (iv) effect of storage bag, storage temperature, storage period and extraction techniques on recovery of nematodes from the soil sample, (v) quantitative estimation of nematode population density by six extraction procedures as affected by season and seasonal fluctuation in nematode population and (vi) evaluation of nematicidal efficiency with two techniques and timings of nematode inoculations, were carried out for evaluating some techniques in Nematology.

Four types of sampling tools were used to test their efficiency. In general, maximum number of nematodes were obtained when the soil samples were collected with the help of a hollow tube-type sampler with 2.5 cm diameter. This was followed by O'Connor's split corer. Sampling with Khurpi and hollow tube-type sampler with 1.0 cm diameter provided lower number of nematodes.

With a few exceptions, irrespective of sampling tools used, 15 probes per sample provided maximum number of nematodes indicating that if there is a wide range in the possible results of our sample, we are the more likely to happen upon 'exceptional' cores which will throw our average off the true value.

Nematodes were distributed in patches of varying densities. It was estimated that a sample of 4 units taken from one random row or one unit taken from 4 random rows consisting of 16 per cent of the total number of units would give the estimate with the error of 20 per cent of the mean. The population was so predominantly heterogenous that we cannot expect to get the estimate with a percentage error below 20 per cent.

Eleven techniques have been compared to evaluate their efficiency in nematode extraction from all samples. The average number of nematodes obtained by the use of various techniques ranged from 27 to 1848.

On the basis of number of nematodes obtained the extraction techniques could be grouped into three categories, namely : efficient, moderately efficient and poor.

Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue, Cobb's modified decanting and sieving + centrifugal flotation, Cobb's modified decanting and sieving + sugar flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue and Cobb's modified decanting and sieving technique might be placed in the efficient category and amongst these Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue was superior to others except in respect of Trichodorus in which case this technique could be categorised <sup>as</sup> moderately efficient to poor.

The techniques which could be placed in moderately efficient category are Oostenbrink's elutriator with double layer of facial tissue paper; sugar flotation sieving, with 12.5 ppm Separan SP<sub>7</sub>; sugar flotation sieving with 12.5 ppm Separan PG<sub>2</sub> and centrifugal flotation.

The techniques which could be placed in poor category are direct soil placement over moistened facial tissue paper; Oostenbrink's elutriator with double layer of cotton wool filter; Oostenbrink's elutriator with single layer of cotton wool filter, Cobb's modified decanting and sieving + sugar flotation sieving + 12.5 ppm Separan PG<sub>2</sub> + 5 ppm methylene blue and Seinhorst's erlenmeyer flask method.

Studies were carried out to see the effect of two types of storage bags, eight storage temperatures, six storage periods and <sup>three</sup> extraction techniques on the recovery of nematodes.

In general, polythene bag was superior to paper bag; 15°C was the best storage temperature; one week storage period was most satisfactory and Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP<sub>7</sub> + 5 ppm methylene blue was the best extraction technique.

Two experiments were designed to compare the efficiency of six nematode extraction methods in measuring seasonal population shifts to plant-parasitic and free-living nematodes. The first experiment was carried out in a field

given to maize-wheat rotation and the second experiment comprised of 8 crop rotations.

Two experiments with different nematodes showed highly significant interactions between each technique and time. Irrespective of crop rotations, the maximum variation in the number of nematode obtained by different methods was 5-6 times, except in respect of Trichodorus and Heterodera larvae in which case the variation was only upto 2-3 times.

In general, irrespective of nematode and time of sampling CSCFSM was superior to CSSF, except in the case of Trichodorus which was found only in respect of 8 crop rotations. CSSF was either superior or equal to CSSSM except in a few cases of Trichodorus. In respect of total plant-parasitic nematodes, however, it was mostly superior. With Hoplolaimus and Pratylenchus while in case of maize-wheat rotation it was superior as in respect of September and July respectively, in case of 8 crop rotations it was better than CSSSM in 50 per cent cases. In case of Tylenchorhynchus, however, irrespective of crop rotations it was only 3-4 times superior to CSSSM. With Helicotylenchus and free-living nematodes it was 6-10 times better than CSSSM.

DT was always inferior to CSCFSM except in the case of Trichodorus and once in the case of Tylenchorhynchus in respect of 8 crop rotations.

With Tylenchorhynchus there were 3 peaks (October, January and April) in case of maize-wheat rotation and 2 peaks (October and April) in respect of 8 crop rotation. The peaks

were appreciable in case of CSCFSM and CSCF<sup>and</sup> to some extent<sup>in</sup> CS in respect of 8 crop rotations. This was not very appreciable in case of maize-wheat rotation except in case of CSCFSM. With Hoplolaimus one peak (April) was observed in maize-wheat rotation and two peaks (September and April) in case of 8 crop rotations. With Pratylenchus there was one peak (in April). With Helicotylenchus also there was only one peak which was noticed during March-April. Heterodera larvae were found in respect of 8 crop rotations, which showed only one peak (in May). With Trichodorus there was not much fluctuation in the population except in August and September. With free-living nematodes there was one peak (in February) in respect of 8 crop rotations and one peak (in May) in case of maize-wheat crop rotation. There was a marked fall from April to July-August in case of total plant-parasitic nematodes. From December to April there was a gradual increase in the population. However, there was a marked peak (in April) only in respect of 8 crop rotations.

Host crops seem to affect the prevalence of nematode genera because seasonal peaks of the nematodes corresponded to the maximum growth of the host crop. The seasonal effects were confounded with the stages of crop growth.

Pot studies were carried out to evaluate the nematicidal efficacy with two techniques and timings of nematode inoculation. On the basis of nematode population in soil/root-tissue and number of galls/plant, both techniques and timings of nematode inoculation could be placed in<sup>the</sup> following order: Inoculation

of 2000 larvae/pot, one week after transplanting/3 weeks after nematicidal application; inoculation of 5 g chopped infested root-gall/pot one week after transplanting/3 weeks after nematicidal application; inoculation of 5 g chopped infested root-gall/pot at the time of nematicidal application; inoculation of 2000 larvae/pot at the time of nematicidal application.

Both the methods of inoculation at the time of nematicidal application recorded either none or only a few nematodes either in the soil or in the root-tissue. Also, the number of galls were few. However, this was not true when nematodes/infested chopped root-galls were inoculated three weeks after the nematicidal application indicating that this timing of inoculation might provide better differentiation in nematicidal efficacy. Further, in comparison to chopped root-inoculation, the nematode population in root-tissue and the number of galls per plant was more, when the nematode larvae were inoculated.

Nematode population in soil would not be<sup>a</sup> reliable criteria for evaluating the efficacy of nematicides. Since the root-tissue observation was more complicated and time consuming, it would be better to count the number of galls/plant. Also, the observations upto three weeks after transplanting would be sufficient to give a reliable estimate of the nematicidal efficacy.

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\*Original not seen.

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APPENDIX - I

Effect of storage temperature, storage bag, extraction technique and storage period (week) on the recovery of Tylenchorhynchus from the soil samples

Storage Temp. (°C)	Polythene bag											
	Extraction techniques											
	Oostenbrink's elutriator				Cobb's modified decanting & sieving tech.							
	Storage period (weeks)											
	1	2	3	4	6	8	1	2	3	4	6	8
0	146.7	86.7	63.3	0	0	0	163.3	93.3	73.3	0	0	0
5	143.3	140.0	163.3	110.0	63.3	40.0	173.3	160.0	180.0	116.7	110.0	53.3
10	145.0	143.3	175.0	123.3	85.0	53.0	163.3	165.0	165.0	135.0	103.0	63.0
15	135.0	145.0	163.3	135.0	103.3	80.0	150.0	163.0	183.0	143.0	125.0	105.0
20	143.3	175.0	153.3	110.0	85.0	43.0	183.3	153.3	165.3	153.3	106.7	81.7
25	113.3	113.3	103.3	53.3	45.3	13.3	163.3	133.3	123.3	75.0	51.7	33.3
30	123.3	83.3	81.3	43.3	33.3	23.3	143.3	103.3	103.3	53.3	43.3	31.3
Room temp. (15-20°C)	133.3	133.3	113.3	103.3	100.3	83.3	155.0	155.0	133.3	121.3	112.2	93.3

Storage Temp. (°C)	Polythene bag											
	Extraction techniques											
	Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7+5 ppm methylene blue											
	Storage period (weeks)											
	1	2	3	4	6	8	1	2	3	4	6	8
0	196.7	106.7	86.7	0	0	0	196.7	106.7	86.7	0	0	0
5	203.3	195.0	218.3	153.3	150.0	61.7	203.3	195.0	218.3	153.3	150.0	61.7
10	195.0	193.3	103.3	145.0	125.0	25.0	195.0	193.3	103.3	145.0	125.0	25.0
15	153.3	175.0	218.0	178.0	153.3	121.7	153.3	175.0	218.0	178.0	153.3	121.7
20	206.7	183.3	193.3	153.0	123.3	85.0	206.7	183.3	193.3	153.0	123.3	85.0
25	185.0	185.0	163.3	83.3	63.3	43.3	185.0	185.0	163.3	83.3	63.3	43.3
30	153.3	133.3	133.3	53.3	50.1	40.3	153.3	133.3	133.3	53.3	50.1	40.3
Room temp. (15-20°C)	173.3	173.3	153.3	140.3	131.3	111.7	173.3	173.3	153.3	140.3	131.3	111.7

CONTD.

Storage temp. (°C)		Paper bag											
		Oostenbrink's elutriator						Cobb's modified decanting & sieving tech.					
		Extraction techniques											
		Storage period (weeks)											
		1	2	3	4	6	8	1	2	3	4	6	8
0	133.3	53.3	123.3	33.3	0	0	0	123.3	63.3	43.3	0	0	0
5	133.3	123.3	56.7	43.3	23.3	0	0	133.3	143.3	43.3	43.3	33.3	0
10	83.3	93.3	93.3	73.3	53.3	33.3	33.3	120.0	133.3	123.3	93.3	73.3	53.3
15	123.3	106.7	68.7	63.3	53.3	23.1	23.1	128.0	126.6	87.7	53.3	48.3	40.3
20	113.3	105.0	73.3	62.2	48.2	43.3	43.3	153.3	133.3	123.3	103.3	80.3	33.3
25	83.3	87.3	83.3	43.3	23.3	8.3	8.3	148.3	83.3	133.3	70.3	28.3	13.3
30	73.3	63.3	56.7	30.0	13.0	6.6	6.6	123.3	93.3	73.3	60.0	33.3	21.0
Room temp. (15-20°C)		103.3	93.3	93.3	80.0	40.3	18.3	126.3	123.3	116.7	90.0	73.3	35.0

Storage temp. (°C)		Paper bag											
		Extraction techniques											
		Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7+5 ppm methylene blue											
		Storage period (weeks)											
		1	2	3	4	6	8	1	2	3	4	6	8
0	143.3	93.3	63.3	63.3	0	0	0	0	43.3	10.5	0	0	0
5	163.3	163.3	83.3	83.3	63.3	63.3	63.3	43.3	93.3	33.3	33.3	33.3	10.5
10	173.3	153.3	113.3	113.3	103.3	103.3	103.3	70.3	70.3	60.7	60.7	60.7	43.3
15	153.3	143.3	103.3	103.3	83.3	83.3	83.3	75.3	75.3	43.3	43.3	43.3	20.8
20	193.3	163.3	150.5	150.5	140.3	140.3	140.3	33.3	33.3	20.8	20.8	20.8	30.3
25	163.3	133.3	106.7	106.7	70.0	70.0	70.0	50.3	50.3	30.3	30.3	30.3	50.0
30	133.3	103.3	83.3	83.3	60.3	60.3	60.3	80.3	80.3	50.0	50.0	50.0	50.0
Room temp. (15-20°C)		123.3	133.3	133.3	153.3	113.3	113.3	80.3	80.3	50.0	50.0	50.0	50.0

APPENDIX - II

Effect of storage temperature, storage bag, extraction technique and storage period (week) on the recovery of Hoplolaimus from the soil samples

		Polythene bag											
		Extraction techniques											
		Cobb's modified decanting and sieving tech.											
		Oostenbrink's elutriator											
		Storage period (weeks)											
Storage temp. (°C)		1	2	3	4	6	8	1	2	3	4	6	8
0	21.7	18.3	13.3	13.3	0	0	0	25.0	21.7	20.0	0	0	0
5	13.3	13.3	15.0	20.0	20.0	10.0	10.0	26.6	23.3	21.0	20.0	15.0	12.0
10	25.0	18.7	20.0	16.7	15.7	9.7	9.7	35.3	28.0	26.6	25.0	21.7	15.0
15	15.0	20.0	15.0	15.0	15.0	6.7	6.7	33.3	23.3	20.0	19.0	18.0	11.7
20	25.0	20.0	18.0	16.0	13.0	0	0	36.0	25.0	18.0	15.5	13.0	0
25	18.3	14.7	13.3	0	0	0	0	23.3	17.3	15.0	0	0	0
30	13.3	13.3	0	0	0	0	0	23.3	13.3	0	0	0	0
Room temp. (15-20°C)	21.3	21.3	21.3	18.5	8.3	8.3	8.0	23.3	23.3	21.3	17.0	13.3	10.3

		Polythene bag											
		Extraction techniques											
		Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP <sub>7</sub> + 5 ppm methylene blue											
		Storage period (weeks)											
Storage temp. (°C)		1	2	3	4	6	8	1	2	3	4	6	8
0	43.3	36.7	26.7	26.7	0	0	0	26.7	36.7	26.7	0	0	0
5	25.3	25.0	20.0	20.0	13.3	10.3	10.3	20.0	25.0	13.3	9.5	9.5	9.5
10	53.3	45.0	43.0	43.0	35.0	33.3	33.3	43.0	45.0	35.0	23.3	23.3	23.3
15	45.0	40.0	25.0	25.0	18.0	19.0	15.0	25.0	40.0	18.0	15.0	15.0	15.0
20	46.0	43.0	38.0	31.0	31.0	20.0	0	38.0	43.0	31.0	0	0	0
25	41.7	25.0	20.0	0	0	0	0	25.0	25.0	0	0	0	0
30	33.3	23.3	0	0	0	0	0	23.3	23.3	0	0	0	0
Room temp. (15-20°C)	23.3	23.3	23.3	20.0	20.0	16.7	10.0	23.3	23.3	20.0	16.7	10.0	10.0

Contd.

Storage temp. (°C)	Paper bag											
	Oostenbrink's elutriator				Extraction techniques							
	Cobb's modified decanting and sieving tech.											
	Storage period (weeks)											
	1	2	3	4	6	8	1	2	3	4	6	8
0	13.3	10.0	8.3	0	0	0	23.3	18.3	13.3	0	0	0
5	8.3	13.3	6.6	3.3	3.3	0	20.0	13.3	18.3	13.3	10.6	0
10	13.3	13.3	8.0	6.6	0	0	26.3	23.3	10.3	8.3	0	0
15	18.3	16.3	18.0	8.3	8.3	0	19.3	18.3	10.0	8.5	8.3	0
20	23.3	18.3	13.3	8.3	3.3	0	30.3	23.3	16.7	10.7	8.3	0
25	16.6	10.6	6.6	0	0	0	21.0	13.3	8.7	0	0	0
30	11.3	8.7	0	0	0	0	23.3	16.0	0	0	0	0
Room temp. (15-20°C)	13.3	8.3	13.3	0	0	0	13.3	8.3	5.0	0	0	0

Storage temp. (°C)	Paper bag											
	Extraction techniques				Extraction techniques							
	Cobb's modified decanting and sieving+centrifugal flotation with 12.5 ppm Separan SP <sub>7</sub> +5 ppm methylene blue											
	Storage period (weeks)											
	1	2	3	4	6	8	1	2	3	4	6	8
0	33.3	23.3	21.6	0	0	0	23.3	18.3	16.7	36.6	10.3	0
5	21.7	20.0	18.0	15.0	8.8	0	20.0	13.3	13.3	25.7	10.0	0
10	38.3	28.3	18.3	13.3	0	0	28.3	13.3	13.3	13.3	0	0
15	43.3	28.3	16.7	13.3	13.3	0	37.7	25.7	10.3	0	0	0
20	43.3	37.7	36.6	25.7	10.0	0	23.3	8.3	0	0	0	0
25	28.3	23.3	10.3	0	0	0	8.3	0	0	0	0	0
30	13.3	8.3	0	0	0	0	0	0	0	0	0	0
Room temp. (15-20°C)	30.3	17.5	0	0	0	0	17.5	0	0	0	0	0

APPENDIX - III

Effect of storage temperature, storage bag, extraction techniques and storage period (week) on the recovery of Pratylenchus from the soil samples

Polythene bag													
Extraction techniques													
Oostenbrink's elutriator													
Storage temp. (°C)	Storage period (weeks)								8				
	1	2	3	4	6	8	1	2		3			
0	31.7	23.3	15.0	0	0	0	0	60.0	26.7	26.7	0	0	0
5	40.0	35.0	30.0	15.0	10.0	0	0	60.7	45.0	36.0	20.0	12.6	0
10	35.0	25.0	20.0	13.3	13.0	8.3	0	33.3	33.3	23.3	20.0	13.0	13.0
15	28.0	25.0	21.7	18.0	18.0	16.0	0	65.0	63.0	35.0	23.5	21.0	15.0
20	35.0	25.0	20.0	18.7	10.7	8.3	0	45.0	35.0	31.6	28.7	16.7	15.3
25	35.0	28.6	15.7	10.0	0	0	0	55.0	41.3	33.3	20.6	0	0
30	23.3	23.3	13.3	6.5	0	0	0	30.0	21.7	20.0	18.7	0	0
Room temp. (15-20°C)	33.3	23.3	21.0	16.0	6.6	3.5	53.3	40.3	33.3	25.5	16.6	12.6	

Polythene bag													
Extraction techniques													
Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7+5 ppm methylene blue													
Storage temp. (°C)	Storage period (weeks)								8				
	1	2	3	4	6	8	1	2		3			
0	70.0	38.0	31.0	0	0	0	0	0	0	0	0	0	0
5	63.3	55.3	50.3	41.6	43.3	25.0	20.0	20.3	20.0	20.0	20.0	20.0	0
10	65.0	65.0	58.3	58.3	35.3	28.0	15.8	25.0	28.0	28.0	20.3	20.3	0
15	83.7	81.3	58.3	38.3	33.3	23.3	15.0	23.3	38.3	23.3	20.3	20.3	0
20	48.3	40.0	21.7	20.6	20.6	15.0	0	15.0	21.7	15.0	0	0	0
25	65.0	45.0	31.7	25.0	25.0	0	0	0	31.7	0	0	0	0
30	41.7	33.3	33.3	25.3	25.3	20.3	18.3	20.3	33.3	20.3	20.3	18.3	18.3
Room temp. (15-20°C)	60.3	43.3	33.3	25.3	25.3	20.3	18.3	20.3	33.3	20.3	20.3	18.3	18.3

Contd.

Storage temp. (°C)		Paper bag										
		Oostenbrink's elutriator				Cobb's modified decanting and sieving tech.						
		Storage period (weeks)										
1	2	3	4	6	8	1	2	3	4	6	8	
0	30.0	13.3	11.3	0	0	0	43.3	23.3	20.3	0	0	0
5	34.3	23.3	13.3	8.3	8.3	0	46.7	26.7	26.7	8.3	8.3	0
10	33.3	23.3	23.3	13.3	10.3	8.0	33.3	23.3	13.3	10.3	8.3	6.3
15	26.3	23.3	18.3	13.3	8.3	6.7	43.3	33.3	13.3	13.3	8.3	6.7
20	33.3	23.3	18.0	17.1	5.1	0	43.3	33.3	23.3	19.0	16.0	0
25	33.3	28.7	8.3	6.3	0	0	40.3	33.3	18.3	13.3	0	0
30	26.3	18.3	8.3	5.0	0	0	23.3	13.3	8.3	0	0	0
Room temp. (15-20°C)	26.3	16.7	8.3	0	0	0	46.6	33.3	28.7	0	0	0

Storage temp. (°C)		Paper bag										
		Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7+5 ppm methylene blue										
		Storage period (weeks)										
1	2	3	4	6	8	1	2	3	4	6	8	
0	33.3	33.3	21.3	0	0	0	0	0	0	0	0	0
5	56.3	28.3	18.3	16.3	11.3	0	11.3	0	0	0	0	0
10	53.3	23.3	23.3	21.7	17.3	10.3	17.3	10.3	10.3	10.3	10.3	10.3
15	58.3	43.3	28.2	23.3	20.3	0	20.3	0	0	0	0	0
20	48.3	23.3	20.0	13.0	8.3	0	13.0	0	0	0	0	0
25	43.3	36.7	18.3	8.3	0	0	8.3	0	0	0	0	0
30	18.3	16.7	13.3	0	0	0	13.3	0	0	0	0	0
Room temp. (15-20°C)	53.3	36.6	30.0	11.7	0	0	11.7	0	0	0	0	0

APPENDIX - IV

Effect of storage temperature, storage bag, extraction technique and storage period (week) on the recovery of *Helicotylenchus* from the soil samples

Storage temp. (°C)	Polythene bag											
	Extraction techniques											
	Oostenbrink's elutriator											
	Cobb's modified decanting and sieving tech.											
	Storage period (weeks)											
	1	2	3	4	6	8	1	2	3	4	6	8
0	186.7	163.3	100.0	0	0	0	286.7	246.3	210.0	0	0	0
5	290.0	213.3	193.3	183.3	153.3	96.0	313.3	296.7	286.7	193.3	170.3	103.3
10	185.3	175.0	165.0	150.0	135.0	103.3	263.3	253.3	205.0	200.0	190.0	135.0
15	303.3	275.0	185.0	161.0	160.3	145.0	423.3	393.3	313.3	220.0	181.7	160.3
20	283.3	266.7	175.0	125.0	103.3	100.0	323.3	303.3	265.0	240.0	203.3	123.3
25	236.7	203.3	163.3	103.3	85.0	33.3	363.3	303.3	243.3	173.3	143.3	43.3
30	213.3	183.3	103.3	103.3	53.3	0	346.7	243.3	143.3	143.3	73.3	0
Room temp. (15-20°C)	233.3	216.7	183.3	153.3	133.3	123.3	303.3	283.3	283.3	233.3	201.3	183.3

Storage temp. (°C)	Polythene bag											
	Extraction techniques											
	Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue											
	Storage period (weeks)											
	1	2	3	4	6	8	1	2	3	4	6	8
0	295.0	255.0	211.7	0	0	0	183.7	0	0	0	0	0
5	350.7	341.7	326.7	283.7	245.0	233.3	233.3	160.0	153.3	153.3	153.3	160.0
10	273.3	263.3	253.3	245.0	318.3	260.0	260.0	188.3	188.3	188.3	188.3	188.3
15	458.3	413.3	345.0	318.3	245.0	213.3	213.3	125.0	125.0	125.0	125.0	125.0
20	365.0	316.7	266.7	245.0	313.3	173.3	173.3	133.3	133.3	133.3	133.3	133.3
25	413.3	383.3	363.3	313.3	163.3	0	153.3	0	0	0	0	0
30	393.3	283.3	166.7	163.3	290.7	250.3	250.3	123.3	123.3	123.3	123.3	123.3
Room temp. (15-20°C)	393.3	313.3	293.3	290.7	250.3	250.3	250.3	123.3	123.3	123.3	123.3	123.3





Cont'd

Storage temp. (°C)	Paper bag											
	Oostenbrink's elutriator				Cobb's modified decanting and sieving test							
	1	2	3	4	5	6	7	8				
0	21.7	10.0	0	0	0	0	16.7	15.0	0	0	0	0
5	13.3	13.0	0	0	0	0	13.3	13.3	0	0	0	0
10	25.0	8.3	0	0	0	0	33.3	13.3	0	0	0	0
15	25.0	16.7	0	0	0	0	30.0	23.3	0	0	0	0
20	18.3	10.3	0	0	0	0	23.3	13.3	0	0	0	0
25	13.3	0	0	0	0	0	15.3	0	0	0	0	0
30	10.3	3.3	0	0	0	0	23.3	13.3	0	0	0	0
Room temp. (15-20°C)	20.3	13.3	0	0	0	0	23.3	15.3	0	0	0	0

Storage temp. (15-20°C)	Paper bag					
	Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue			Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue		
	1	2	3	4	5	6
0	13.3	6.7	0	0	0	0
5	17.7	6.7	0	0	0	0
10	28.3	23.3	0	0	0	0
15	24.1	15.0	0	0	0	0
20	13.3	8.3	0	0	0	0
25	18.3	0	0	0	0	0
30	18.3	10.3	0	0	0	0
Room Temp. (15-20°C)	18.3	11.3	0	0	0	0



Cont'd

		Paper bag											
		Costenbrink's elutriator					Cobb's modified decanting and sieving tech.						
		Extraction techniques											
		Storage period (weeks)											
		1	2	3	4	6	8	0	2	3	4	6	8
Storage temp. (°C)	0	336.7	223.3	128.3	108.7	108.3	233.3	430.0	373.3	173.3	158.3	141.7	316.7
	5	365.0	283.3	141.7	136.7	115.0	48.3	485.0	388.3	346.6	188.3	213.3	108.3
	10	371.7	315.0	220.0	225.0	121.7	160.0	561.7	396.7	315.0	358.0	125.0	253.3
	15	400.3	295.0	248.3	178.3	143.3	58.3	568.0	453.3	350.0	286.7	198.3	110.0
	20	505.0	383.3	225.0	126.7	76.7	21.7	556.7	431.7	391.7	266.7	116.7	26.7
	25	326.7	381.7	246.7	103.3	56.7	14.7	536.7	318.3	186.7	145.0	116.7	15.7
	30	413.3	281.7	135.0									
Room temp. (15-20°C)		438.3	273.3	323.2	203.3	155.0	95.0	541.3	365.0	351.3	320.0	220.0	155.0

		Paper bag							
		Extraction techniques							
		Cobb's modified decanting and sieving + centrifugal flotation with 12.5 ppm Separan SP7 + 5 ppm methylene blue							
		Storage period (weeks)							
		1	2	3	4	6	8	0	2
Storage temp. (°C)	0	471.3	391.7	218.3	168.3	205.0	183.3	0	133.3
	5	585.0	450.0	251.7	241.7	190.0	160.0	0	160.0
	10	655.0	480.0	356.7	323.3	278.3	198.3	0	198.3
	15	753.3	555.0	388.3	341.7	251.7	141.7	0	141.7
	20	688.8	555.0	428.0	271.7	116.7	61.7	0	61.7
	25	640.0	455.0	386.7	178.3	146.3	40.2	0	40.2
	30	586.7	365.0	216.7				0	
Room temp. (15-20°C)		600.0	483.3	445.0	365.0	295.0	216.7	0	216.7

