MATERIALS AND METHODS

A survey was conducted for plant parasitic nematodes associated with cabbage and cauliflower during August 1964 to September 1968. A mixture of roots and soil weighing about ½ to 1 kg was collected in alkathene bags from around the roots of cabbage and cauliflower from different districts of Uttar Pradesh. A tag containing relevant information was introduced in each bag and later they were tied with a rubber band. Every effort was made to isolate nematodes from each soil sample on the day of collection. Those soil samples which could not be processed were stored in a refrigerator running at 5-10°C, till the isolation was made.

The soil was processed by a modification of Cobb's sifting and gravity method. The soil sample was put in a large bucket containing water and stirred until all clods were broken. The heavy soil particles sank to the bottom and the nematodes remained suspended in water. The water was then poured through the course sieve (60 mesh), leaving the heavy particles in the bucket. The whole aliquot was then passed through 200 and 300 mesh sieves. The catch of all the sieves were mixed up and the quantity of water was reduced by decantation allowing sufficient time for the nematodes to settle down. The suspension containing the nematodes were poured over a Green's filter paper no. 50 appended on a coarse sieve and submerged in water in a
large trough. Occasionally Cotton-wool filter in place of Green's filter paper was also used (Oostenbrink, 1960). Care was taken not to allow the debris to float-off the edges of the filter. After 24 hours most of the nematodes wriggled out at the bottom of the trough in clear water. These were later concentrated by decantation.

The roots were removed from the soil with utmost care and washed slowly in running water to get rid of the adhering soil particles. They were then chopped into small pieces, the largest piece not exceeding 1 cm in length. These pieces were transferred to a large size petriplate filled with water. After 24 hours the aliquot was passed through 60, 250 and 300 meshes. The sieve on the top removed the root debris, while the nematodes were collected in the clear water. At times 4-5 g root pieces with small amount of water were placed in Waring blender and the blender was allowed to run for 30-50 seconds. Whenever it was considered necessary to make permanent slides the nematodes were killed by slow heating and fixed in FA 4:10. Permanent mounts of nematodes were prepared by Siddiqi's (1964) technique after passing through lactophenol. Baker's (1953) modified technique was also used.

Root samples were carefully washed in running water and immediately immersed in boiling 0.1 per cent
cotton blue lactophenol for about three minutes and were allowed to cool. The roots were then washed in running water to remove excess of stain. Infested portions of the roots were studied after preparing a temporary mount in lactophenol solution.

*T. brassicae* collected from the type locality was raised by inoculating cabbage and cauliflower seedlings grown in 12-inches clay pots filled with autoclaved soil-sand mixture. Great care was taken to avoid external contamination of other parasitic forms. On comparison with the type specimens of *T. brassicae*, some minor differences in morphological structures of diagnostic value at specific level were observed, such as position of excretory pore, shape and size of the spear, number of tail annules and size of spicules. Instead of raising a new species, it was considered to take all these characters under variations. *(Fig. 1)*

The seedling of the test plants were raised in wooden trays containing autoclaved soil-sand-organic manure mixture in the ratio of 3:1:1. After 3 weeks they were transplanted in 6 inches test pots. For certain *plants* crops where transplantation was not possible, the seeds were sown directly in the test pots.

The nematodes extracted from the pots containing culture of *T. brassicae* served as inoculum. For experiments
Figure 1.

*Tylenchorhynchus brassicae*

A. Oesophageal region of male.

B. Female, entire.

C. Oesophageal region of female.

D. Tail end of male.

E-F. Variations in female tail.
dealing with host tolerance, the seedlings were inoculated each with 10, 100, 1000 and 10000 specimens of *T. brassicae*, while in host range tests, each plant was inoculated with 1000 nematodes. For counting the nematodes 1 ml of nematode suspension was pipetted in Peter's counting slide and the number of nematodes counted. Calculations were made for total suspension. The size and weight of controlled and inoculated plants as well as the final population of nematodes was determined 90 days after inoculation of the plants.

Cabbage and cauliflower of the same age and the same leaf number were grown in autoclaved soil-sand-organic manure mixture having 33 per cent moisture holding capacity contained in six inches glazed crocks. Constant soil moisture was maintained by adding requisite amount of water for the values above 33 per cent or the pots were allowed to dry for values below 33 per cent. The pots were weighed from time to time for the maintenance of constant soil moisture levels. The surface of the pots were covered with plastic sheets to check evaporation.

Effect of soil temperature on the population of *T. brassicae* was studied after transferring the seedlings of cabbage and cauliflower in six inches pots placed in temperature tanks, each running at 15, 20, 25, 30, 35 and
40°C respectively with air temperature of glass-house at 25°C.

The effect of different temperatures and moisture levels on the longevity of *T. brassicae* was studied by inoculating 1000 nematodes in soil-sand mixture contained in six inches pots without the host plant. Constant temperature and moisture were maintained as mentioned above.

The decline in population in the absence of host plant under glass house condition was also studied by inoculating 10, 100, 1000 and 10000 nematodes each in soil-sand mixture contained in 6 inches pots. The nematode population was estimated for 8 months at monthly interval.

To determine the seasonal fluctuations in the population of *T. brassicae* soil samples were collected from two different fields of cabbage and cauliflower. Three soil samples each weighing approximately ½ kg were collected upto the depth of 10-30 cm from each of the six beds selected for study. Soil temperature and soil moisture was recorded at the time of collecting the sample. The three samples thus collected from each bed were thoroughly mixed. A sample of 250 g soil out of this mixture was processed for the isolation of nematodes and the number of *T. brassicae* was counted as given on page 10.
To observe the seasonal fluctuations in the population of *T. brassicae* at different depths from field "A" three shafts were dug to the depth of 60 cm from one wall of each, soil samples were collected with the help of metallic cylinders of 10 cm in diameter. In all four samples were taken from each shaft starting from the bottom to prevent contamination by falling soil particles. The soil temperature as well as moisture was recorded at different depths.

The field experiments were conducted in test plots each of 64 sq. ft. harbouring a high population of *T. brassicae* (1560/250 g soil). The amount of oilcakes of *Azadirachta indica* Juss., *Madhuca indica* Gmel., *Ricinus communis* L., *Arachis hypogaea* L., *Brassica nigra* L (Koch) and inorganic fertilizers applied to each plot was calculated on the basis of 80 lb of nitrogen per acre as recommended for cabbage and cauliflower (Yawaker et al. 1962). Plots treated with inorganic fertilizer i.e. a mixture of ammonium sulphate, superphosphate and potassium sulphate served as control.

For pot tests organic amendments and inorganic fertilizers at rates indicated above were added to autoclaved soil-sand mixture contained in 8 inches pots. The field as well as pots were watered. After a waiting period
fifteen days, 3-week old seedlings raised in autoclaged soil in each plot were transplanted with a spacing of 2 ft. in a row and the rows were 2 ft. apart. One seedling was transplanted per pot. Later, it was inoculated with 1000 specimens of *T. brassicae*.

The oilcakes were deoiled by using petroleum ether (B.P. 40°C), except in castor cake where chloroform was used as a solvent. The deoiling was done in Soxlet's apparatus by the solvent extraction process. Each oilcake was packed in a filter paper of a convenient size and was placed in the apparatus. The extraction was allowed to continue for about eight hours. The residue obtained after this treatment have been named as deoiled cakes.

The extracts of oilcakes and deoiled cakes were prepared by adding 50 g of each of the oiled and deoiled manure in 200 g of distilled water which was thoroughly mixed in Waring blender for two minutes and then filtered. Filterate thus obtained has been termed as Standard solution "S". Later different dilutions viz., S/2, S/10, S/50, S/100 and S/1000 were prepared in distilled water. To 10 ml solution contained in 2 inches petridishes of each of the above dilution, 1000 specimens of *T. brassicae* were transferred. They were then kept in an incubator running at 20°C. The counting of living and dead nematodes was made after 12, 48 and 96 hours. Nematodes placed in distilled water served as control.
The effect of different organic additives on the population of *T. brassicae* in the absence of host was studied in 8 inches pots. To each pot 2 kg soil heavily infested with *T. brassicae* (8000 specimens/kg) and treated with organic amendments at rates indicated on page 12 was transferred. Pots were watered at regular intervals.

DD and Vapam were applied with a hand gun injector to properly prepared beds at the rate of 40 gallons and 25 gallons per acre respectively. After a waiting period of three weeks, following the application of the nematicide seeds of cabbage and cauliflower were sown.

For glass-house tests, taking all these precautions the pots containing soil-sand organic manure mixture were also treated with these nematicides at the same rate.

To test the efficacy of granular pesticides the recommended dose of Nemaphos was dissolved in water, and the solution thus obtained was sprayed on soil surface, with Solvirex, Thimet 10G and Rogor G were mixed in soil.

The field experiments were conducted in plots measuring 64 sq. ft. with three replicates, while the pot experiments were based on ten replicates. After the application of the pesticides, the cabbage and cauliflower seedlings were transplanted on the following day.
The solution of DD and Vapam were prepared by adding 1 ml of each nematicides in 100 ml of distilled water, while the standard solutions of different pesticides were prepared by dissolving 1 g of the pesticide in 100 ml of water. Later, different dilutions viz., 0.002, 0.001, 0.0002, 0.0001 and 0.00001 were prepared by diluting the standard solution with distilled water. For estimating the percentage mortality of *T. brassicae*, the procedure adopted was the same as given for oilcakes.

For determining the long term effect of DD, Vapam, Nemaphos, Thimet 10G, Solvirex and Rogor G, the seed beds were treated with these pesticides as mentioned above. Later, 5-week old seedlings of cabbage and cauliflower were carefully uprooted from treated as well as untreated beds and were transplanted in the field. The nematode population of the field and weight of the plant was determined at the time of transplanting and also at the time of harvest of the crop.

Two kg autoclaved soil was filled in 8 inches pots and each was inoculated with 10,000 specimens of *T. brassicae*. Immediately after inoculation, the pots were either treated with DD or Vapam or the soil was autoclaved. Pots inoculated with *T. brassicae* and not subjected to the above treatment served as control. There were five replicates for each
treatment. Seeds were sown after the waiting period in each of the above categories of treatments. The population of nematodes and weight of the plant was determined as in field experiments.